Published in final edited form as:

Bioorg Med Chem. 2010 May 15; 18(10): 3570–3574. doi:10.1016/j.bmc.2010.03.054.

Aplysinopsin Analogs: Synthesis and Anti-proliferative Activity of Substituted (Z)-5-(N-benzylindol-3-ylmethylene)imidazolidine-2,4-diones

Y. Thirupathi Reddy^a, P. Narsimha Reddy^a, Srinivas Koduru^b, Chendil Damodaran^b, and Peter A. Crooks^{a,*}

^aDepartment of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA

^bDepartment of Clinical Sciences, College of Health Sciences, University of Kentucky, Lexington, KY 40536, USA

Abstract

A series of substituted (Z)-5-(N-benzylindol-3-ylmethylene)imidazolidine-2,4-dione (**3**) analogs structurally related to aplysinopsin, and that incorporate a variety of substituents in both the indole and N-benzyl moieties have been synthesized under microwave irradiation and conventional heating methods. These analogs were evaluated for their anti-proliferative activity against MCF-7 and MDA-231 breast cancer cell lines, and A549 and H460 lung cancer cell lines. Two analogs, **3f** and **3j** had IC₅₀ values of 4.4 and 5.2 μM, respectively, compared to 5-fluorouracil (IC₅₀ = 15.2 μM) against MCF-7 cells.

1. Introduction

Aplysinopsins (Fig. 1, A) are indole-derived marine natural products. The parent aplysinopsin was isolated for the first time by Kazlauskas *et al.*¹ as the major metabolite of eight Indo-Pacific sponge species, which are representatives of the genera *Thorecta*. The N-1-unsubstituted aplysinopsins have generated considerable interest due to their potentially useful medicinal properties.² Aplysinopsin has been reported as a potent cytotoxic agent against the Kβ-cell line by Hollenbeak *et al.*³, and Kondo *et al.* have reported the anti-cancer activity of aplysinopsin and methyl-aplysinopsins against L-1210- and Kβ-cell lines.⁴ Wen-Tai *et al.* reported the synthesis and biological evaluation of N-heterocyclic indolyl-glyoxylamides as orally active anticancer agents.⁵ Recently, Peng-Fei and co-workers reported the synthesis of a series of 4α-O- and 4β-N-indol-3-yl-glyoxyl-substituted derivatives of podophyllotoxin as potent cytotoxic agents against Hela, SKOV3, K562, and K562ADR cells.⁶ James and coworkers⁷ have also reported synthesis and SAR studies on a series of N-benzylindole and indolizine-glyoxylamides (Fig. 1, B) that exhibit significant *in vitro* anti-proliferative activities against a variety of cancer cell lines, including hematologic and solid tumor cell lines (e.g., leukemia, breast, colon and uterine). Recently, we have reported the synthesis and thermal sensitization activity of a series of novel substituted (Z)-2-(N-benzylindol-3-ylmethylidene)quinuclidin-3-ol analogs. In this series, (Z)-(±)-2-[N-

© 2010 Elsevier Ltd. All rights reserved.

*Corresponding author: Tel.: +1-859-257-1718; fax: +1-859-257-7585; pcrooks@email.uky.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

(4-chlorobenzyl)- indole-3-ylmethylidene]quinuclidin-3-ol (Fig. 1, C) was identified as a potent thermal sensitizer, capable of lowering the threshold for Hsf1 activation and increasing the thermal sensitivity of cancer cells to ionizing radiation.⁸ Very recently, we have reported the synthesis and radio-sensitization activity of a small library of (*Z*)-5-((*N*-benzyl-1*H*-indol-3-yl)methylene)imidazolidine-2,4-diones and 5-((*N*-benzyl-1*H*-indol-3-yl)methylene) pyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione derivatives⁹. In this series, 4-[(3-((2,4,6-trioxotetrahydropyrimidin-5-(6*H*)-ylidene)methyl)-1*H*-indol-1-yl)methyl]benzonitrile (Fig. 1, D), methyl-4-[(3-((2,4,6-trioxotetrahydropyrimidin-5-(6*H*)-ylidene)methyl)-1*H*-indol-1-yl)methyl]benzoate (Fig. 2, E), and 5-((*N*-4-nitrobenzyl-1*H*-indol-3-yl)methylene)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (Fig. 1, F) were identified as potent radio-sensitizing agents with additional synergistic anti-angiogenic properties, whereas the (*Z*)-5-((*N*-benzyl-1*H*-indol-3-yl)methylene)- imidazolidine-2,4-dione analogs exhibited very poor radio-sensitization activity.⁹

These observations prompted us to synthesize a series of substituted(*Z*)-5-(*N*-benzylindol-3-ylmethylene)imidazolidine-2,4-diones that were expected to function as potent cytotoxic agents against lung and breast cancer cells.

