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Microwave assisted synthesis and in vitro cytotoxicities of substituted (*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

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ABSTRACT

The synthesis of several novel substituted (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)methylene-1-methyl-1*H*-imidazol-4(5*H*)-ones structurally related to aplysinopsin have been carried out under microwave irradiation and conventional heating methods. The analogs **3a**, **3b**, **3d**–**3g**, **3k** and **3l** were evaluated for their in vitro cytotoxic activity against an NCI 60 human tumor cell line panel. Compound **3f** exhibited good growth inhibitory properties against all but four of the human cancer cell lines examined, and afforded LC_{50} values <10 μM for 30% of the cell lines in the panel. Compound **3e** was an effective inhibitor of leukemia, CNS, melanoma, and breast cancer cell growth, but generally less effective as a cytotoxic agent. Thus, the aplysinopsin analog **3f** was regarded as a useful lead compound for further structural optimization.

In the past several decades researchers have been challenged by the task of identifying effective clinical agents to treat cancer, which is the second leading cause of death in the United States.[1] The World Cancer Congress (WCC) has released a report stating that 8 million people died from cancer in 2008, and 12 million people were suffering from cancer during the same time period. Anticancer drugs such as cisplatin, 5-fluorouracil, paclitaxel, and docetaxel, are some of the major chemotherapeutic agents currently being used to treat cancer.[2] However, research is still needed to discover newer, more effective anticancer agents. Indole-derived aplysinopsin analogs (Fig. 1. structure **A**) have been reported to be cytotoxic agents against cancer cells.[3,4] Li et al.[5] have synthesized and studied a series of structurally related *N*-heterocyclic indolylglyoxylamides (Fig. 1. structure **B**) and found that such compounds possess interesting activity against several cancer cell lines, including multidrug resistance (MDR) cell lines. James et al.[6] also reported structure–activity relationship (SAR) studies on a series of *N*-benzylindole and indolizine glyoxylamides (Fig. 1. structure **C**) that exhibit substantial in vitro anti-proliferative activity against various cancer cell lines, including hematologic and solid tumor cell lines (i.e., leukemia, breast, colon, and uterine). As the part of a drug discovery program to discover and develop small molecules as potential anticancer agents, we identified (*Z*)-2-(*N*-benzylindol-3-ylmethylidene)quinuclidin-3-ol and (*Z*)-(±)-2-[*N*-(4-chlorobenzyl)indole-3-yl-methylidene]quinuclidin-3-ol as potent thermal-sensitizers capable of lowering the threshold for Hsf1 activation and thermal sensitivity. These compounds were considered as potential thermal radiosensitization agents.[7]

In continuation of our work on the design and synthesis of substituted (*Z*)-5-(*N*-benzyl-1*H*-indol-3-yl)methylene derivatives we focused on a series of novel (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)methylene-1-methyl-1*H*-imidazol-4(5*H*)ones structurally related to aplysinopsin that incorporated electron donating and electron withdrawing substituents in both the indolic ring and the phenyl ring of the *N*-benzyl moiety.

The aromatic substituted *N*-benzylindole-3-carboxaldehydes were synthesized in 85–90% yield by treating the appropriately substituted indole-3-carboxaldehyde with various substituted benzyl halides under phase-transfer catalytic (PTC) conditions utilizing triethylbenzyl ammonium chloride (TEBA) and a mixture of dichloromethane in 50% w/v aqueous NaOH solution (Scheme 1). Aldol condensation of the appropriate *N*-benzylindole-3-carboxaldehyde with creatinine, in the presence of CH_3COOH/sodium acetate utilizing either conventional heating or microwave irradiation methodologies (Scheme 1), afforded a series of novel substituted (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)-methylene-1-methyl-1*H*-imidazol-4(5*H*)-one analogs. Microwave irradiation using an open vessel in a domestic microwave oven (1100 W; Kenmore) at atmospheric pressure was found to be more advantageous than conventional heating, and afforded product yields in the range of 85–91% compared to 70–83% for the latter method (Table 1). In addition, reaction times were very short with

