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Synthesis and anti-proliferative activity of aromatic substituted 5-((1-benzyl-1*H*-indol-3-yl)methylene)-1,3-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione analogs against human tumor cell lines

Nikhil Reddy Madadi, Narsimha Reddy Penthala, Venumadhav Janganati, Peter A. Crooks *

Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

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ABSTRACT

Based on previous SAR studies on *N*-benzylindole and barbituric acid hybrid molecules, we have synthesized a series of aromatic substituted 5-((1-benzyl-1*H*-indol-3-yl)methylene)-1,3-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione analogs (**3a–i**) and evaluated them for their in vitro growth inhibition and cytotoxicity against a panel of 60 human tumor cell lines. Compounds **3c**, **3d**, **3f** and **3g** were identified as highly potent anti-proliferative compounds against ovarian, renal and breast cancer cell lines with GI_{50} values in low the nanomolar range. The 4-methoxy-*N*-benzyl analog (**3d**) was the most active compound with GI_{50} values of 20 nM and 40 nM against OVCAR-5 ovarian cancer cells and MDA-MB-468 breast cancer cells, respectively. Two other analogs, **3c** (the 4-methyl-*N*-benzyl analog) and **3g** (the 4-fluoro-*N*-benzyl analog) exhibited equimolar potency against MDA-MB-468 cells GI_{50} = 30 nM). Analog **3f** (the 4-chloro-*N*-benzyl analog) exhibited a GI_{50} value of 40 nM against renal cancer cell line A498. These results suggest that aromatic substituted *N*-benzylindole dimethylbarbituric acid hybrids may have potential for development as clinical candidates to treat a variety of solid tumors.

Cancer is the second most life threatening disease after cardiovascular disease, affecting more than six million people per year worldwide.[1] Drastic changes in life style during the end of the 19th century has increased the risk of humans developing different types of cancers. Also, considerable effort has been put into identifying molecules with anti-cancer properties from both natural and synthetic sources.

Indole and barbituric acids derivatives are known to have a wide range of beneficial biological activities such as anti-cancer,[2] anti-inflammatory,[3] anti-convulsant,[4] anti-psychotic,[5] anti-hypertensive,[6] and anti-bacterial properties.[7] Singh et al. have synthesized and evaluated some novel *N*-benzyl indole-barbituric acid hybrid molecules against a panel of 60 human tumor cell lines. They identified compound **1** (Fig. 1) as a promising lead compound with significant tumor growth inhibitory activity (GI_{50}) against a variety of human cancer cell lines; the molecule also had good maximum tolerable dose (MTD) characteristics.[8] Our laboratory has also reported on several novel indole barbiturates as anti-cancer and radio-sensitization agents[9,10] (e.g., compound **2**, Fig. 1). Recently,[11] we have reported that *N*-aroyl indole thiobarbituric acids (e.g., compound **3**, Fig. 1) possess both anticancer and anti-inflammatory properties. Several of these analogs are also inhibitors of DNA repair and replication stress response polymerases.[12]

In our continuing studies on improving the potencies of newly identified anti-cancer leads, we now report on the synthesis and antiproliferative properties of some aromatic substituted 5-(indolin-3-ylmethylene)-1,3-dimethylpyrimidine-2,4,6-triones as second generation indole barbituric acid hybrids.

A series of *N*-benzylindole-3-carboxaldehydes (**2a–i**) were synthesized by reacting an appropriate indole carboxaldehyde (**1a–c**) with various aromatic substituted benzyl halides utilizing the phase transfer catalyst triethylbenzyl ammonium chloride (TEBAC) in a mixture of 50% w/v aq NaOH solution and dichloromethane. The resulting *N*-benzyl products were obtained in 80–85% yield.[5] The *N*-benzylindole-3-carboxaldehydes (**2a–i**) (1 mmol) were each then reacted with *N*,*N*-dimethylbarbituric acid (1.2 mmol) in methanol at room temperature to afford a series of 5-((1-benzyl-1*H*-indol-3-yl)methylene)-1,3-dimethyl-pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione analogs (**3a–i**) (Scheme 1 and Table 1) in 75–90% yield. The synthesized compounds were fully characterized by ^{1}H NMR and ^{13}C NMR spectrometric analysis.[13]

In vitro screening of the above compounds was carried out against a panel of 60 human tumor cell lines utilizing the procedure described by Rubinstein et al.[14] Compounds **3a–i** were initially screened at 10^{-5} M to determine growth inhibition and cytotoxic properties (Table 2). Compounds **3c**, **3d**, **3f** and **3g** showed more than 60% growth inhibition in at least eight cell lines

* Corresponding author. Tel.: +1 501 686 6495; fax: +1 501 686 6057.
E-mail address: pacrooks@uams.edu (P.A. Crooks).