2. Results and discussion

2.1. Chemistry

The appropriate substituted (*Z*)-5-(*N*-benzylindol-3-yl-methylene)imidazolidine-2,4-dione (**3a-m**) was synthesized *via* the general synthetic route shown in Scheme 1.

The substituted *N*-benzylindole-3-carboxaldehyde derivatives **2a-m** were prepared in 85–90% yield utilizing literature procedures⁸ by treating the appropriate commercially available indole-3-carboxaldehyde (**1a-e**) with various substituted benzyl halides under phase-transfer catalytic (PTC) conditions utilizing triethylbenzylammonium chloride and 50% (w/v) NaOH aqueous solution/dichloromethane (1:1 v/v). Aldol condensation of the resulting product (**2a-m**) with hydantoin in the presence of ammonium acetate in acetic acid under microwave irradiation or under reflux conditions, afforded the corresponding substituted (*Z*)-5-(*N*-benzylindol-3-ylmethylene)imidazolidine-2,4-dione derivatives **3a-m**.

The desired products were formed in very good yields (80–85%) within 40–60 seconds utilizing the previously reported microwave irradiation method (Method-A).⁹ The products were also obtained in good yields (74–80%) utilizing conventional heating procedure (Method-B, 115–116 °C) within 8–12 hrs. A comparison between these two methods with respective to reaction time and yield of product is shown in Table 1. All the synthesized compounds were characterized by ¹H-NMR and ¹³C-NMR spectrometry. The geometry of the double bond in the representative compounds **3b** and **3e** was established from X-ray crystallographic data.^{10,11}

2.2. Evaluation of biological activity

MCF-7, MDA-231, A549 and H460 cancer cells lines were purchased from the American Type Culture Collection (ATCC) (Manassas, VA) and were utilized to test the antitumor activity of the above indole-derived analogs. Breast cancer cell lines, MCF-7 and MDA-231 were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% L-glutamine, whereas lung cancer cell lines A549 and H460 were maintained and propagated in RPMI 1640 medium containing 2 μM L-glutamine, 4.5 g/L glucose, 10 μM HEPES, 1.0 μM sodium pyruvate and 10% FBS. All cell cultures were maintained at 37 °C in a 5% CO₂/95% air-humidified atmosphere. Anti-proliferative activity of the indole-derived analogs **3a-m** were assessed in cell viability assays (trypan blue dye exclusion)¹² on both breast (MCF-7 and MDA-231) and lung (A549 and H460) cancer cells at 24 hours post-

treatment at 5 concentrations of drug (1, 5, 10, 20 and 40 μM) and with vehicle (DMSO) alone. All assays were performed in triplicate. Results were expressed as percentage growth inhibition compared to control values.¹³ IC_{50} values for growth inhibition were derived from a nonlinear regression model based on sigmoidal dose-response curves and computed using GraphPad Prism 5 (Graphpad).

We initially evaluated the abilities of compounds **3a-m** to inhibit the growth of the two distinct human lung cancer cell lines A549 and H460 (Table 2). All thirteen compounds exhibited IC_{50} values $>14 \mu\text{M}$ in both cancer cell lines, and generally showed poorer growth inhibition against A549 lung cancer cells and H460 lung cancer cells when compared to the positive control drug, 5-fluorouracil (5-FU, $\text{IC}_{50} = 5.4$ and $2.7 \mu\text{M}$, respectively).