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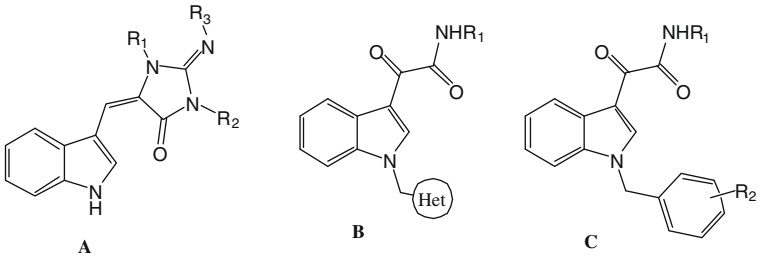
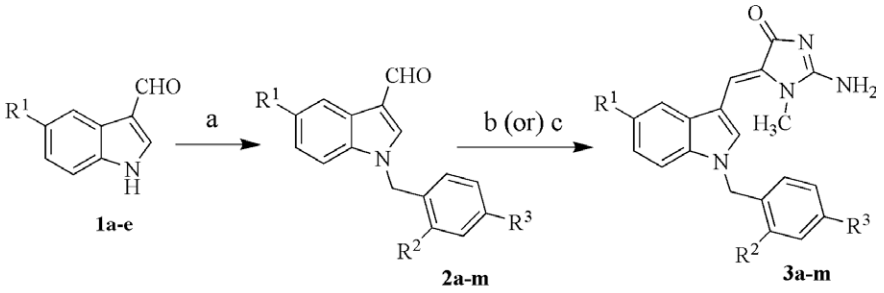


Figure 1. Cytotoxic indole-derived aplysinopsin analogs (**A**–**C**).



3a	$R^1=R^2=R^3=H$,	**3g**	$R^1=R^2=H$, $R^3=F$
3b	$R^1=R^2=H$, $R^3=CN$	**3h**	$R^1=Cl$, $R^2=R^3=H$
3c	$R^1=R^2=H$, $R^3=NO_2$	**3i**	$R^1=Br$, $R^2=H$, $R^3=OCH_3$
3d	$R^1=R^2=H$, $R^3=Cl$	**3j**	$R^1=CH_3$, $R^2=R^3=H$
3e	$R^1=R^3=H$, $R^2=Br$	**3k**	$R^1=Br$, $R^2=R^3=H$
3f	$R^1=R^2=H$, $R^3=COOCH_3$	**3l**	$R^1=OCH_3$, $R^2=R^3=H$
		3m	$R^1=R^2=H$, $R^3=CH_3$

Scheme 1. Synthesis of (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)methylene-1-methyl-1*H*-imidazol-4(5*H*)-one analogs. Reagents and conditions: (a) appropriate benzyl halide, aqueous NaOH solution, triethylbenzyl ammonium chloride, DCM, rt; (b) creatinine (1.1 mol equiv), NaOAc (1.2 mol equiv), AcOH, MWI, 30–60 s; (c) creatinine (1.1 mol equiv), NaOAc (1.2 mol equiv), AcOH, reflux, 7–10 h.

microwave irradiation (30–60 s) compared to conventional heating (7–10 h). All the synthesized compounds were fully characterized by ^{1}H NMR, ^{13}C NMR and mass spectral analysis.[9]

The in vitro screening studies involved a two-stage process with preliminary evaluation of compounds **3a**, **3b**, **3d**–**3g**, **3k** and **3l** against a 60 human tumor cell line panel at a single dose of 10 μM, according to the procedures described by Rubinstein et al.[8] The human tumor cell line panel included leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines. Only compounds which showed more than 60% growth inhibition in at least eight of the 60 tumor cell lines were selected for further dose–response studies; the remaining compounds were not further investigated.

Table 1
Reaction times and yields of novel substituted (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)methylene-1-methyl-1*H* imidazol-4(5*H*)-ones

Compds	Method A		Method B	
	Yield (%)	Time (s)	Yield (%)	Time (h)
	(Microwave condition)		(Conventional heating)	
3a	90	40	79	7
3b	85	60	71	8
3c	88	60	83	10
3d	86	30	73	9
3e	91	40	82	7
3f	87	60	75	10
3g	86	30	70	7
3h	89	40	77	9
3i	87	60	81	10
3j	88	50	78	8
3k	86	60	80	10
3l	87	50	74	9
3m	89	60	72	10