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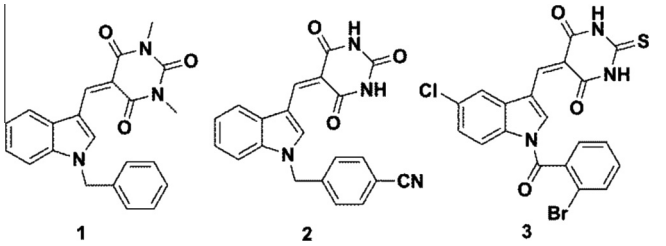
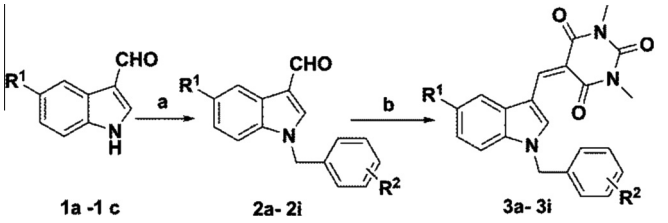


Figure 1. Lead compounds from previous studies (**1**–**3**).[8–10]



Scheme 1. Reagents and conditions: (a) appropriate benzyl halide, 50% NaOH, CH_2Cl_2, TEBAC, 2 h; (b) dimethylbarbituric acid in methanol, room temp, 75–90% yield.

Table 1
5-((1-Benzyl-1*H*-indol-3-yl)methylene)-1,3-dimethyl-pyrimi- dine-2,4,6(1*H*,3*H*,5*H*)-trione analogs (**3a**–**3i**)

Compound	R^1	R^2
3a	$COOCH_3$	*p*-SO_2Ph
3b	H	*o*-Br
3c	H	*p*-CH_3
3d	H	*p*-OCH_3
3e	H	*p*-CN
3f	H	*p*-Cl
3g	H	p-F
3h	Cl	p-F
3i	$COOCH_3$	*p*-$COOCH_3$

Table 2
Percentage growth inhibition of five human cancer cell lines by compounds (**3a**–**3i**)[a] at 10 μM

Cell line	Percentage growth inhibition								
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**
NCI-H226	98	−32	−63	−55	−72	−82	−78	−79	88
OVCAR-5	108	75	−23	−38	6	−23	−33	−19	98
A498	83	74	−91	−92	68	−94	−85	−95	105
TK-10	100	105	−4	−12	62	−26	−15	19	113
MDA-MB-468	88	56	−62	−65	−71	−71	−71	−70	99

[a] If $(OD_{test} - OD_{tzero}) \geq 0$. Then PG is shown as positive. If $(OD_{test} - OD_{tzero}) < 0$, PG is shown as negative, implying cell death.

from the panel of sixty cell lines, and were selected for a complete dose response study at five different concentrations, viz. 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} M.

The growth inhibitory or cytotoxicity effect of the test compounds in the above cellular assay is measured by determining percentage cell growth (PG) inhibition. Optical density (OD) measurements of SRB-derived color just before exposing the cells to the test compound (OD_{tzero}) and after 48hrs exposure to the test compound (OD_{test}) or the control vehicle (OD_{ctrl}) are recorded.[15]

Growth percentage is calculated utilizing one of the two formulas below. A negative growth percentage implies cytotoxicity (Table 2).

