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Halenaquinone and xestoquinone derivatives, inhibitors of Cdc25B phosphatase from a *Xestospongia* sp.

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Abstract—Separation of an extract of a *Xestospongia* sp., guided by bioassay against Cdc25B, led to the isolation of nine compounds, halenaquinone (**1**), xestoquinone (**2**), adociaquinones A (**3**) and B (**4**), 3-ketoadociaquinones A (**5**) and B (**6**), tetrahydrohalenaquinones A (**7**) and B (**8**), and 13-*O*-methyl xestoquinol sulfate (**9**). The structures of the new natural products **6** and **9** were established on the basis of extensive one- and two-dimensional NMR studies. Compounds **1**, **4**, and **6** inhibited recombinant human Cdc25B in vitro with IC_{50} values of 0.7, 0.07, and 0.2 μM, respectively, and were 19- to 150-fold less active against two related protein phosphatases. Compound **4** blocked cell cycle progression through mitosis.

1. Introduction

The dual specificity protein–tyrosine phosphatase (dsPTPase), CDC25, plays a pivotal role in the regulation of the cell cycle. The human genome encodes three Cdc25 homologues, Cdc25A, B, and C. Cdc25A is thought to activate CDK2/cyclin E and thereby trigger the G1/S transition of the cell cycle. Cdc25B appears to play a role in G2 by regulating CDK2/cyclin A and CDK1/cyclin A. Cdc25C activates the CDK1/cyclin B enzyme by dephosphorylating Thr14 and Tyr15 residues on CDK1, thereby triggering the G2/M transition.[1,2] Cdc25 has been a screening target to identify anti-mitotic natural products,[3] and some compounds have been reported as Cdc25 inhibitors.[4–10]

Bioassay-directed separation of an extract of a sponge of the genus *Xestospongia* showed reproducible activity against the catalytic domain of human Cdc25B and fractionation against this enzyme led to the isolation of nine compounds with inhibitory activity: halenaquinone (**1**),[11] xestoquinone (**2**),[12] adociaquinones A (**3**) and B (**4**),[13] 3-ketoadociaquinones A (**5**) and B (**6**),[13] tetrahydrohalenaquinones A (**7**)[13,14] and B (**8**),[14,15] and 13-*O*-methyl xestoquinol sulfate (**9**). Compounds **6** and **9** were new natural products, although **6** has previously been synthesized. The structures of the known compounds **1**–**5**, **7**, and **8** were identified by comparison of their spectral data with previously published values, while the structures of **6** and **9** were elucidated on the basis of their spectroscopic data. Several of the isolated

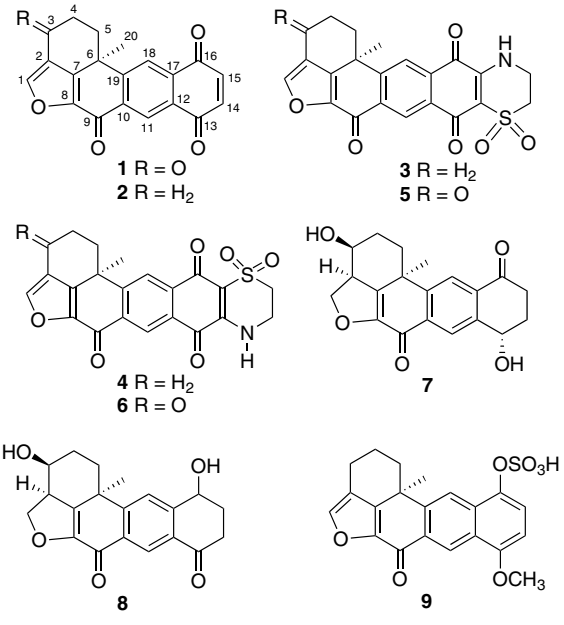

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Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **6** and **9**