The two most active compounds (**3e** and **3f**) from the preliminary 60 cell screen were subsequently 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. Except for EVVX (non-small cell lung), UACC-257 (melanoma), A498 (renal) and CAKI-1 (renal) cell lines, compound **3f** afforded GI_{50} values in the range 1.46–9.46 μM against all the other cell lines utilized, with 85% of these GI_{50} values falling in the range 1.46–2.93 μM. Of particular interest was the effect of **3f** on cell lines HCC-2998 (colon; GI_{50} = 2.99 μM; LC_{50} = 4.71 μM), SF-539, and SNB-75 (CNS; GI_{50} = 1.54 μM and 1.46 μM, respectively; LC_{50} = 5.56 μM and 5.47 μM, respectively), SK-MEL-28 and SK-MEL-5 (melanoma; GI_{50} = 1.78 μM and 1.69 μM, respectively; LC_{50} = 6.14 μM and 5.75 μM, respectively), 786-0 and ACHN (renal; GI_{50} = 1.75 μM and 1.76 μM, respectively; LC_{50} = 5.85 μM and 6.14 μM, respectively), and T-47D and MDA-MB-468 (breast; GI_{50} = 1.50 μM and 1.63 μM, respectively; LC_{50} = 5.74 μM and 6.78 μM, respectively).

Table 2
Antitumor growth inhibitory activity ($GI_{50}/\mu M$)[a] and cytotoxicity ($LC_{50}/\mu M$)[b] data for compounds **3e** and **3f** in five dose studies against an NCI 60-cancer cell line panel

Panel/cell line	Compound **3e**		Compound **3f**	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}
Leukemia				
CCRF-CEM	2.54	36.2	2.91	>100
HL-60(TB)	1.85	21.0	2.67	>100
K-562	2.45	77.4	2.23	>100
MOLT-4	4.57	>100	2.42	>100
RPMI-8226	1.19	65.9	3.25	>100
SR	1.96	72.7	2.48	>100
Non-small cell lung				
A549/ATCC	28.7	>100	5.05	>100
EKVX	58.1	>100	>100	>100
HOP-62	8.78	51.2	1.99	33.0
HOP-92	2.98	44.5	1.51	7.75
NCI-H226	1.37	69.9	2.26	>100
NCI-H23	8.07	54.8	3.23	58.8
NCI-H322M	11.0	>100	2.56	>100
NCI-H460	3.95	56.8	2.55	>100
NCI-H522	5.38	>100	1.93	>100
Colon				
COLO 205	2.21	12.3	1.91	nd
HCC-2998	6.95	47.2	2.99	4.71
HCT-116	2.42	30.9	1.79	7.70
HCT-15	19.8	>100	2.27	>100
HT29	2.18	6.94	2.17	nd
KM12	5.71	50.8	3.18	>100
SW-620	3.68	43.3	2.20	>100
CNS				
SF-268	5.38	59.8	2.56	>100
SF-295	4.96	>100	2.36	>100
SF-539	1.73	6.94	1.54	5.56
SNB-19	4.50	43.8	2.39	31.0
SNB-75	1.58	18.6	1.46	5.47
U251	3.01	66.2	1.81	7.72
Melanoma				
LOX IMVI	1.97	9.37	1.89	8.66
MALME-3M	3.30	73.8	1.70	8.33
M14	4.46	47.8	1.90	7.25
MDA-MB-435	4.97	68.6	1.93	nd
SK-MEL-2	5.73	>100	2.70	>100
SK-MEL-28	4.14	38.4	1.78	6.14
SK-MEL-5	3.38	42.3	1.69	5.75
UACC-257	12.9	66.9	17.9	>100
Ovarian				
IGR-OV1	20.0	>100	5.91	>100
OVCAR-3	1.95	7.35	2.29	7.98
OVCAR-4	2.41	25.2	2.18	>100
OVCAR-5	2.12	29.0	1.90	18.5
OVCAR-8	10.1	>100	3.45	>100
NCI/ADR-RES	>100	>100	9.46	>100
SK-OV-3	11.9	49.9	1.98	8.68
Renal				
786-0	6.15	46.2	1.75	5.85
A498	13.9	53.1	14.1	53.2
ACHN	11.8	59.0	1.76	6.14
CAKI-1	68.1	>100	35.1	>100
RXF 393	2.77	53.7	1.95	8.71
SN12C	6.07	76.3	1.74	8.59
TK-10	6.84	>100	3.16	>100
UO-31	7.95	>100	3.20	>100
Prostate				
PC-3	10.4	48.1	6.27	>100
DU-145	82.1	68.8	2.25	14.8
Breast				
MCF7	2.25	48.1	2.45	77.8
MDA-MB-231/ATCC	4.07	46.7	2.01	14.3
HS 578T	3.97	>100	2.17	>100
BT-549	2.05	>100	1.73	>100
T-47D	1.68	13.1	1.50	5.74
MDA-MB-468	1.69	6.90	1.63	6.78

