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Novel substituted (Z)-5-((N-benzyl-1H-indol-3-yl)methylene)imidazolidine-2,4-diones and 5-((N-benzyl-1H-indol-3-yl)methylene)pyrimidine-2,4,6(1H,3H,5H)-triones as potent radio-sensitizing agents

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Abstract

A series of (Z)-5-((N-benzyl-1H-indol-3-yl)methylene)imidazolidine-2,4-dione (**9a–9m**) and 5-((N-benzyl-1H-indol-3-yl)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (**10a–10i**) derivatives that incorporate a variety of aromatic substituents in both the indole and N-benzyl moieties have been synthesized. These analogs were evaluated for their radiosensitization activity against the HT-29 cell line. Three analogs, **10a**, **10b**, and **10c** were identified as the most potent radiosensitizing agents.

Keywords

Aplysinopsin analogs; Radiosensitizing agents; Indolyl barbiturates; Indolyl hydantoins

Rectal cancer is one of the most frequent cancers in Western countries, and over 40,000 Americans were diagnosed with rectal cancer in 2008.¹ Radiation therapy has been used widely as adjuvant therapy with or without chemotherapy to improve treatment outcomes. Postoperatively administered radio-chemotherapy provides improved local control and overall survival in locally advanced disease. Debulking of tumors using ionizing radiation provides another opportunity to decrease recurrence events due to an improved ability to obtain radial margins.² The colorectal adenocarcinoma cells (HT-29) used in the present study express the cancer stem cell markers CD44 and EpCAM,³ and are extremely resistant to radiation.⁴ Failure to control primary disease leads to progression to devastating invasive disease, which is associated with an increased risk of metastasis and cancer mortality.^{5,6} Unfortunately, in cases of aggressive disease, ionizing radiation does not always provide local-regional control. Hence, there is an urgent need to develop radiosensitizers.

Therapeutic hyperthermia (temperatures greater than 42 °C) is one of the most potent radiation sensitizers identified to date.⁷ Cytotoxicity exerted by heat shock is believed to be the consequence of changes in protein conformation, which includes protein unfolding and aggregation.⁸ Several studies have confirmed this hypothesis.^{9–12} Chromosomal aberrations induced by ionizing radiation are a consequence of misrepaired or unrepaired DNA double strand breaks, which contribute significantly to radiation-induced cell death.¹³ Hyperthermia suppresses the rejoining of DNA double strand breaks induced by ionizing radiation, but does not affect the formation of radiation-induced breaks.¹⁴ Thus, heat shock enhances the formation of radiation-induced chromosomal aberration by inhibiting the repair of DNA double strand breaks. Although many recent clinical studies have demonstrated the therapeutic benefit of adjuvant hyperthermia in treating aggressive cancers such as chest wall, cervical, bladder, and head and neck,¹⁶ technical problems in heat delivery has severely hampered the clinical use of hyperthermia.^{10,15}

In order to overcome the problem of inadequate thermal dosing, we have used an unbiased compound discovery program that employed a forward chemical genetics screen to identify small molecule indole-based compounds that increase (two-fold) thermal sensitivity and thermal radiosensitization of tumor cells at the clinically achievable temperature of 41 °C. We have used indomethacin as a structural platform to design novel thermal-sensitizers. Previously, (Z)-2-(*N*-benzyl-indol-3-ylmethylene)quinuclidin-3-one, (Z)-2-(*N*-benzylindol-3-ylmethylene)quinuclidin-3-ol, (Z)-2-(*N*-benzenesulfonylindol-3-ylmethylene)quinuclidin-3-one, (Z)-2-(*N*-benzenesulfonylindol-3-yl-methylene)quinuclidin-3-ol, and (Z)-2-(*N*-(4-chlorobenzyl) indol-3-ylmethylene)quinuclidin-3-ol (**1–5**, respectively; Fig. 1) were identified as potent thermal-sensitizers that could lower the threshold needed for thermal sensitivity to radiation treatment.^{17,18}

The indole nucleus plays an important role in the activity of these compounds, as lack of an indole moiety produced inactive compounds.¹⁷ Also, absence of the *N*-benzyl group also results in inactive or less active compounds. *N*-Benzenesulfonyl derivatives (**3** and **4**) were found to be less potent than *N*-benzyl or *N*-4-chlorobenzyl derivatives (**2** and **5**).

Aplysinsin analogs (**6**; Fig. 1) have structural similarities to compounds **1–5**, and are reported to be potent cytotoxic agents against various cancer cells. These observations prompted us to design, synthesize, and evaluate the radiosensitization activity of a series of (Z)-5-((*N*-benzyl-1*H*-indol-3-yl)methylene)imidazolidine-2,4-diones (**9a–9m**) and 5-((*N*-benzyl-1*H*-indol-3-yl)methylene) pyrimidine-2,4,6(1*H*,3*H*,5*H*)triones (**10a–10i**), and also to undertake a more detailed investigation of the structure–activity relationships of these analogs that incorporate a variety of different aromatic substituents in both the *N*-benzyl moiety and the indole moiety. The synthetic routes to these two groups of analogs are illustrated in Scheme 1.

