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# Synthesis and in vitro screening of novel *N*-benzyl aplysinopsin analogs as potential anticancer agents

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ABSTRACT

A series of novel substituted (*Z*)-5-((1-benzyl-1*H*-indol-3-yl)methylene)imidazolidin-2,4-diones (**3a**–**f**) and (*Z*)-5-((1-benzyl-1*H*-indol-3-yl)methylene)-2-iminothiazolidin-4-ones (**3g**–**o**) have been synthesized utilizing microwave irradiation. These analogs were evaluated for in vitro cytotoxicity against a panel of 60 human tumor cell lines. Compound **3i** exhibits potent growth inhibition against melanoma UACC-257 ($GI_{50}$ = 13.3 nM) and OVCAR-8 ovarian ($GI_{50}$ = 19.5 nM) cancer cells while possessing significant cytotoxicity ($LC_{50}$ = 308 nM and $LC_{50}$ = 851 nM, respectively) against the same cell lines within this series of compounds. A second analog, **3a**, had $GI_{50}$ values of 307 and 557 nM against SK-MEL-2 melanoma and A498 renal cancer cell lines, and exhibited $GI_{50}$ values ranging from 0.30 to 6 μM against 98% of all cancer cell lines in the 60-cell panel. Thus, (*Z*)-5-((5-chloro-1-(4-fluorobenzyl)-1*H*-indol-3-yl)methylene)-2-iminothiazolidin-4-one (**3i**) and (*Z*)-methyl 1-(4-cyanobenzyl)-3-((2,5-dioxoimidazolidin-4-ylidene)methyl)-1*H*-indole-6-carboxylate (**3a**) can be regarded as useful lead compounds for further structural optimization as antitumor agents.



Considerable effort has been focused on the development of anti-cancer agents that contain an indole moiety as their main structural motif.[1–5] We have recently reported on the antitumor activity of a number of novel indole barbituric acid analogs and related compounds that act as both radiosensitizing agents and anticancer agents (e.g., Fig. 1. Structure **A**).[6,7] Recently, our laboratory reported on the cytotoxicity of a series of (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)-methylene-1-methyl-1*H*-imidazol-4(5*H*)-ones against human tumor cell lines (e.g., Fig. 1. Structure **B**).[8] In this respect, Li et al. have synthesized and studied a series of structurally related *N*-heterocyclic indolyl glyoxylamides (Fig. 1. Structure **C**), and found that such compounds possess marked activity against several cancer cell lines, including multidrug resistance (MDR) cell lines.[9] *N*-Benzylindole and indolizine glyoxyl amides (Fig. 1. Structure **D**) have also been shown to exhibit substantial in vitro antiproliferative activity against various cancer cell lines, including hematologic and solid tumor cell lines, that is, leukemia, breast, colon, and uterine.[10]

In our continuing efforts to develop small molecules that function as novel anticancer agents we have now synthesized a series of (*Z*)-5-((1-benzyl-1*H*-indol-3-yl)methylene)imidazolidin-2,4-diones (**3a**–**f**) and a series of (*Z*)-5-((1-benzyl-1*H*-indol-3-yl)methylene)-2-iminothiazolidin-4-ones (**3g**–**o**) as second generation analogs of the aplysinopsin analog B (Fig. 1).

In the synthesis of analogs **3a**–**o**, a series of substituted *N*-benzylindole-3-carboxaldehydes were initially synthesized in 85–90% yield by reacting the appropriate indole-3-carboxaldehyde with various substituted benzyl halides under phase-transfer catalytic (PTC) conditions using triethylbenzyl ammonium chloride (TEBA) and 50% w/v aqueous NaOH solution in dichloromethane (Scheme 1). Aldol condensation of the appropriate *N*-benzylindole-3-carboxaldehyde with hydantoin or 2-iminothiazolidin-4-one, in the presence of $CH_3COOH$ and sodium acetate under microwave irradiation conditions (1100 W; Kenmore, 30–60 s) (Scheme 1), afforded a series of (*Z*)-5-((1-benzyl-1*H*-indol-3-yl)methylene)imidazolidin-2,4-diones (**3a**–**f**) and (*Z*)-5-((1-benzyl-1*H*-indol-3-yl)methylene)-2-iminothiazolidin-4-ones (**3g**–**o**) (Table 1), respectively, in yields ranging from 85–93%. All the synthesized compounds were fully characterized by $^1$H NMR and $^{13}$C NMR.[12]