If $(OD_{test} - OD_{tzero}) \geq 0$, then

Table 3
Growth inhibition (GI_{50}/μM)[a] and cytotoxicity (LC_{50}/μM)[b] data for compounds **3c**, **3d**, **3f** and **3g** against human cancer cells[c]

Panel/cell line	**3c**		**3d**		**3f**		**3g**	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}
Leukemia								
K-562	2.23	>100	2.18	>100	NA	>100	NA	>100
SR	1.89	>100	5.43	>100	70.3	>100	>100	>100
Non-small cell lung cancer								
HOP-62	7.44	>100	6.22	>100	10.7	>100	7.70	>100
NCI-H226	1.95	>100	1.44	53.9	1.26	>100	2.56	>100
NCI-H23	8.19	>100	4.67	>100	10.2	>100	51.5	>100
NCI-H460	**0.91**	>100	**0.81**	82.8	**0.40**	>100	**0.37**	>100
Colon cancer								
COLO 205	1.89	>100	**0.63**	45.3	2.78	29.5	1.44	>100
HCT-116	5.45	>100	5.01	>100	4.47	>100	6.26	>100
SW-620	NA	>100	6.89	>100	>100	>100	>100	>100
CNS cancer								
SF-295	2.02	>100	1.54	>100	1.21	>100	1.97	>100
SNB-19	26.8	>100	15.8	>100	18.3	>100	26.7	>100
Melanoma								
LOX IMVI	27.8	>100	5.45	>100	17.3	>100	>100	>100
MDA-MB-435	NA	>100	3.29	>100	>100	>100	>100	>100
SK-MEL-2	3.36	>100	2.78	6.25	2.84	75.8	6.35	>100
SK-MEL-5	9.17	>100	5.08	64.4	>100	>100	>100	>100
UACC-257	2.02	>100	1.54	38.2	2.01	>100	2.52	>100
UACC-62	2.42	>100	**0.90**	68	2.06	>100	2.89	>100
Ovarian cancer								
IGROV1	2.95	>100	1.93	>100	2.88	>100	4.33	>100
OVCAR-5	**0.07**	>100	**0.02**	>100	**0.16**	>100	**0.11**	>100
OVCAR-8	>100	>100	13.4	88.6	21.3	>100	>100	>100
NCI/ADR-RES	6.23	>100	3.59	>100	16.3	>100	>100	>100
SK-OV-3	23.7	>100	5.03	>100	6.08	>100	15.3	>100
Renal cancer								
786-0	22.1	>100	11.2	>100	6.90	>100	22.7	>100
A498	**0.12**	**0.69**	**0.06**	5.27	**0.04**	**0.64**	**0.07**	**0.67**
RXF 393	15.2	>100	14.8	>100	12.5	>100	14.9	>100
SN12C	>100	>100	12.5	>100	92.1	>100	>100	>100
TK-10	**0.28**	>100	**0.10**	72.5	**0.18**	50.4	**0.59**	>100
Breast cancer								
MCF7	6.76	>100	5.26	>100	>100	>100	>100	>100
MDA-MB-231/ ATCC	13.6	>100	5.86	>100	12.2	>100	23.6	>100
BT-549	13.5	>100	4.94	>100	12.5	>100	24.1	>100
T-47D	5.00	>100	3.53	>100	3.53	>100	5.38	>100
MDA-MB-468	**0.03**	**0.62**	**0.04**	**0.76**	**0.06**	**0.70**	**0.03**	**0.50**

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.
[c] GI50 and LC50 values below 1 μM are bolded.

$PG = 100 \times (OD_{test} - OD_{tzero})/(OD_{ctrl} - OD_{tzero})$ and
percentage growth is shown as positive.
If $(OD_{test} - OD_{tzero}) < 0$, then
$PG = 100 \times (OD_{test} - OD_{tzero})/OD_{tzero}$ and
percentage growth is shown as negative, which implies cell death.[15]

The four compounds selected for full dose response studies were effective against lung cancer cell line NCI-H226, renal cancer cell line A498 and breast cancer cell line MDA-MB-468 in the single dose screen (Table 2). Activities of all four compounds against tumor cell line 498 were good, with ~−90 percentage growth at 10 μM. Although compounds **3h** and **3e** were not selected for complete dose–response studies, compound **3h** was effective against the A498 cell line (−95 percentage growth), while compound **3e** was active against the MDA-MB-468 cell line (−71 percentage growth) (Table 2).