The analogs were also tested for their anti-proliferative activity against ER positive MCF-7 and ER negative MDA-231 breast cancer cell lines. All the compounds showed growth inhibition against MCF-7 cell lines with IC_{50} values ranging from $4.4 \mu\text{M}$ to $27.3 \mu\text{M}$. The 2'-bromo (**3f**, $\text{IC}_{50} = 4.4 \mu\text{M}$), 5-methyl (**3j**, $\text{IC}_{50} = 5.2 \mu\text{M}$), 5-methoxy (**3l**, $\text{IC}_{50} = 7.2 \mu\text{M}$), and 5-bromo (**3k**, $\text{IC}_{50} = 7.6 \mu\text{M}$) analogs were identified as the most potent cytotoxic agents against MCF-7 cells, and were significantly more cytotoxic than 5FU ($\text{IC}_{50} = 15.2 \mu\text{M}$).¹⁴ The 4'-cyano (**3b**, $\text{IC}_{50} = 14.1 \mu\text{M}$), 4'-nitro (**3c**, $\text{IC}_{50} = 10.9 \mu\text{M}$), 5-chloro (**3h**, $\text{IC}_{50} = 9.2 \mu\text{M}$), and 5-COOCH₃ (**3e**, $\text{IC}_{50} = 15.4 \mu\text{M}$) analogs also exhibited cytotoxicity against MCF-7 cell lines comparable to 5-FU. Analogs **3a-m** generally exhibited lower anti-proliferative potency against MDA-231 cells. Only one compound, the 5-methoxy analog (**3l**, $\text{IC}_{50} = 7.0 \mu\text{M}$) exhibited moderate anti-proliferative activity against MDA-231 cells, which was similar to that obtained for 5-FU ($\text{IC}_{50} = 3.5 \mu\text{M}$).¹⁵

3. Conclusions

In conclusion, a small library of (*Z*)-2-(*N*-benzylindol-3-ylmethylene)imidazolidine-2,4-dione analogs that incorporate a variety of aromatic substituents in both the indole and *N*-benzyl moieties have been synthesized under both microwave irradiation and conventional heating procedures, and evaluated for their anti-proliferative activity against breast cancer and lung cancer cells in culture. The compounds all contain an *N*-benzylindole nucleus linked to a hydantoin moiety *via* a double bond with *Z*-geometry. Several analogs exhibited IC_{50} values $<8 \mu\text{M}$ as anti-proliferative agents against the MCF-7 cell line, and those analogs were more active than 5-FU ($\text{IC}_{50} = 15.2 \mu\text{M}$). We consider analogs **3f**, **3j**, **3k**, and **3l** to be lead compounds worthy of consideration for further structural optimization and development as potential anticancer agents for the treatment of breast cancer.

4. Experimental Procedure

The purity of the compounds synthesized was assessed by thin layer chromatography (TLC) performed on microscopic slides, $2 \times 7.5 \text{ cm}$, coated with silica gel G as stationary phase and the spots were visualized by exposure to iodine vapors or to UV light. Melting points were measured with a Fischer Scientific apparatus and are reported uncorrected. All the synthesized final compounds (**3a-m**) had melting points above 300°C . ^1H NMR and ^{13}C NMR spectra of the final compounds (**3a-m**) were recorded in DMSO- d_6 and those of intermediate compounds (**2a-m**) were recorded in CDCl_3 on a Varian 300 MHz spectrometer utilizing TMS as internal standard. Chemical shift values are reported in *ppm* downfield on the δ scale. High resolution electron impact (EI) ionization mass spectra were recorded at 25eV on a JEOL JMS-700T MStation (magnetic sector instrument) at a resolution of greater than 10000. Perfluorokerosene (pfk) was used to produce reference masses.

Representative Synthetic Procedure: Microwave irradiation method (Method A)

A mixture of the appropriate substituted *N*-benzylindole-3-carboxaldehyde (1 mmol), hydantoin (1.1 mmol) and ammonium acetate (1.1 mmol) in acetic acid (1 ml) was irradiated in a domestic microwave oven (1100 W; Kenmore) for 40–60 sec. During this time period, intermittent removal of reaction vessel from the oven was carried out every 10 seconds, in order to cool the reaction mass to room temperature. The reaction mixture was allowed to cool to room temperature, water (10 ml), was added, and the mixture was basified with saturated NaHCO₃ solution and stirred for 10 minutes. The precipitate thus obtained was collected by filtration, washed with cold water, and finally washed with methanol and dried to afford the crude product. Crystallization from methanol and ethyl acetate (1:1) afforded a yellow crystalline product.