The in vitro screening studies involved a two-stage process in the evaluation of compounds (**3a**–**o**) against a 60 human tumor cell line panel. In the first stage of the screen, compounds which showed more than 60% growth inhibition in at least eight of the 60 tumor cell lines in single dose (10 μM) studies, were selected for the second stage five dose–response studies, according to the procedures described by Rubinstein et al.[11]

The human tumor cell line panel included leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines. From the single dose–response studies the imidazolidine-2,4-dione analog (**3a**) showed >60% growth inhibition in 50 of the 60 cancer cell lines utilized. The remainder of the

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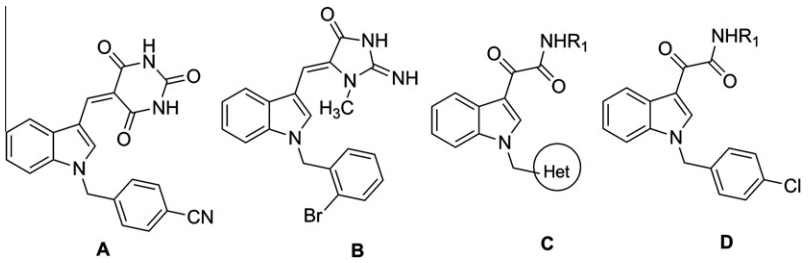
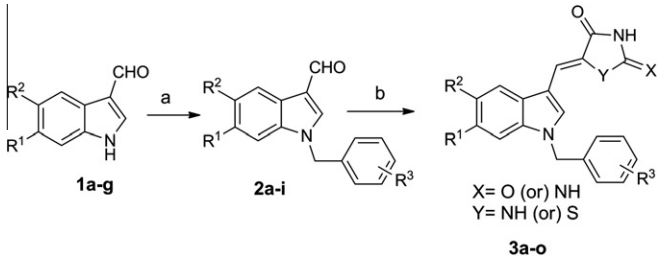



**Figure 1.** Cytotoxic indole-derived aplysinopsin analogs (**A**–**D**).




**Scheme 1.** Reagents and conditions: (a) Appropriate benzyl halides, 50% NaOH, $CH_2Cl_2$, TEBA, 2 h; (b) Hydantoin or 2-iminothiazolidin-4-one, sodium acetate, acetic acid, microwave irradiation, 30–60 s, 85–93% yield.

**Table 1**
*N*-Benzyl aplysinopsin analogs (**3a**–**o**)

| S.No. | $R^1$ | $R^2$ | $R^3$ | X | Y |
|---|---|---|---|---|---|
| **3a** | $COOCH_3$ | H | *p*-CN | O | NH |
| **3b** | H | $COOCH_3$ | *p*-CN | O | NH |
| **3c** | H | H | *p*-CN | O | NH |
| **3d** | H | H | *H* | O | NH |
| **3e** | Cl | H | H | O | NH |
| **3f** | $OCH_3$ | H | H | O | NH |
| **3g** | H | H | *o*-F | NH | S |
| **3h** | H | Cl | *o*-F | NH | S |
| **3i** | H | Cl | *p*-F | NH | S |
| **3j** | H | Br | *o*-F | NH | S |
| **3k** | H | Br | *p*-F | NH | S |
| **3l** | $COOCH_3$ | H | *p*-CN | NH | S |
| **3m** | $COOCH_3$ | H | *p*-$COOCH_3$ | NH | S |
| **3n** | H | $COOCH_3$ | *p*-CN | NH | S |
| **3o** | H | $COOCH_3$ | *p*-$COOCH_3$ | NH | S |

analogs in this series did not qualify for five dose–response screening. In the 2-iminothiazolidin-4-one series, analog **(3i)** showed more than 60% growth inhibition in 14 of the 60 cancer cell lines. The other compounds in the same series (**3g**–**h** and **3j**–**o)** did not qualify for five dose testing.