The appropriate substituted *N*-benzylindole-3-carboxaldehyde **8** was prepared in 85–90% yield by the reaction of the corresponding indole-3-carboxaldehyde **7** with various substituted benzyl halides under phase-transfer catalytic (PTC) conditions utilizing triethylbenzylammonium chloride and a 50% w/v aqueous NaOH solution in dichloromethane.

The substituted (Z)-5-((N-benzyl-1*H*-indol-3-yl)methylene)imidazolidine-2,4-dione derivatives (**9a–9m**) were prepared in 80–85% yield by the condensation of **8** with hydantoin in the presence of NH₄OAc in acetic acid under microwave irradiation conditions. The condensation of **8** with barbituric acid in methanol at room temperature afforded the corresponding 5-((N-benzyl-1*H*-indol-3-yl)methylene)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione derivatives (**10a–10i**) in 85–90% yield. All the synthesized compounds were characterized by ¹H NMR and ¹³C NMR spectrometry and HRMS analysis.¹⁹ The geometry of the double bond in the substituted (Z)-5-((N-benzyl-1*H*-indol-3-yl)methylene)imidazolidine-2,4-dione derivatives (**9a–9m**) was established from X-ray crystallographic data.^{20,21}

Colony formation assays of tumor cells were used to assess the potency of newly synthesized analogs.¹⁷ HT-29 cells growing exponentially in T25 flasks (*n* = 4) were exposed to DMSO/analog for 30 min, irradiated (4 Gy) at room temperature using a Cs¹³⁷ source, and then incubated at 37 °C for 90 min. Analog was then washed off, and cells were incubated in fresh medium for 14 days. Viable cells formed colonies that were stained with crystal violet and counted. The plating efficiency was defined as the ratio of the number of colonies counted divided by the initial number of cells exposed to vehicle control (DMSO) for 2 h. Vehicle treatment (2 h) was not toxic to cells, as survival was not significantly different from plating efficiency measured in the absence of vehicle (*p* > 0.05 Student's *t*-test) and was arbitrarily set at 1.0. All analogs were tested at 25 μM. The percentage of surviving cells following exposure to analog alone was 70% or greater. Induction of heat shock protein 70 (Hsp70) and activation of heat shock factor 1 (Hsf1) was studied with analog **10a**; these results will be published elsewhere. The surviving fraction of cells irradiated in the absence or presence of analog (2 h/37 °C) was calculated from the ratio of the number of colonies counted divided by the initial number of cells treated. The surviving fraction is corrected for both plating efficiency and analog toxicity. Radiosensitization is defined as a decrease in survival following exposure to radiation plus compound that exceeds 25%.

(Z)-5-((N-Benzyl-1*H*-indol-3-yl)methylene)imidazolidine-2,4-dione derivatives (**9a–9m**, Table 1) that incorporate a variety of aromatic substituents in both the indole and *N*-benzyl moieties did not show any radiosensitization activity against the HT-29 cell line at 25 μM concentration.

The 5-((N-benzyl-1*H*-indol-3-yl)methylene)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione derivatives (**10a–10i**, Table 2) exhibited greater radiosensitization activity over the corresponding (Z)-5-((N-benzyl-1*H*-indol-3-yl)methylene)imidazolidine-2,4-dione derivatives. Introduction of a variety of aromatic substituents into the indole moiety of 5-((N-benzyl-1*H*-indol-3-yl)methylene)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione derivatives (**10e**, **10f**, and **10g**) did not increase radiosensitization activity compared to solvent control (*p* < 0.05 Student's *t*-test). However, introduction of electron withdrawing groups, such as 4-CN, 4-NO₂, or 4-COOCH₃ into the *N*-benzyl moiety (i.e., compounds **10a**, **10b**, and **10c**) significantly increased radiosensitization activity compared to solvent control (*p* < 0.05 Student's *t*-test).

The goal of the present study was to develop analogs that mimic heat shock which could be used as radiosensitizers at physiological temperatures. As discussed above, our initial indole analogs (**1–5**) exhibited thermal sensitization only when the cells were heated at 41 °C. In

the present study, the second generation indole derivatives exhibited radio-sensitization at physiological temperature, 37 °C. Compound **10a** was studied in detail to demonstrate its ability to induce Hsp70 protein expression and to activate Hsf1. Also, several other cancer cell lines, including pancreatic cancer (Panc1), lung cancer (H460), and breast cancer (MCF-7) cell lines were radiosensitized in the presence of compound **10a** (unpublished data). These results also provide evidence to support the hypothesis that radiosensitization by heat shock is independent of cancer cell type.