Conventional heating method (Method B)

A mixture of the appropriate substituted *N*-benzylindole-3-carboxaldehyde (1 mmol), hydantoin (1.1 mmol) and ammonium acetate (1.1 mmol) was stirred in acetic acid (5 ml) at 115–116 °C for 8–12 hrs. The reaction mixture was cooled to room temperature, water (10 ml) added, and the mixture was basified with saturated NaHCO₃ solution and stirred for 10 minutes. The precipitate thus obtained was collected by filtration, washed with cold water, and finally washed with methanol and dried to afford the crude product. Crystallization from methanol and ethyl acetate (1:1) afforded a yellow crystalline product.

5. Analytical data for the synthesized compounds

1-(2-Bromobenzyl)-1*H*-indole-3-carbaldehyde (2f)

M.P. 127–129 °C; ¹H NMR (CDCl₃): δ 5.45 (s, 2H, CH₂), 6.77–6.80 (t, 1H, Ar-H), 7.21–7.24 (m, 3H, Ar-H), 7.33–7.37 (m, 2H, Ar-H), 7.64–7.67 (d, 1H, Ar-H) 7.72 (s, 1H, C2H), 8.34–8.37 (d, 1H, Ar-H), 10.03 (s, 1H, CHO); ¹³C NMR (CDCl₃): δ 51.12, 110.42, 118.82, 122.33, 122.98, 123.28, 124.42, 125.45, 128.19, 128.72, 130.01, 138.29, 134.67, 137.45, 138.62, 184.63. HRMS (+EI; mean value): m/z found 313.0098, calcd. C₁₆H₁₂BrNO (+EI; mean value): 313.0102.

5-Bromo-1-(4-methoxybenzyl)-1*H*-indole-3-carboxaldehyde (2i)

M.P. 101–103 °C; ¹H NMR (CDCl₃): δ 3.79 (s, 3H, OCH₃), 5.25 (s, 2H, CH₂), 6.86–6.89 (d, 2H, Ar-H), 7.10–7.13 (d, 2H, Ar-H), 7.18–7.21 (d, 2H, Ar-H), 7.35–7.39 (dd, 1H, Ar-H), 8.46 (s, 1H, C2H), 9.92 (s, 1H, CHO); ¹³C NMR (CDCl₃): δ 50.94, 55.59, 111.98, 114.70 (2C), 116.84, 117.80, 124.94, 126.70, 127.14, 127.21, 128.95 (2C), 136.13, 138.88, 159.82, 184.34. HRMS (+EI; mean value): m/z found, 343.0205, calcd. C₁₇H₁₄BrNO₂ (+EI; mean value): 343.0208.

(*Z*)-5-((1-Benzyl-1*H*-indol-3-yl)methylene)imidazolidine-2,4-dione (3a)

¹H NMR (DMSO-*d*₆): δ 5.56 (s, 2H, CH₂), 6.73 (s, 1H, CH), 7.13–7.23 (m, 5H, Ar-H), 7.29–7.31 (d, 2H, Ar-H), 7.51–7.54 (d, 1H, Ar-H), 7.75–7.77 (d, 1H, Ar-H), 8.32 (s, 1H, C2H), 10.15 (bs, 1H, NH), 11.07 (bs, 1H, NH); ¹³C NMR (DMSO-*d*₆): δ 49.76, 100.92, 108.09, 110.58, 118.28, 120.38, 122.43, 123.81, 127.15, 127.35, 127.46, 128.46, 129.95, 135.48, 137.28, 155.08, 165.08. HRMS (+EI; mean value): m/z found 317.1162, calcd. C₁₉H₁₅N₃O₂ (+EI; mean value) 317.1164.

(*Z*)-4-((3-((2,5-Dioxoimidazolidin-4-ylidene)methyl)-1*H*-indol-1-yl)methyl)benzonitrile (3b)

¹H NMR (DMSO *d*₆): δ 5.56 (s, 2H, CH₂), 6.74 (s, 1H, CH), 7.13–7.23 (m, 2H, Ar-H), 7.44–7.53 (m, 3H, Ar-H), 7.79–7.83 (m, 3H, Ar-H), 8.30 (s, 1H, C2H), 10.15 (bs, 1H, NH), 11.07 (bs, 1H, NH); ¹³C NMR (DMSO-*d*₆): δ 50.07, 101.46, 109.25, 111.06, 111.22,

119.21, 119.29, 121.34, 123.42, 124.95, 128.10, 128.70, 130.63, 133.26, 136.25, 143.71, 155.94, 165.87. HRMS (^{+}EI ; mean value): m/z found, 342.1116, calcd. $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_2$ (^{+}EI ; mean value): 342.1117.