Further evaluation of lead compounds **3c**, **3d**, **3f** and **3g** in the five dose screen showed that these compounds were very effective

against five particular cancer cell lines: NCI-H460, OVCAR-5, A498, TK-10 and MDA-MB-468, with GI_{50} values in the nanomolar range. Breast cancer cell line MDA-MB-468 appeared to be the most sensitive to the growth inhibition effects of these compounds; **3c**, **3d**, **3f** and **3g** exhibited GI_{50} values of 30 nM, 40 nM, 60 nM, and 30 nM, respectively, with LC_{50} values of 620 nM, 760 nM, 700 nM, and 500 nM, respectively, against this cell type. Compounds **3c**, **3d**, **3f** and **3g** also exhibited good growth inhibition against renal cancer cell line A498, with GI_{50} values of 120 nM, 60 nM, 40 nM, and 70 nM, respectively, and LC_{50} values of 690 nM, 527 nM, 640 nM, and 670 nM, respectively. All four compounds were active against renal cancer cell line TK-10 with GI_{50} values of 280 nM, 100 nM, 180 nM, and 590 nM, respectively, and also exhibited growth inhibitory effects against ovarian cancer cell line OVCAR-5 (GI_{50} = 70 nM, 20 nM, 160 nM, and 110 nM, respectively) and non-small cell lung cancer cell line NCI-H460 (GI_{50} = 910 nM, 810 nM, 400 nM, and 370 nM, respectively). Compound **3d** also inhibited the growth of colon cancer cell line COLO 205 (GI_{50} = 630 nM) and melanoma cell line UACC-62 (GI_{50} = 900 nM) (see Table 3).

In conclusion, a series of novel aromatic substituted 5-((1-benzyl-1*H*-indol-3-yl)-methyl-ene)-1,3-dimethylpyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione analogs have been synthesized and evaluated for growth inhibition properties against a panel of 60 human cancer cell lines, and their GI_{50} and LC_{50} values have been determined. Four lead compounds (**3c**, **3d**, **3f** and **3g**) have been identified with GI_{50}'s in the nanomolar range against 5 different cell lines. All four compounds exhibited GI_{50} values in the range 30–60 nM and 40–120 nM against breast cancer MDA-MB-468 and renal cancer A49 cell lines, respectively; compounds **3c** and **3d** afforded GI_{50} values of 70 nM and 20 nM, respectively, against the ovarian cancer cell line OVCAR-5. The above four compounds generally have superior GI_{50} values compared to the previous lead compound **1** against most of the cell lines in the 60 tumor cell line panel. The biggest difference was the GI_{50} value of compound **1** against renal cancer cell line A49 (GI_{50} = 300 nM) compared to GI_{50} values over the range 40–120 nM for compounds **3c**, **3d**, **3f**, and **3g**. These novel aromatic substituted 5-((1-benzyl-1*H*-indol-3-yl)-methyl-ene)-1,3-dimethylpyrim-idine-2,4,6-(1*H*,3*H*,5*H*)-triones represent promising new analogs that may have clinical potential in treating a variety of solid tumors.