In conclusion, novel compounds containing an *N*-benzylindole nucleus linked to a barbituric acid moiety via a double bond, and incorporating an electron withdrawing substituent such as –CN, –NO₂, or –COOCH₃ at the 4-position of the *N*-benzyl group, exhibit potent radiosensitizing properties. The present study demonstrates for the first time that novel indole derivatives that mimic heat shock can be designed and synthesized.

Acknowledgments

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References and notes

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. *CA Cancer J Clin.* 2008; 58:71. [PubMed: 18287387]
2. Rodel C, Sauer R. *Surg. Oncol.* 2004; 13:93. [PubMed: 15572091]
3. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C, Clarke MF. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:10158. [PubMed: 17548814]
4. Gao X, Saha D, Kapur P, Anthony T, Livingston EH, Huerta SJ. *Surg. Oncol.* 2009; 100:149.
5. Fredriksson I, Liljegren G, Arnesson LG, Emdin SO, Palm-Sjovall M, Fornander T, Holmqvist M, Holmberg L, Frisell J. *Eur. J. Cancer.* 2002; 38:1860. [PubMed: 12204668]
6. Haasbeek CJ, Uitterhoeve AL, van der Velden J, Gonzalez DG, Stalpers LJ. *Radiother. Oncol.* 2008; 89:197. [PubMed: 18237803]
7. Kampinga HH, Dikomey E. *Int. J. Radiat. Biol.* 2001; 77:399. [PubMed: 11304434]
8. Lepock JR. *Int. J. Hyperthermia.* 2005; 21:681. [PubMed: 16338849]
9. Dewey WC, Westra A, Miller HH, Nagasawa H. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* 1971; 20:505. [PubMed: 5316696]
10. Lepock JR, Frey HE, Rodahl AM, Kruuv J. *J. Cell. Physiol.* 1988; 137:14. [PubMed: 3170654]
11. Massicotte-Nolan P, Glofcheski DJ, Kruuv J, Lepock JR. *Radiat. Res.* 1981; 87:284. [PubMed: 7267996]
12. Nguyen VT, Morange M, Bensaude O. *J. Biol. Chem.* 1989; 264:10487. [PubMed: 2499580]
13. Thacker J. *Int. J. Radiat. Biol.* 1994; 66:591. [PubMed: 7983451]
14. Iliakis G, Seaner R, Okayasu R. *Int. J. Hyperthermia.* 1990; 6:813. [PubMed: 2394929]
15. Nielsen OS, Horsman M, Overgaard J. *Eur. J. Cancer.* 2001; 37:1587. [PubMed: 11527682]
16. Horsman MR, Overgaard J. *Clin. Oncol. (R. Coll. Radiol.).* 2007; 19:418. [PubMed: 17493790]
17. Sekhar KR, Sonar VN, Muthusamy V, Sasi S, Laszlo A, Sawani J, Horikoshi N, Higashikubo R, Bristow RG, Borrelli MJ, Crooks PA, Lepock JR, Roti Roti JL, Freeman ML. *Cancer Res.* 2007; 67:695. [PubMed: 17234780]
18. Sonar VN, Thirupathi Reddy Y, Sekhar KR, Sasi S, Freeman ML, Crooks PA. *Bioorg. Med. Chem. Lett.* 2007; 17:6821. [PubMed: 17980582]