(Z)-5-((1-(4-Nitrobenzyl)-1*H*-indol-3-yl)methylene)-imidazolidine-2,4-dione (3c)

^1H NMR ($\text{DMSO}-d_6$): δ 5.61 (s, 2H, CH_2), 6.74 (s, 1H, CH), 7.13–7.53 (m, 4H, Ar-H), 7.79–7.82 (d, 2H, Ar-H), 8.18–8.21 (d, 2H, Ar-H), 8.31 (s, 1H, C2H), 10.16 (bs, 1H, NH), 11.08 (bs, 1H, NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 49.80, 101.48, 109.35, 111.19, 119.22, 121.36, 123.44, 124.47, 124.99, 128.12, 128.95, 130.66, 136.26, 145.7, 147.56, 155.88, 165.81. HRMS (^{+}EI ; mean value): m/z found, 362.1013, calcd. $\text{C}_{19}\text{H}_{14}\text{N}_4\text{O}_4$ (^{+}EI ; mean value): 362.1015.

(Z)-5-((1-(4-chlorobenzyl)-1*H*-indol-3-yl)methylene)imidazolidine-2,4-dione (3d)

^1H NMR ($\text{DMSO}-d_6$): δ 5.44 (s, 2H, CH_2), 6.73 (s, 1H, CH), 7.12–39 (m, 4H, Ar-H), 7.52–7.55 (d, 2H, Ar-H), 7.77–7.80 (d, 2H, Ar-H), 8.30 (s, 1H, C2H), 10.15 (bs, 1H, NH), 11.06 (bs, 1H, NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 48.97, 100.78, 108.21, 110.49, 118.31, 120.42, 122.49, 123.93, 127.32, 128.42, 129.02, 129.81, 132.07, 135.40, 136.26, 155.05, 165.01. HRMS (^{+}EI ; mean value): m/z found, 351.0776, calcd. $\text{C}_{19}\text{H}_{14}\text{ClN}_3\text{O}_2$ (^{+}EI ; mean value): 351.0775.

(Z)-Methyl-4-((3-((2,5-dioxoimidazolidin-4-ylidene)-methyl)-1*H*-indol-1-yl)methyl)benzoate (3e)

^1H NMR ($\text{DMSO}-d_6$): δ 3.81 (s, 3H, OCH_3), 5.54 (s, 2H, CH_2), 6.74 (s, 1H, CH), 7.15–7.23 (m, 2H, Ar-H), 7.40–7.42 (d, 2H, Ar-H), 7.50–7.52 (d, 1H, Ar-H), 7.79–7.81 (d, 1H, Ar-H), 7.91–7.93 (d, 2H, Ar-H), 8.32 (s, 1H, C2H), 10.15 (bs, 1H, NH), 11.06 (bs, 1H, NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 49.52, 52.2, 101.44, 109.26, 111.26, 119.22, 121.35, 123.44, 124.01, 127.29, 128.95, 129.35, 131.23, 136.28, 142.64, 155.93, 165.85, 167.12. HRMS (^{+}EI ; mean value): m/z found, 375.1213, calcd. $\text{C}_{21}\text{H}_{17}\text{N}_3\text{O}_4$ (^{+}EI ; mean value): 375.1218.

(Z)-5-((1-(2-Bromobenzyl)-1*H*-indol-3-yl)methylene)-imidazolidine-2,4-dione (3f)

^1H NMR ($\text{DMSO}-d_6$): δ 5.51 (s, 2H, CH_2), 6.69–6.70 (d, 1H, Ar-H), 6.74 (s, 1H, CH), 7.16–7.30 (m, 4H, Ar-H), 7.44–7.47 (d, 1H, Ar-H), 7.69–7.72 (d, 1H, Ar-H), 7.846–7.85 (d, 1H, Ar-H), 8.24 (s, 1H, C2H), 10.13 (bs, 1H, NH), 11.06 (bs, 1H, NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 49.85, 100.75, 108.48, 10.39, 118.45, 120.58, 121.73, 122.70, 124.16, 127.23, 128.02, 128.26, 129.49, 130.17, 132.59, 135.76, 136.05, 155.08, 165.05. HRMS (^{+}EI ; mean value): m/z found 395.0267, calcd. $\text{C}_{19}\text{H}_{14}\text{BrN}_3\text{O}_2$ (^{+}EI ; mean value) 395.0269.