No.	6		9	
	^{1}H	^{13}C	^{1}H	^{13}C
1	8.89 s	151.0	7.68 s	146.5
2		122.5		121.8
3		191.6	2.90 br dd (17.7, 5.2), 2.66 m	19.7
4	3.08 ddd (18.7, 13.1, 5.0), 2.65 dd (18.7, 5.1, 1.3)	32.3	2.17 m 2.32 m	17.9
5	2.93 ddd (12.8, 5.0, 1.3), 2.19 ddd (13.1, 12.8, 5.1)	36.5	2.74 dt (13.0, 3.4), 1.81 ddd (13.3, 13.0, 4.2)	32.9
6		36.3		37.8
7		147.3		149.8
8		144.1		148.6
9		170.1		174.4
10		137.4		131.4
11	8.73 s	124.9	9.15 s	125.5
12		132.3		124.7
13		178.2		155.3
14		111.4	6.90 d (8.5)	104.8
15		148.6	7.60 d (8.5)	123.3
16		173.6		143.0
17		131.2		132.0
18	8.34 s	124.2	8.45 s	120.5
19		153.3		146.5
20	1.64 s	29.7	1.55 s	33.9
21	3.40 m	48.3		
22	3.88 m	40.4		
NH	9.27 t (3.2)			
OMe			4.05 s	56.4

compounds have shown activity in other bioassays. Thus halenaquinone (**1**) was cytotoxic against KB and P388 cell lines,[13,16] while halenaquinone (**1**) and xestoquinone (**2**) showed cardiotonic properties[12,17–20] and inhibitory activity against Ca^{2+} ATPase,[21–23] phosphatidylinositol 3-kinase,[24,14] protein–tyrosine kinase,[25] and mammalian topoisomerase I.[26] Adociaquinones A (**3**) and B (**4**) were reported to be cytotoxic toward P388, HCT, KB16, and HEP-3B cell lines,[13,27,28] Adociaquinone B (**4**) was the most active among the isolates in the Cdc25B assay.

2. Results and discussion

After solvent partitioning, the CH_2Cl_2 extract retained the bulk of activity in the Cdc25B assay. This fraction was first separated on a reversed phase C_{18} flash column using 55% MeOH to furnish 12 fractions (I–XII). Compounds **1** and **2** were purified by reversed phase C_{18} HPLC from fractions IX and XI, respectively, while compounds **3**–**8** were obtained using phenyl HPLC from fractions III–V, VII, and VIII. Compound **9** was purified by reversed phase C_{18} flash chromatography of the aqueous MeOH extract followed by reversed-phase HPLC. The structures of compounds **1**–**5**, **7** and **8** were identified by comparison of their spectral data with previously published values.[11–15]

3-Ketoadociaquinone B (**6**) was isolated as a yellowish powder. Its molecular formula was assigned as $C_{22}H_{15}NO_7S$ based on its HR–FAB-MS spectrum. The ^{1}H NMR data for **6** in $CDCl_3$ (20% CD_3OD) matched those of the semi-synthesized product 3-ketoadociaquinone B, which was originally derived from halenaquinone.[13] The structure of **6** was confirmed by analysis of the 2D NMR spectra (^{1}H–^{1}H COSY, HMQC, and HMBC). Since there were no ^{13}C NMR data available for **6** in the literature, such data were included in Table 1.

Compound **9** was also isolated as a yellowish powder. There were one olefinic and two aromatic singlets (δ_H 9.15, s; 8.45, s; 7.68, s), two aromatic doublets (δ_H 7.60, d, $J = 8.5$ Hz; 6.90, d, $J = 8.5$ Hz), one methoxyl singlet (δ_H 4.05, s), one methyl singlet (δ_H 1.55, s), and signals attributed to three methylenes (δ_H 2.90, br dd, $J = 17.7$, 5.2 Hz; 2.74, dt, $J = 13.0$, 3.4 Hz; 2.66, m; 2.32, m; 2.17, m; 1.81, ddd, $J = 13.3$, 13.0, 4.2 Hz). The ^{1}H–^{1}H COSY spectrum exhibited two spin-coupling systems (CH_2–CH_2–CH_2 and CH=CH). In the HMBC spectrum of **9**, both H-18 (δ_H 8.45) and H-20 (δ_H 1.55) correlated with C-6 (δ_C 37.8). In addition, H-11 (δ_H 9.15) showed long-range correlations with both C-9 (δ_C 174.4) and C-13 (δ_C 155.3). The ^{1}H and ^{13}C NMR data for **9**, which are summarized in Table 1, compared favorably with the published values for xestoquinol sulfate,[15] with the major difference between them being the presence of signals for an extra methoxy group in **9**. The evidence for the connectivity of the methoxy group to C-13 was obtained by observation of a ^{3}J HMBC correlation from the methoxy singlet at δ_H 4.05 to C-13 at δ_C 155.3, and of a ROESY correlation between the signal at δ_H 4.05 and H-14 at δ_H 6.90. Therefore, **9** was deduced to be a 13-*O*-methyl derivative of xestoquinol sulfate.