nd: Not determined.
[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 **3f** exhibited generally poor LC_{50} values against leukemia and non-small cell lung cancer cell lines. With the exception of the NCI/ADR-RES ovarian cancer cell line, compound **3e** exhibited growth inhibitory effects against all the cell lines tested, with GI_{50} values ranging from 1.19 to 82.1 μM, and with 77% of the cells affording GI_{50} values falling in the range 1.19–4.57 μM. Good growth inhibitory activity was observed against leukemia (GI_{50} = 1.19–4.57 μM), CNS (GI_{50} = 1.58–5.38 μM), and breast (GI_{50} = 1.68–4.07 μM) cell line sub-panels. Generally, **3e** exhibited poorer LC_{50} values compared to those obtained for **3f**. Most notable were the effects of **3e** against HT29 (colon; GI_{50} = 2.18 μM; LC_{50} = 6.94 μM), SF-539 (CNS; GI_{50} = 1.73 μM; LC_{50} = 6.94 μM), OVCAR-3 (ovarian; GI_{50} = 1.95 μM; LC_{50} = 7.35 μM), and MDA-MB-468 (breast; GI_{50} = 1.69 μM; LC_{50} = 6.90 μM) cell lines. The results indicate that introduction of a 2-bromo group (**3e**) or a 4-carboxymethyl moiety (**3f**) into the *N*-benzyl moiety enhanced the activity of *N*-benzyl-indolecreatinine (**3a**), compared to the introduction of 4-cyano (**3b**), 4-chloro (**3d**), and 4-fluoro (**3g**) groups.

In summary, a series of novel substituted (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)methylene-1-methyl-1*H*-imidazol-4(5*H*)-one analogs have been synthesized and evaluated for anticancer activity against a panel of 60 human cancer cell lines. Compounds **3e** and **3f** were identified as molecules of interest from a single dose assay, and were then evaluated for dose-dependent growth inhibition and cytotoxicity in all 60 human cancer cell lines. Compound **3f** exhibited good growth inhibitory properties against all but four of the human cancer cell lines examined, and afforded LC_{50} values <10 μM for 30% of the cell lines in the panel. Compound **3e** was an effective inhibitor of leukemia, CNS, melanoma, and breast cancer cell growth, but was generally less effective as a cytotoxic agent. Thus, the aplysinopsin analog **3f** was regarded as a useful lead compound for further structural optimization in the search for anticancer agents with clinical potential.

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9. *Analytical data for compound* **3e**: ^{1}H NMR (DMSO-d_6): δ 3.28 (s, 3H, N–CH_3), 5.53 (s, 2H, CH_2), 6.53 (s, 1H, CH), 6.76–6.79 (d, 1H, *J* = 6.9 Hz C_4–H), 7.17–7.27 (m, 4H, Ar–H), 7.45–7.47 (t, 1H, *J* = 7.5 Hz C_5–H), 7.68–7.70 (t, 1H, *J* = 6 Hz, C_6–H), 7.71 (br s, 2H, NH_2), 7.95–7.98 (d, 1H, *J* = 8.1 Hz, C_7–H), 9.12 (s, 1H, C_2–H) ppm ^{13}C NMR (DMSO-d_6): δ 27.81, 49.59, 103.49, 109.08, 110.16, 118.30, 119.80, 121.97, 122.10, 127.93, 128.11, 128.61, 129.49, 131.19, 131.42, 132.62, 135.49, 136.17, 164.97, 175.11 ppm ES-API LC–MS *m*/*z*: 409.8 and 410.8 (MH^+).
Analytical data for compound **3f**: ^{1}H NMR (DMSO-d_6): δ 3.49 (s, 3H, N–CH_3), 3.81 (s, 3H, OCH_3), 5.70 (s, 2H, CH_2), 7.20 (s, 1H, CH), 7.23–7.26 (m, 2H, C_4–H and C_7–H), 7.30–7.32 (d, 2H, *J* = 8.4 Hz, Ar–H), 7.52–7.55 (t, 1H, *J* = 9.0 Hz, C_5–H), 7.90–7.93 (d, 2H, *J* = 8.1 Hz, Ar–H), 8.09–8.12 (t, 1H, *J* = 9.3 Hz, C_6–H), 9.03 (s, 1H, C_2–H). 9.31 (br s, 2H, NH_2) ppm ^{13}C NMR (DMSO-d_6): δ 28.74, 49.36, 52.16, 108.23, 110.93, 113.56, 118.75, 120.96, 123.67, 127.09, 128.25, 128.76, 129.48, 133.59, 135.64, 142.55, 151.73, 161.55, 165.65 ppm ES-API LC–MS *m*/*z*: 388.90 (MH^+).