(Z)-5-((1-(4-Fluorobenzyl)-1*H*-indol-3-yl)methylene)imidazolidine-2,4-dione (3g)

^1H NMR ($\text{DMSO}-d_6$): δ 5.43 (s, 2H, CH_2), 6.73 (s, 1H, CH), 7.12–40 (m, 4H, Ar-H), 7.55–7.58 (d, 2H, Ar-H), 7.77–7.80 (d, 2H, Ar-H), 8.32 (s, 1H, C2H), 10.16 (bs, 1H, NH), 11.06 (bs, 1H, NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 49.02, 101.07, 108.61, 110.67, 118.42, 120.63, 122.72, 124.05, 127.61, 128.92, 129.33, 129.98, 132.27, 135.56, 136.79, 155.27, 165.12. HRMS (^{+}EI ; mean value): m/z found, 335.1065, calcd. $\text{C}_{19}\text{H}_{14}\text{FN}_3\text{O}_2$ (^{+}EI ; mean value) 335.1070.

(Z)-5-((1-Benzyl-5-chloro-1*H*-indol-3-yl)methylene)imidazolidine-2,4-dione (3h)

^1H NMR ($\text{DMSO}-d_6$): δ 5.44 (s, 2H, CH_2), 6.73 (s, 1H, CH), 7.22–7.29 (m, 4H, Ar-H), 7.51–7.55 (d, 2H, Ar-H), 8.10–8.14 (d, 2H, Ar-H), 8.37 (s, 1H, C2H), 10.44 (bs, 2H, 2NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 49.95, 100.47, 108.02, 112.23, 118.06, 122.43, 124.34, 125.25, 127.14, 127.56, 128.52, 131.27, 134.02, 137.04, 155.14, 165.01. HRMS (^{+}EI ; mean value): m/z found, 351.0773, calcd. $\text{C}_{19}\text{H}_{14}\text{ClN}_3\text{O}_2$ (^{+}EI ; mean value): 351.0775.

(Z)-5-((5-Bromo-1-(4-methoxybenzyl)-1H-indol-3-yl)methylene)imidazolidine-2,4-dione (3i)

¹H NMR (DMSO d₆): δ 3.70 (s, 3H, OCH₃), 5.35 (s, 2H, CH₂), 6.72 (s, 1H, CH), 6.87–6.90 (d, 2H, Ar-H), 7.27–7.29 (d, 2H, Ar-H), 7.52–7.56 (d, 2H, Ar-H), 8.02 (s, 1H, Ar-H), 8.33 (s, 1H, C2H), 10.48 (bs, 2H, 2NH); ¹³C NMR (DMSO-d₆): δ 50.34, 55.96, 100.63, 104.06, 108.38, 111.02, 111.12, 126.54, 127.13, 128.17, 128.78, 130.43, 132.07, 137.58, 154.08, 155.15, 165.03. HRMS (+EI; mean value): m/z found, 425.0372, calcd. C₂₀H₁₆BrN₃O₃ (+EI; mean value): 425.0375.

(Z)-5-((1-Benzyl-5-methyl-1H-indol-3-yl)methylene)imidazolidine-2,4-dione (3j)

¹H NMR (DMSO d₆): δ 2.39 (s, 3H, CH₃), 5.39 (s, 2H, CH₂), 6.73 (s, 1H, CH), 7.00–7.03 (d, 1H, Ar-H), 7.28–7.30 (m, 4H, Ar-H), 7.41–7.43 (d, 2H, Ar-H), 7.57 (s, 1H, Ar-H), 8.28 (s, 1H, C2H), 10.5 (bs, 2H, NH), ¹³C NMR (DMSO-d₆): δ 21.10, 49.77, 101.04, 107.59, 110.29, 117.85, 123.47, 123.93, 127.04, 127.38, 127.59, 128.39, 129.24, 129.91, 133.89, 137.34, 155.02, 165.03. HRMS (+EI; mean value): m/z found 331.1319, calcd. C₂₀H₁₇N₃O₂ (+EI; mean value) 331.1321.