Some structure–activity data could be generated from the initial single dose studies. In the *para*-cyano-*N*-benzylindoleimidazolidine-2,4-dione series (**3a**–**c**), the 6-carbomethoxyindole analog **3a** exhibited the best growth inhibition, and moving this substituent to the 5-indolic carbon (analog **3b**) resulted in a complete loss of growth inhibition, indicating a significant regiospecific requirement for this substituent. However, in the *N*-benzyl substituted indole-2-iminothiazolidin-4-one series (**3g**–**o**), both the 5- and 6-carbomethoxyindole analogs **3o** and **3m** exhibited growth inhibition against colon KM12 and CNS U251 cells, although these analogs contained a *para*-carbomethoxy-*N*-benzyl moiety rather that a *para*-cyano-*N*-benzyl moiety. In addition, unlike **3m**, analog **3o** was active against colon HCT-116 cells and lung NCI-H460 cells. Interestingly, the presence of a 5-indolic halogeno substituent (Cl or Br) and an indolic *N*-*para*-fluorobenzyl or *N*-*ortho*-fluorobenzyl moiety in the indole-2-iminothiazolidin-4-one series afforded compounds **3i**, **3j**, and **3k** with growth inhibitory activity against colon HCT-116 cells. Analog **3k** was also active against melanoma LOX IMVI cells. Analog **3i** exhibited the best growth inhibitory properties in this series.

The two most active compounds (**3a** and **3i**) from the preliminary 60 cell screen were evaluated in five dose–response studies for their in vitro cytotoxic effects on growth parameters against each of the 60 human tumor cell lines. Dose-response curves were created by plotting cytotoxic effect against the $\log_{10}$ of the drug concentration for each cell line. Cytotoxic effects of each compound were determined as $GI_{50}$, and $LC_{50}$ values, which represent the molar drug concentration required to cause 50% growth inhibition, and the concentration that kills 50% of the cells, respectively. The results are presented in Table 2.

**Table 2**
Antitumor activity ($GI_{50}/\mu M$)[a] and toxicity ($LC_{50}/\mu M$)[b] data of compounds selected for five dose studies for the NCI 60-cell lines screen

| Panel/cell line | Compound **3a** | | Compound **3i** | |
|---|---|---|---|---|
| | $GI_{50}$ | $LC_{50}$ | $GI_{50}$ | $LC_{50}$ |
| *Leukemia cancer* | | | | |
| CCRF-CEM | 3.06 | >100 | >100 | >100 |
| HL-60(TB) | 2.54 | >100 | >100 | >100 |
| K-562 | 0.97 | >100 | 77.6 | >100 |
| MOLT-4 | 1.38 | >100 | >100 | >100 |
| RPMI-8226 | 3.61 | >100 | >100 | >100 |
| *Non-small cell lung cancer* | | | | |
| A549/ATCC | 4.44 | >100 | 0.77 | >100 |
| EKVX | 4.22 | >100 | >100 | >100 |
| HOP-62 | 3.58 | 73.0 | >100 | >100 |
| HOP-92 | na | na | >100 | |
| NCI-H226 | 5.94 | >100 | 80.7 | >100 |
| NCI-H23 | 4.07 | >100 | 60.1 | >100 |
| NCI-H322M | 0.72 | >100 | 71.0 | >100 |
| NCI-H460 | 2.91 | >100 | >100 | >100 |
| NCI-H522 | na | na | 11.0 | >100 |
| *Colon cancer* | | | | |
| COLO 205 | 3.16 | >100 | >100 | >100 |
| HCC-2998 | 2.43 | 85.0 | >100 | >100 |
| HCT-116 | 1.21 | >100 | 5.41 | >100 |
| HCT-15 | na | na | >100 | >100 |
| HT29 | 5.99 | >100 | 20.8 | >100 |
| KM12 | 3.73 | >100 | 7.49 | >100 |
| SW-620 | 2.27 | >100 | 38.3 | >100 |
| *CNS cancer* | | | | |
| SF-268 | 4.22 | >100 | 32.0 | >100 |
| SF-295 | 3.09 | >100 | >100 | >100 |
| SF-539 | 2.39 | >100 | 4.40 | >100 |
| SNB-19 | 3.77 | >100 | >100 | >100 |
| SNB-75 | 4.60 | >100 | 2.47 | >100 |
| U-251 | 2.03 | 59.1 | 2.94 | >100 |
| *Melanoma cancer* | | | | |
| LOX IMVI | 1.43 | >100 | 2.32 | >100 |
| MALME-3M | 2.01 | >100 | 23.5 | >100 |
| M14 | 1.43 | >100 | 57.2 | >100 |
| MDA-MB-435 | 2.76 | >100 | 32.4 | >100 |
| SK-MEL-2 | 0.30 | 30.6 | >100 | >100 |
| SK-MEL-28 | 3.79 | >100 | >100 | >100 |