The activities of compounds **1**–**9** in the Cdc25B assay are shown in Table 2. Compounds **1**–**6**, which had a naphthoquinone moiety, were more active than compounds

Table 2. IC_{50} values (μM ± SEM; $n = 4$) of compounds **1**–**9** against recombinant human Cdc25B, VHR, and PTP1B

Compound number	Cdc25B Catalytic domain	Cdc25B Full length	VHR	PTP1B
1	0.75 ± 0.06	0.70 ± 0.08	64.0 ± 1.1	25.0 ± 0.4
2	8.1 ± 0.3	12.5 ± 0.4	107.0 ± 4.0	55.0 ± 1.6
3	0.50 ± 0.05	NT	NT	NT
4	0.08 ± 0.01	0.07 ± 0.01	10.6 ± 0.6	4.0 ± 0.1
5	0.38 ± 0.02	NT	NT	NT
6	0.13 ± 0.02	0.21 ± 0.01	9.0 ± 0.2	3.9 ± 0.2
7	9.8 ± 1.1	NT	NT	NT
8	18.8 ± 0.9	NT	NT	NT
9	22.1 ± 1.8	NT	NT	NT

7–**9** against the catalytic domain of Cdc25B. Furthermore, when comparing the relative bioactivities of **1**–**6**, compounds **3**–**6** were more potent than **1** and **2**. The dihydro-benzothiazine dioxide in compounds **3**–**6** appears to be an important framework for this enhanced activity. A 6-fold difference in IC_{50} values was seen between the xestoquinone-based regioisomers **3** and **4**, while the halenaquinone-based regioisomers **5** and **6** exhibited only a 3-fold difference in IC_{50} values. Compounds **4** and **6** were the most potent inhibitors of the Cdc25B catalytic domain and were subjected to further analyses. With these compounds, the IC_{50} values for full length recombinant Cdc25B approximated that seen with the catalytic domain (Table 2) and were firmly in the nanomolar range. Furthermore, both **4** and **6** showed remarkable 19- to 150-fold selectivity for Cdc25B when compared to either the closely related dual specificity phosphatase VHR or the tyrosine phosphatase PTP1B.

Because Cdc25B has a prominent role in controlling mitosis,[2–4] we next examined the ability of the most potent and selective Cdc25B inhibitor, **4**, to block cell progression during mitosis using a temperature sensitive mutant cell line tsFT210, which permits cell synchronization without the use of chemical agents.[29] As illustrated in Figure 1, even a brief 6 h treatment with compound **4** caused a concentration-dependent block in progression through mitosis as indicated by the increase in G2/M phase cells. These results are consistent with inhibition of cellular Cdc25.

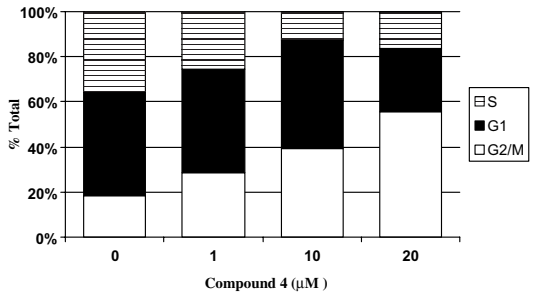

Figure 1. Effects of **4** on cell cycle distribution of tsFT210 cells. Cells were arrested at G2/M and then released in the presence or absence of various concentrations of **4** for 6 h. Cell cycle distribution was determined by flow cytometry.

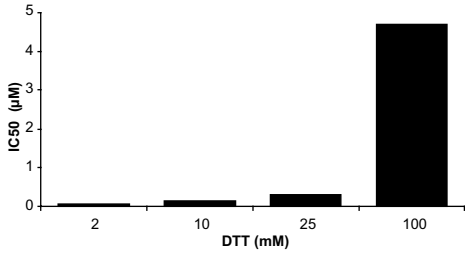

Figure 2. Effect of dithiothreitol on in vitro IC_{50} values of compound **4** for human full length Cdc25B.

The active-site cysteines of the Cdc25 phosphatases are highly susceptible to oxidation because of their low pKa and quinones are known to be potential cellular oxidants.[30,31] Thus, we examined the ability of the reductant dithiothreitol (DTT) to abrogate inhibition of Cdc25B by compound **4**. Incubation of Cdc25B with up to 25 mM DTT had only a modest affect on inhibition, but incubation with 100 mM caused almost a 70-fold increase in the IC_{50} value (Fig. 2). Although it is obvious from our studies[29] (Table 2) that selectivity exists among quinones concerning their ability to inhibit Cdc25B, the current results with DTT suggest that selective oxidation of the catalytic cysteine could be one possible mechanism for Cdc25B inhibition by **4**.