(Z)-5-((1-Benzyl-5-bromo-1H-indol-3-yl)methylene)imidazolidine-2,4-dione (3k)

¹H NMR (DMSO d₆): δ 5.44 (s, 2H, CH₂), 6.72 (s, 1H, CH), 7.24–7.31 (m, 4H, Ar-H), 7.50–7.54 (d, 2H, Ar-H), 8.03–8.04 (d, 2H, Ar-H), 8.35 (s, 1H, C2H), 10.46 (bs, 2H, 2NH); ¹³C NMR (DMSO-d₆): δ 50.66, 101.42, 108.56, 113.40, 114.01, 121.68, 124.99, 125.81, 127.81, 128.39, 129.30, 129.79, 131.75, 134.95, 137.62, 155.89, 165.93. HRMS (+EI; mean value): m/z found 395.0268, calcd. C₁₉H₁₄BrN₃O₂ (+EI; mean value) 395.0269.

(Z)-5-((1-(4-Methoxybenzyl)-1H-indol-3-yl)methylene)-imidazolidine-2,4-dione (3l)

¹H NMR (DMSO-d₆): δ 3.80 (s, 3H, OCH₃), 5.38 (s, 2H, CH₂), 6.74 (s, 1H, CH), 6.80–6.83 (d, 1H, Ar-H), 7.22–7.32 (m, 6H, Ar-H), 7.41–7.44 (d, 1H, Ar-H), 8.29 (s, 1H, C2H), 10.10 (bs, 1H, NH), 11.02 (bs, 1H, NH); ¹³C NMR (DMSO-d₆): δ 49.56, 55.59, 100.76, 104.19, 108.58, 111.08, 111.52, 126.74, 127.24, 128.38, 128.88, 130.54, 132.13, 137.66, 154.11, 155.15, 165.06. HRMS (+EI; mean value): m/z found 347.1267, calcd. C₂₀H₁₇N₃O₃ (+EI; mean value) 347.1270.

(Z)-5-((1-(4-Methylbenzyl)-1H-indol-3-yl)methylene)-imidazolidine-2,4-dione (3m)

¹H NMR (DMSO-d₆): δ 2.37 (s, 3H, CH₃), 5.36 (s, 2H, CH₂), 6.74 (s, 1H, CH), 7.00–7.05 (d, 2H, Ar-H), 7.23–7.29 (m, 3H Ar-H), 7.39–7.69 (d, 2H, Ar-H), 7.54 (s, 1H, Ar-H), 8.26 (s, 1H, C2H), 10.48 (bs, 2H, NH), ¹³C NMR (DMSO-d₆): δ 20.97, 49.93, 101.71, 108.27, 110.96, 118.56, 120.83, 122.86, 123.91, 127.51, 127.69, 129.34, 134.48, 135.72, 137.15, 155.45, 165.59. HRMS (+EI; mean value): m/z found, 331.1318, calcd. C₂₀H₁₇N₃O₂ (+EI; mean value): 331.1321.

Acknowledgments

This research was supported by NIH/National Cancer Institute grant PO1 CA104457.

References and notes

1. Kazlauskas R, Murphy PT, Quinn RJ, Wells RJ. Tetrahedron Lett. 1977; 18:61–64.
2. Bialonska D, Zjawiony JK. Marine Drugs. 2009; 7:166–183. [PubMed: 19597579]
3. Hollenbeak KH, Schmitz FJ. Lloydia. 1977; 40:479. [PubMed: 562965]
4. Kondo K, Nishi J, Ishibashi M, Kobayashi J. J Nat Prod. 1994; 57:1008–1011. [PubMed: 7964781]