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13. *General experimental procedure:* In a 50 ml round bottom flask the appropriate indole carboxaldehyde (1 mmol), benzyl halide (1.1 mmol), triethylbenzyl ammonium chloride (0.01 mmol) and 50% w/v aq NaOH were added to 5 volumes of DCM. The reaction mixture was stirred at room temperature and monitored by TLC. When the reaction was completed, water was added and the mixture extracted into DCM. The organic layer was concentrated under reduced pressure at 40 °C and the residue was purified by flash chromatography using methanol/DCM as mobile phase to afford the corresponding *N*-benzylindole-3-carboxaldehydes in 80–85% yield. The *N*-benzylindole-3-carboxaldehyde (1 mmol) and *N*,*N*-dimethylbarbituric acid (1.2 mmol) were added to 10 volumes of methanol and the resulting mixture stirred at room temperature. The final product, the appropriate 5-((1-benzyl-1*H*-indol-3-yl)methylene)-1,3-dimethyl-pyrimidine-2,4,6(1*H*, 3*H*,5*H*)-trione crashed out of the solution once the reaction was complete (1–2 h). The final product was filtered and recrystallized from methanol to afford the 5-((1-benzyl-1*H*-indol-3-yl)methylene)-1,3-dimethyl-pyrimidine-2,4,6-(1*H*,3*H* 5*H*)-trione in 75–90% yield. *Analytical data for compound* ***3d****:* Yellow solid; yield, 90%; mp >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.36 (s, 6H, *N*-CH_3), 3.70 (s, 3H, –OCH_3), 5.45 (s, 2H, –CH_2), 6.89–6.91 (d, *J* = 8.8 Hz, 2H, ArH), 7.23–7.31 (m, *J* = 30 Hz, 4H, ArH), 7.61–7.63 (d, *J* = 7.6 Hz, 1H, ArH), 8.09–8.12 (d, *J* = 7.6 Hz, 1H, ArH), 8.45 (s, 1H, ArH), 9.93 (s, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 49.69, 55.44, 111.85, 111.96, 114.52, 117.74, 121.46, 121.53, 122.97, 124.01, 125.23, 128.99, 129.45, 137.31, 141.21, 159.31, 185.09. HRMS (ESI): *m*/*z* calcd for $C_{23}H_{22}N_3O_4$ [M−H] 404.1610; found 404.1606. *Compound* ***3g****:* Yellow solid; yield 85%; mp >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.24 (s, 6H, –*N*–CH_3), 5.70 (s, 2H, –CH_2), 7.17–7.22 (t, *J* = 17.6 Hz, 2H, ArH), 7.34–7.40 (m, *J* = 25.6 Hz, 4H, ArH), 7.67-7.69 (d, *J* = 8Hz, 1H, ArH), 7.87-7.89 (d, *J* = 8.4Hz, 1H, ArH), 8.75 (s, 1H, ArH), 9.66 (s, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6): 28.22, 28.85, 50.02, 109.33, 111.43, 112.61, 118.34, 118.43, 123.63, 124.47, 129.26, 129.65, 130.42, 133.00, 135.87, 136.77, 142.42, 144.15, 151.66, 162.03, 163.38. HRMS (ESI): *m*/*z* calcd for $C_{22}H_{19}N_3O_3F$ [M−H] 392.1410; found 392.1389. *Compound* ***3f****:* Yellow solid; yield, 90%; mp >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.24–3.25 (d, *J* = 3.6 Hz, 6H, *N*-CH_3), 5.71 (s, 2H, –CH_2), 7.31–7.35 (m, *J* = 16.8 Hz, 4H, ArH), 7.41–7.43 (d, *J* = 8 Hz, 2H, ArH), 7.63–7.65 (d, *J* = 8 Hz, 1H, ArH), 7.87–7.89 (d, *J* = 6.4 Hz, 1H, ArH), 8.75 (s, 1H, ArH), 9.66 (s, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 28.22, 28.27, 28.85, 28.90, 50.02, 109.34, 111.43, 112.61, 118.35, 118.44, 123.63, 124.47, 129.26, 129.66, 130.43, 133.00, 135.88, 136.77, 142.42, 144.16, 151.66, 162.03, 163.39. HRMS (ESI): *m*/*z* calcd for $C_{22}H_{19}N_3O_3Cl$ [M−H] 408.1115; found 408.1122. *Compound* ***3c****:* Yellow solid; yield, 90%; mp >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, 3H, –CH_3), 3.23–3.24 (d, *J* = 3.2 Hz, 6H, *N*–CH_3), 5.62 (s, 2H, –CH_2), 7.14–7.16 (d, *J* = 7.6 Hz, 2H, ArH), 7.20–7.22 (d, *J* = 7.6 Hz, 2H, ArH), 7.32–7.33 (t, *J* = 5.6 Hz, 2H, ArH), 7.63-7.65 (d, *J* = 7.6 Hz, 1H, ArH), 7.85–7.86 (d, *J* = 6.8 Hz, 1H, ArH), 8.73 (s, 1H, ArH), 9.64 (s, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 28.21, 28.84, 109.16, 111.34, 118.47, 123.46, 123.75, 127.68, 127.92, 129.72, 129.82, 129.90, 130.47, 133.78, 136.90, 137.67, 142.38, 142.56, 144.32, 151.72, 162.09, 163.47. HRMS (ESI): *m*/*z* calcd for $C_{23}H_{22}N_3O_3$ [M−H] 388.1661; found 388.1636.
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