3. Experimental

3.1. General procedures

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 and a Unity 400 spectrometer in CD_3OD (**9**) and DMSO-d_6 (**6**). Mass spectra were obtained on a JEOL JMS-HX-110 instrument. The chemical shifts are given in δ (ppm) and coupling constants are reported in Hertz. A Horizon™ Flash Chromatograph from BioTage Inc. was used for flash column chromatography. HPLC was performed on a Shimadzu LC-10AT instrument with a semi-preparative C_{18}, a phenyl Varian Dynamax (5 μ, 250 × 10 mm), and a preparative C_{18} Varian Dynamax column (8 μ, 250 × 21.4 mm). Finnigan LTQ LC/MS with a C_{18} Hypersil column (5 μ, 100 × 2.1 mm) was used for the analysis of compound **9** [20% MeCN, 20% MeCN, 100% MeCN, 100% MeCN: 0 min, 5 min, 20 min, 30 min; flow rate: 0.2 mL/min; t_R: 20 min].

3.2. Marine sponge material

The sponge species (Phylum: Porifera, Class: Demospongiae, Order: Petrosiidae Genus *Xestospongia*) was collected by the Coral Reef Research Foundation at 18 m depth in Manado, Indonesia on May 15, 1993.

The voucher specimen is deposited at the Smithsonian Institution and a photograph of the sponge collection are available as supporting data.

3.3. In vitro phosphatase assays

Bioassay-directed fractionation was conducted with an epitope-tagged (histidine$_6$) catalytic domain of human recombinant Cdc25B, which contained amino acids 275–539 of the full length protein and has been previously described.[32] The histidine$_6$-tagged catalytic domain and full length Cdc25B were isolated and purified from *E. coli* with Ni-NTA resin as described previously.[32] Human recombinant VHR and PTP1B phosphatase were purchased from BIOMOL (Plymouth Meeting, PA). Activities of all phosphatases were measured using the substrate *O*-methyl fluorescein phosphate (Sigma, St. Louis, MO) in a 96-well microtiter plate assay based on previously described methods.[4,5,29,32] The final incubation mixtures (25 μL) were prepared with a Biomek 2000 laboratory automation workstation (Beckman Coulter, Inc., Fullerton, CA). Fluorescence emission from the product was measured after a 20 or 60 min incubation period at ambient temperature with a multiwell plate reader (Applied Biosystems Cytofluor II; Foster City, CA; excitation filter, 485 nm/bandwidth 20 nm; emission filter, 530 nm/bandwidth 25 nm). For the initial bioassay-directed fractionation, samples were evaluated at one concentration (0.2–1.0 μg/mL) and subsequent fractionations were examined with a minimum of six concentrations to determine the concentration required to inhibit enzyme activity by 50% (IC_{50}). In some studies with full length Cdc25B, the final concentration of DTT in the enzyme buffer, which contained 30 mM Tris (pH 8.5), 75 mM NaCl, 1 mM EDTA and 0.033% bovine serum albumin, was adjusted from the standard of 2 mM to 100 mM. DTT concentrations $\geq$100 mM had no effect on Cdc25B enzyme activity.

3.4. Flow cytometry

tsFT210 cells were plated at 1×10^6 cells/mL, maintained at 32 °C and treated as previously described.[29] Briefly, cell proliferation was blocked at the G2/M phase by incubation at 39.4 °C for 17 h. Cells were released from the block by incubation at 32 °C and immediately treated with various concentrations of compound **4** or the vehicle 0.1% dimethyl sulfoxide. After 6 h, cells were harvested with phosphatase-buffered saline and stained with 500 μL of propidium iodide/Rnase staining buffer supplied by BD Pharmigen (San Diego, CA). Flow cytometry analysis was conducted with a FACSCalibur flow cytometer (BD Parmigen).