5. Wen-Tai L, Der-Ren H, Ching-Ping C, Chien-Wei S, Chen-Long H, Tung-Wei C, Chi-Hung L, Yee-Ling C, Ying-Ying C, Yue-Kan L, Huan-Yi T, Chu-Chung L, Jeng-Shin S, Hua-Chien C, Shu-Jen C, Se-Hui W, Chiung-Tong C. *J Med Chem*. 2003; 46(9):1706–1715. [PubMed: 12699388]
6. Peng-Fei Y, Hong C, Jing W, Chun-Xian H, Bo C, Min L, Na Y, Zhi-Yong L, Mao-Sheng C. *Chem Pharm Bull*. 2008; 56(6):831–834. [PubMed: 18520089]
7. James DA, Koya K, Li H, Liang G, Xia Z, Ying W, Wu Y, Sun L. *Bioorg Med Chem Lett*. 2008; 18:1784. [PubMed: 18308566]
8. Sonar VN, Thirupathi Reddy Y, Sekhar KR, Sasi S, Freeman ML, Crooks PA. *Bioorg Med Chem Lett*. 2007; 17:6821. [PubMed: 17980582]
9. Thirupathi Reddy Y, Sekhar KR, Sasi N, Narsimha Reddy P, Freeman ML, Crooks PA. *Bioorg Med Chem Lett*. 2010; 20(2):600–602. [PubMed: 20005706]
10. Penthala NR, Reddy TRY, Parkin S, Crooks PA. *Acta Crystallographica, Section E*. 2008; 64E:o2122.
11. Penthala NR, Reddy TRY, Parkin S, Crooks PA. *Acta Crystallographica, Section E*. 2009; 65E:o62.
12. Kumar R, Srinivasan S, Koduru S, Pahari P, Rohr J, Kyprianou N, Damodaran C. *Cancer Prev Res*. 2009; 2:234.
13. Koduru S, Sowmyalakshmi S, Kumar R, Rohr J, Gomathinayagam R, Damodaran C. *BMC Cancer*. 2009; 9:41. [PubMed: 19183448]
14. Zrinka R, Branka Z, Silvana RM, Katja E, Marijeta K, Kresimir P, Jan B, Erik DC, Mladen M. *Molecules*. 2006; 11:837. [PubMed: 18007390]
15. Ling P, Yun Z, De YK, Wei DZ. *Phytother Res*. 2009 (published online).

Bioorg Med Chem. Author manuscript; available in PMC 2013 July 29.

Figure 2A

Figure 2B

Figure 2. Breast (MDA-231 and MCF-7) and lung (A548 and H460) cancer cell lines were treated with varying concentrations of **3f** (Figure 2A) and **3j** (Figure 2B) for 24 h and Trypan Blue Exclusion assays were performed to determine cell viability. Each data point represents the mean $\pm$ SD for four wells.

Scheme 1.

Reagents and conditions: (a) appropriate benzylhalide, aqueous NaOH solution, triethylbenzylammonium chloride, DCM, RT; (b) Method A: hydantoin, ammonium acetate in acetic acid, microwave irradiation, 40–60 seconds, 80–85% yield; (c) Method-B: hydantoin, ammonium acetate in acetic acid, 115–116 °C, 8–12 hours, 74–85% yield.

Table 1 Synthesis of (*Z*)-5-(*N*-benzylindol-3-ylmethylene)imidazolidine-2,4-diones **3a-m**.

Entry	Method-A ⁹		Method-B	
	Yield (%)	Time (sec)	Yield (%)	Time (hrs)
3a	84	40	80	8
3b	82	60	75	10
3c	85	40	76	10
3d	83	40	74	10
3e	81	50	75	12
3f	80	60	80	9
3g	81	60	74	12
3h	80	60	76	12
3i	85	40	79	10
3j	84	60	76	12
3k	83	50	75	12
3l	80	60	78	10
3m	82	50	79	10

Table 2IC₅₀ values for (*Z*)-5-(*N*-benzylindol-3-ylmethylene)imidazolidine-2,4-dione derivatives **3a-m**.

Entry	Lung cancer cell lines		Breast cancer cell lines	
	A-549 (IC ₅₀)	H-460 (IC ₅₀)	MCF-7 (IC ₅₀)	MDA-231 (IC ₅₀)
3a	>40	14.2	27.3	15.7
3b	>40	19.1	14.1	17.6
3c	>40	>40	10.9	23.3
3d	25.4	25.4	21.4	25.3
3e	>40	30.8	15.4	18.4
3f	39.5	16.9	4.4	21.8
3g	27.8	16.3	22.3	32.8
3h	29.7	26.9	9.2	18.0
3i	25.2	22.3	16.4	23.2
3j	27.7	14.6	5.2	20.1
3k	>40	>40	7.6	12.4
3l	>40	28.4	7.2	7.0
3m	34.8	26.1	20.2	22.1
5-FU ^{14,15}	5.4	2.7	15.2	3.5