19. *Analytical data and yields for four of the most active compounds: (9a):* ^1H NMR (DMSO- d_6): δ 5.56 (s, 2H), 6.73 (s, 1H), 7.13–7.23 (m, 2H), 7.44–7.46 (d, 2H), 7.51–7.53 (d, 1H), 7.56 (m, 3H), 8.30 (s, 1H), 10.15 (br s, 1H), 11.07 (br s, 1H); ^{13}C NMR (DMSO- d_6): δ 50.0, 101.4, 109.2, 111.0, 111.2, 119.2, 119.3, 121.3, 123.4, 124.9, 128.1, 128.7, 130.6, 133.2, 136.2, 143.7, 155.9, 165.8. HRMS (EI $^+$): m/z found 342.1117, calcd $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_2$ (EI $^+$) 342.1116; Yield: 93%: **(10a)**: ^1H NMR (DMSO- d_6): δ 5.81 (s, 2H), 7.33–7.36 (m, 2H), 7.43–7.45 (d, 2H), 7.61–7.64 (m, 1H), 7.82–7.84 (d, 2H), 7.90–7.93 (m, 1H), 8.68 (s, 1H), 9.63 (s, 1H), 11.07 (br s, 1H), 11.15 (br s, 1H); ^{13}C NMR (DMSO- d_6): δ 49.7, 109.4, 110.5, 110.8, 111.8, 117.9, 118.4, 123.0, 123.8, 128.0, 129.6, 132.6, 136.1, 141.7, 141.8, 142.6, 159.2, 162.9, 164.1. HRMS (EI $^+$): m/z found 370.1066, calcd $\text{C}_{21}\text{H}_{14}\text{N}_4\text{O}_3$ (EI $^+$) 370.1067; Yield: 95%: **(10b)**: δ 3.82 (s, 3H), 5.79 (s, 2H), 7.33–7.36 (m, 2H), 7.40–7.42 (d, 2H), 7.61–7.63 (m, 1H), 7.91–7.93 (m, 1H), 7.94–7.95 (d, 2H), 8.68 (s, 1H), 9.62 (s, 1H), 11.06 (br s, 1H), 11.14 (br s, 1H); ^{13}C NMR (DMSO- d_6): δ 49.9, 52.1, 109.2, 110.7, 111.8, 117.8, 122.9, 123.7, 127.4, 129.0, 129.5, 129.6, 136.2, 141.4, 141.7, 142.6, 150.1, 162.8, 164.1, 165.5. HRMS (EI $^+$): m/z found 403.1168, calcd $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}_5$ (EI $^+$) 403.1167; Yield: 94%: **(10c)**: ^1H NMR (DMSO- d_6): δ 5.81 (s, 2H), 7.33–7.36 (m, 2H), 7.43–7.45 (d, 2H), 7.61–7.64 (m, 1H), 7.82–7.84 (d, 2H), 7.90–7.93 (m, 1H), 8.68 (s, 1H), 9.63 (s, 1H), 11.07 (br s, 1H), 11.15 (bs, 1H); ^{13}C NMR (DMSO- d_6): δ 49.7, 109.4, 110.5, 110.8, 111.8, 117.9, 118.4, 123.0, 123.8, 128.0, 129.6, 132.6, 136.1, 141.7, 141.8, 142.6, 159.2, 162.9, 164.1. HRMS (EI $^+$): m/z found 390.0964, calcd $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_5$ (EI $^+$) 390.0965; Yield: 93%:
20. Penthala NR, Yerramreddy TR, Parkin S, Crooks PA. *Acta Crystallogr. Sect. E.* 2008; 64:2122.
21. Penthala NR, Yerramreddy TR, Parkin S, Crooks PA. *Acta Crystallogr. Sect. E.* 2009; E65:62.

Figure 1. Chemical structures of the potent thermal radiosensitizing agents (1–5) and potent aplysinopsin anti-cancer agents (6).

Scheme 1.

Reagents and conditions: (a) substituted benzyl halide, 50% NaOH aq, triethylbenzylammonium chloride, DCM, rt; (b) NH₄OAc, AcOH, microwave irradiation, 40–60 s; (c) MeOH, rt, 4–6 h.

Table 1

Relative survival of cultured HT-29 cells and the relative potency of substituted (Z)-5-((N-benzyl-1*H*-indol-3-yl)methylene)-imidazolidine-2,4-diones determined following exposure to 25 μ M concentration of analog and 4 Gy radiation

Analog	Relative survival ^a of HT-29 cells	Relative potency ^b
9a	1.53	0.65
9b	1.30	0.77
9c	1.14	0.88
9d	1.44	0.69
9e	1.00	1.00
9f	1.18	0.85
9g	0.91	1.10
9h	1.38	0.72
9i	1.29	0.77
9j	1.18	0.85
9k	1.06	0.94
9l	1.38	0.72
9m	1.06	0.94
Vehicle	1.00	1.00

^aRelative survival represents survival following radiation plus analog divided by that produced by irradiation alone.

^bRelative potency is defined as the reciprocal of relative survival value.

Table 2

Relative survival of cultured HT-29 cells and the relative potency of substituted 5-((*N*-benzyl-1*H*-indol-3-yl)methylene) pyrimidine-2,4,6(1*H*,3*H*,5*H*)trione analogs determined following exposure to 25 μ M concentration and 4Gy radiation

Analog	Relative survival ^a of HT-29 cells	Relative potency ^b
10^a	0.23 ^c	4.35 ^c
10^b	0.64 ^c	1.56 ^c
10^c	0.44 ^c	2.27 ^c
10^d	1.35	0.74
10^e	1.38	0.72
10^f	1.73	0.58
10^g	0.91	1.10
10^h	1.18	0.85
10ⁱ	1.14	0.88
Vehicle	1.00	1.00

^aRelative survival represents survival following radiation plus analog divided by that produced by irradiation alone.

^bRelative potency is defined as the reciprocal of relative survival value.

^cThese analogs produced significant radiosensitization ($p < 0.05$ Student's *t*-test).