3.5. Extract preparation

The frozen sponge was pulverized at the National Cancer Institute in dry ice by use of a worm-fed grinder (hamburger mill). The powder produced was allowed to stand at −30 °C until the CO_2 sublimed, and the mass was then extracted at 4 °C with de-ionized water (1 L) by stirring (30 rpm) for 30 min. The mixture was centrifuged at rt and the supernatant lyophilized to give the aqueous extract. The insoluble portion from the centrifugation was lyophilized and then statically extracted overnight at rt with 1 L of a 1:1 ratio of MeOH–CH_2Cl_2. The organic phase was filtered off, the pellet washed with a 10% volume of fresh MeOH and the combined organic phases reduced to dryness at <35 °C by rotary evaporation, and then finally dried under high vacuum at rt to give the organic extract as a gum. An extract of this sponge was received from the National Cancer Institute as sample number C011119 (3.16 g).

3.6. Isolation

Extract C011119 (2.3 g; IC_{50} 4 μg/mL against Cdc25B) was suspended in aqueous MeOH (MeOH–H_2O, 9:1, 200 mL) and extracted with hexanes (3 × 200 mL portions). The aqueous layer was then diluted to 70% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 200 mL portions). The CH_2Cl_2 extract (400 mg; IC_{50}: 2 μg/mL Cdc25B) was fractionated by column chromatography on a Horizon Flash Chromatograph over C_{18}Si gel using H_2O–MeOH (45:55 followed by 100% MeOH) to furnish twelve fractions (I–XII). Fractions IX and XI yielded compounds **1** (20 mg) and **2** (10 mg), respectively. The use of phenyl HPLC (75% MeOH) provided compounds **3** (0.8 mg, t_R: 17.5 min) and **4** (1.2 mg, t_R: 15.5 min) from fractions VII and VIII, while **5** (1.0 mg, t_R: 13.5 min) and **6** (1.4 mg, t_R: 11.5 min) were purified in the same way from fractions III–V, respectively. Compounds **7** (0.8 mg, t_R: 29 min) and **8** (0.8 mg, t_R: 28 min) were also separated from fractions III–V utilizing phenyl HPLC (55% MeOH). The aqueous MeOH extract (1 g) was fractionated by column chromatography on a Horizon Flash Chromatograph over C_{18}Si gel using 1:1 H_2O–MeOH to furnish eight fractions (A–H). Additional C_{18} chromatography (10% MeCN–40% MeCN in 30 min) of fraction VIII yielded **9** (2.2 mg, t_R: 23 min).

3.6.1. 3-Ketoadociaquinones B (6). Yellowish powder; $[\alpha]_D^{23}$ +13 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 273 (3.6) nm; IR ν_{max} 3401, 2961, 2921, 2849, 1686, 1676, 1207, 1134, 798, 721 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HR–FAB-MS *m/z* 438.0676 $[M+H]^+$ (calcd for $C_{22}H_{15}NO_7S$, 438.0647).

3.6.2. 13-*O*-Methyl xestoquinol sulfate (9). Yellowish powder; $[\alpha]_D^{23}$ +28 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 331 (4.0), 310 (4.0), 273 (4.1), 223 (4.5) nm; IR ν_{max} 3415, 2955, 2924, 2855, 1629, 1611, 1572, 1459, 1377, 1243, 1039, 825 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; Finnigan LC-ESI MS *m/z* 415 [M+H]; HR–FAB-MS *m/z* 437.0665 $[M+Na]^+$ (calcd for $C_{21}H_{18}O_7S$ + Na, 437.0671).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.11.039. A photograph of the sponge collection is available as a jpg file at http://www.sciencedirect.com.