**Table 2** (*continued*)

| Panel/cell line | Compound **3a** | | Compound **3i** | |
|---|---|---|---|---|
| | $GI_{50}$ | $LC_{50}$ | $GI_{50}$ | $LC_{50}$ |
| SK-MEL-5 | 3.25 | >100 | >100 | >100 |
| UACC-257 | 4.20 | >100 | 0.013 | 0.30 |
| UACC62 | 1.27 | 51.0 | >100 | >100 |
| *Ovarian cancer* | | | | |
| IGR-OV1 | 0.66 | >100 | 3.02 | >100 |
| OVCAR-3 | 2.05 | >100 | >100 | 30.1 |
| OVCAR-4 | 4.13 | >100 | 2.49 | >100 |
| OVCAR-5 | 5.51 | >100 | >100 | >100 |
| OVCAR-8 | 3.75 | >100 | 0.019 | >100 |
| NCI/ADR-RES | >100 | >100 | >100 | >100 |
| SK-OV-3 | 0.86 | 79.8 | >100 | >100 |
| *Renal cancer* | | | | |
| 786-0 | 2.36 | >100 | 37.4 | >100 |
| A498 | 0.55 | >100 | 31.5 | >100 |
| ACHN | 2.08 | >100 | >100 | >100 |
| CAKI-1 | 2.74 | >100 | >100 | >100 |
| RXF 393 | 0.58 | >100 | 1.62 | >100 |
| SN12C | 2.80 | >100 | >100 | >100 |
| TK-10 | 3.87 | >100 | 5.64 | >100 |
| UO-31 | 2.70 | >100 | >100 | >100 |
| *Prostate cancer* | | | | |
| PC-3 | 2.44 | >100 | 23.6 | >100 |
| DU-145 | na | na | 5.24 | >100 |
| *Breast cancer* | | | | |
| MCF7 | 3.55 | >100 | >100 | >100 |
| MDA-MB-231/ATCC | 3.32 | >100 | 6.45 | >100 |
| HS 578T | 0.97 | >100 | 3.67 | >100 |
| BT-549 | 1.24 | >100 | 98.8 | >100 |
| T-47D | 1.50 | >100 | >100 | >100 |
| MDA-MB-468 | 4.14 | >100 | >100 | >100 |

NA: Not analyzed.

[a] $GI_{50}$: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells.

[b] $LC_{50}$: Lethal concentration, concentration of drug lethal to 50% of cells.

Compound **3i** exhibited growth inhibitory properties against 52% of all cancer cell lines in the panel, with $GI_{50}$ values in the range of 0.19–98.8 μM. (Table 2). Good growth inhibitory activity was observed against UACC-257 melanoma ($GI_{50}$ = 13.3 nM), OVCAR-8 ovarian cancer ($GI_{50}$ = 19.5 nM), and A549/ATCC non-small cell lung cancer ($GI_{50}$ = 774 nM) cell lines, and moderate growth inhibition was observed against RXF 393 renal cancer ($GI_{50}$ = 1.62 μM), LOX IMVI melanoma ($GI_{50}$ = 2.32 μM), SNB-75 and U251 CNS cancer ($GI_{50}$ = 2.47 and 2.94 μM, respectively), and OVCAR-4 ovarian cancer ($GI_{50}$ = 2.49 μM) cell lines.