References and notes

1. Dunphy, W. G.; Kumagai, A. *Cell* **1991**, *67*, 189.
2. Galaktionov, K.; Lee, A. K.; Eckstein, J.; Draetta, G.; Meckler, J.; Loda, M.; Beach, D. *Science* **1995**, *269*, 1575.
3. Baratte, B.; Meijer, L.; Galaktionov, K.; Beach, D. *Anticancer Res.* **1992**, *12*, 873.
4. Lyon, M. S.; Ducruet, A. P.; Wipf, P.; Lazo, J. S. *Nature Reviews Drug Disc.* **2002**, *1*, 961.
5. Wipf, P.; Hopkins, C. R.; Phillips, E. O.; Lazo, J. S. *Tetrahedron* **2002**, *58*, 6367.
6. Loukaci, A.; Saout, I. L.; Samadi, M.; Leclerc, S.; Damiens, E.; Meijer, L.; Debitus, C.; Guyot, M. *Bioorg. Med. Chem.* **2001**, *9*, 3049.
7. Dirk, B.; Susanne, M.; Ajay, B.; Oliver, M.; Folker, L.; Herbert, W. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 307.
8. Peng, H.; Zalkow, L. H.; Abraham, R. T.; Powis, G. *J. Med. Chem.* **1998**, *41*, 4677.
9. Gunasekera, S. P.; McCarthy, P. J.; Kelly-Borges, M.; Lobkovsky, E.; Clardy, J. *J. Am. Chem. Soc.* **1996**, *118*, 8759.
10. Brohm, D.; Philippe, N.; Metzger, S.; Bhargava, A.; Müller, O.; Lieb, F.; Waldmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 13171.
11. Roll, D. M.; Scheuer, P. J.; Matsumoto, G. K.; Clardy, J. *J. Am. Chem. Soc.* **1983**, *105*, 6177.
12. Nakamura, H.; Kobayashi, J.; Kobayashi, M.; Ohizumi, Y.; Hirata, Y. *Chem. Lett.* **1985**, *6*, 713.
13. Schmitz, F. J.; Bloor, S. J. *J. Org. Chem.* **1988**, *53*, 3922.
14. Alvi, K. A.; Rodriguez, J.; Diaz, M. C.; Moretti, R.; Wilhelm, R. S.; Lee, R. H.; Slate, D. L.; Crews, P. *J. Org. Chem.* **1993**, *58*, 4871.
15. Kobayashi, J.; Hirase, T.; Shigemori, H.; Ishibashi, M.; Bae, M. A.; Tsuji, T.; Sasaki, T. *J. Nat. Prod.* **1992**, *55*, 994.
16. Park, S. K.; Oh, C.; Scheuer, P. J. *J. Korean Chem. Soc.* **1995**, *39*, 301.
17. Kobayashi, M.; Nakamura, H.; Kobayashi, J.; Ohizumi, Y. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 82.
18. Kobayashi, M.; Muroyama, A.; Nakamura, H.; Kobayashi, J.; Ohizumi, Y. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 90.
19. Ohizumi, Y. *Jpn. J. Clin. Med.* **1993**, *51*, 1299.
20. Ohizumi, Y. *Jpn. J. Pharmacol.* **1992**, *100*, 259.
21. Nakamura, M.; Kakuda, T.; Oba, Y.; Ojika, M.; Nakamura, H. *Bioorg. Med. Chem.* **2003**, *11*, 3077.
22. Ito, M.; Hirata, Y.; Nakamura, H.; Ohizumi, Y. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 976.
23. Sakamoto, H.; Furukawa, K.; Matsunaga, K.; Nakamura, H.; Ohizumi, Y. *Biochemistry* **1995**, *34*, 12570.
24. Fujiwara, H.; Matsunaga, K.; Saito, M.; Hagiya, S.; Furukawa, K. I.; Nakamura, H.; Ohizumi, Y. *Eur. J. Pharmacol.* **2001**, *413*, 37.
25. Lee, R. H.; Slate, D. L.; Moretti, R.; Alvi, K. A.; Crews, P. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 765.
26. Bae, M. A.; Tsuji, T.; Kondo, K.; Hirase, T.; Ishibashi, M.; Shigemori, H.; Kobayashi, J. *Biosci. Biotech. Biochem.* **1993**, *57*, 330.
27. Concepcion, G. P.; Foderaro, T. A.; Eldredge, G. S.; Lobkovsky, E.; Clardy, J.; Barrows, L. R.; Ireland, C. M. *J. Med. Chem.* **1995**, *38*, 4503.
28. Shen, Y. C.; Lo, K. L.; Kuo, Y. H.; Chakraborty, R. *Chin. Pharmacol. J. (Taipei, Taiwan)* **2002**, *54*, 207.
29. Lazo, J. S.; Nemoto, K.; Pestell, K. E.; Cooley, K.; Southwick, E. C.; Mitchell, D. A.; Furey, W.; Gussio, R.; Zaharevitz, D. W.; Joo, B.; Wipf, P. *Mol. Pharmacol.* **2002**, *61*, 720.
30. Sohn, J.; Rudolph, J. *Biochemistry* **2003**, *42*, 10060.
31. Bova, M. P.; Mattson, M. V.; Vasile, S.; Tam, D.; Holsinger, L.; Bremer, M.; Hui, T.; McMahon, G.; Rice, A.; Fukuo, J. M. *Arch. Biochem. Biophys.* **2004**, *429*, 30.
32. Kar, S.; Lefterov, I. M.; Wang, M.; Lazo, J. S.; Scott, C. N.; Wilcox, C. S.; Carr, B. I. *Biochemistry* **2003**, *42*, 10490.