Compound **3a** exhibited growth inhibitory properties against 98% of all cancer cell lines in the panel, with $GI_{50}$ values in the range of 0.30–6 μM (Table 2). Compound **3a** exhibited good growth inhibitory activity against SK-MEL-2 melanoma cell lines ($GI_{50}$ = 307 nM; $LC_{50}$ = 30.6 μM), A498 ($GI_{50}$ = 557 nM; $LC_{50}$ = >100 μM) and RXF 393 renal cancer ($GI_{50}$ = 585 nM; $LC_{50}$ = >100 μM), IGROV1 and SK-OV-3 ovarian cancer ($GI_{50}$ = 667 and 866 nM, respectively), NCI-H322M non-small cell lung cancer ($GI_{50}$ = 721 nm, $LC_{50}$ = >100), HS 578T breast cancer ($GI_{50}$ = 971 nM; $LC_{50}$ = >100 μM), and K-562 leukemia cell lines ($GI_{50}$ = 972 nM; $LC_{50}$ = >100 μM), and showed moderate growth inhibitory activity against HCT-116 colon cancer ($GI_{50}$ = 1.21 μM), BT-549 and T-47D breast cancer ($GI_{50}$ = 1.24 and 1.50 μM, respectively), UACC-62, LOX IMVI and M14 melanoma ($GI_{50}$ = 1.27, 1.43 and 1.43 μM, respectively), and MOLT-4 leukemia ($GI_{50}$ = 1.38) cell lines.

Of particular significance was the observation that the introduction of an indolic 6-carboxymethyl group and an *N*-*para*-cyanobenzyl moiety afforded an analog (**3a)** which exhibited effective growth inhibition in all but one of the cancer cell lines tested. In addition, in the 2-iminothiazolidin-4-one series of compounds, the 5-chloro-(*p*-fluoro-*N*-benzyl)indole analog (**3i)** exhibited very potent growth inhibition of UACC-257 melanoma and OVCAR-8 ovarian cancer cell lines, affording $GI_{50}$ values of 13.3 and 19.5 nM, respectively.

In conclusion, a series of novel substituted (*Z*)-5-((1-benzyl-1*H*-indol-3-yl)methylene)imidazolidin-2,4-diones (**3a**–**f**) (*Z*)-5-((1-benzyl-1*H*-indol-3-yl)methylene)-2-iminothiazolidin-4-ones (**3g**–**o**) have been synthesized and evaluated for their anticancer activity against a panel of 60 human tumor cell lines. Two analogs, (*Z*)-methyl 1-(4-cyanobenzyl)-3-((2,5-dioxoimidazolidin-4-ylidene)methyl)-1*H*-indole-6-carboxylate (**3a**) and (*Z*)-5-((5-chloro-1-(4-fluorobenzyl)-1*H*-indol-3-yl)methylene)-2-iminothiazolidin-4-one (**3i**) emerged as useful lead compounds for further structural optimization as novel anticancer agents.

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12. *Analytical data for compound* **3a**. MF: $C_{22}H_{16}N_4O_4$, mp: >300 °C, $^1$H NMR (300 MHz, DMSO-$d_6$): δ 3.82 (s, 3H, $OCH_3$), 5.68 (s, 2H, $CH_2$), 6.71 (s, 1H, CH), 7.38–7.40 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.72–7.93 (m, 4H, Ar-H), 8.13 (s, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 10.27 (br s, 1H, NH), 11.15 (br s, 1H, NH) ppm; $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 50.00, 52.78, 100.55, 109.65, 111.12, 112.99, 119.26, 121.84, 124.58, 125.76, 128.45 (2C), 131.55, 133.34 (2C), 133.87, 135.66, 143.56, 155.93, 161.86, 165.79, 167.29 ppm.
*Analytical data for compound* **3i**. MF: $C_{19}H_{13}ClFN_3OS$, mp: >300 °C, $^1$H NMR (300 MHz, DMSO-$d_6$): δ 5.55 (s, 2H, $CH_2$), 7.14–7.33 (m, 5H, Ar-H & CH), 7.57–7.58 (d, 1H, *J* = 5.4 Hz, Ar-H), 7.81 (s, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.95 (s, 1H, $C_2$-H), 9.00 (br s, 1H, NH), 9.24 (br s, 1H, NH) ppm; $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 49.66, 111.29, 113.74, 114.45, 116.06, 116.33, 120.81, 121.98, 125.34, 126.24, 129.74, 130.02, 131.53, 133.84, 135.41, 160.54, 163.77, 174.96, 181.07 ppm.