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Synthesis and in vitro evaluation of *N*-alkyl-3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)indolin-2-one analogs as potential anticancer agents

Narsimha Reddy Penthala, Thirupathi Reddy Yerramreddy, Nikhil Reddy Madadi, Peter A. Crooks *

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

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ABSTRACT

A series of novel 3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)indolin-2-one analogs (**3**) have been synthesized under microwave irradiation and conventional heating methods. These analogs were evaluated for in vitro cytotoxicity against a panel of 57 human tumor cell lines. Compound **3o** had GI_{50} values of 190 nM and 750 nM against A549/ATTC non-small cell lung cancer and LOX IMVI melanoma cell lines, respectively, and both **3n** and **3o** exhibited GI_{50} values ranging from 2 to 5 μM against CCRF-CEM, HL-60(TB), K-562, MOLT-4, and RPMI-8226 leukemia cell lines. These results indicate that *N*-4-methoxybenzyl-3-hydroxy-(2-imino-3-methyl-5-oxo-4-yl)indolin-2-one analogs may be useful leads for anticancer drug development.

Many isatin analogs have been reported as potent and selective cytotoxic agents against cancer cells. Vine et al.[1] reported that the substitution of bromo groups at the C-5 and C-7 positions of isatin afforded an analog (Fig. 1, structure **A**) that exhibited potent cytotoxic activity. These investigators also reported that introduction of *N*-benzyl, *N*-phenethyl, *N*-phenethan-1-one, and *N*-2-naphthylmethyl moieties into the 5,7-dibromoisatin molecule (Fig. 1, structures **B**–**E**, respectively) significantly increased hydrophobicity and increased cytotoxicity toward lymphoma cells. These isatin analogs were also potent against a wide range of human cancer cell lines, including MDA-MB-231 metastatic breast adenocarcinoma cells and U937 human monocyte-like histiocytic lymphoma cells.[1–3] Hall et al.[4] studied the MDR1-selective mechanism of action of several isatin-β-thiosemicarbazones (Fig. 1, structure **F**) against a parental HeLa-derived cervical cancer cell line (KB-3-1), while Perrow et al.[5] have described the cytotoxicity of a number of isatin derivatives conjugated to a cell targeting moiety via a spacer group (Fig. 1, structure **G**). In view of the general anticancer properties of these isatin analogs, and as part of an investigation devoted to the development of new anticancer agents derived from structural modification of indoles,[6–8] the design and synthesis of a novel series of 3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)indolin-2-one analogs (**3a–w**) was undertaken. The resulting analogs were then evaluated for their cytotoxic activity against a panel of 57 human tumor cell lines.

The simple and *N*-alkyl substituted isatins (**1a–j**) were all prepared utilizing literature methods.[9–11] A series of novel substituted 3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)indolin-2-one derivatives (**3a–w**) were synthesized by condensation of the appropriate substituted *N*-alkyl isatin with creatinine, in the presence of sodium acetate and acetic acid via both microwave irradiation and conventional heating methodologies (Scheme 1). Of these two methods, microwave irradiation was found to be advantageous over conventional heating, since the product yields were 83–94% for the former method, but only 70–83% for the latter method. In addition, the time course of the reaction was very fast using microwave irradiation (20–40 s) compared to 6–8 h for conventional heating (Table 1). All the synthesized compounds were characterized by ^{1}H NMR and ^{13}C NMR spectrometry.[16] The geometry of the hydroxyl position in the representative compounds **3a**, **3b** and **3t** was established as *trans* to the 4′-methyne hydrogen from X-ray crystallographic data.[12–14]

From the X-ray diffraction and ^{1}H NMR data, analogs **3a–3w** were mixtures of *RR* and *SS* isomers. This is consistent with the mechanism of the aldol condensation reaction of **1** with **2**, which proceeds via the formation of the *E*-enolate, as per the Zimmerman–Traxler model, which favors *anti* products, and is predicted to lead to the formation of equimolar *RR* and *SS* enantiomers. We also determined from the crystal structures of **3a**, **3b** and **3t** that the 3-hydroxy group was *trans* to the 4′-methyne hydrogen, which may explain the inability of these analogs to undergo facile dehydration.

The single dose in vitro cytotoxicity screening assays of the analogs were carried out in accordance with the procedures described in Rubinstein et al.[15] The human tumor cell line panel includes leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines. From the preliminary results of the in vitro single dose screen, analogs containing an *N*-benzyl group in the isatin moiety exhibited good growth inhibition

* Corresponding author. Tel.: +1 859 257 1718; fax: +1 859 257 7585.
E-mail address: pcrooks@email.uky.edu (P.A. Crooks).

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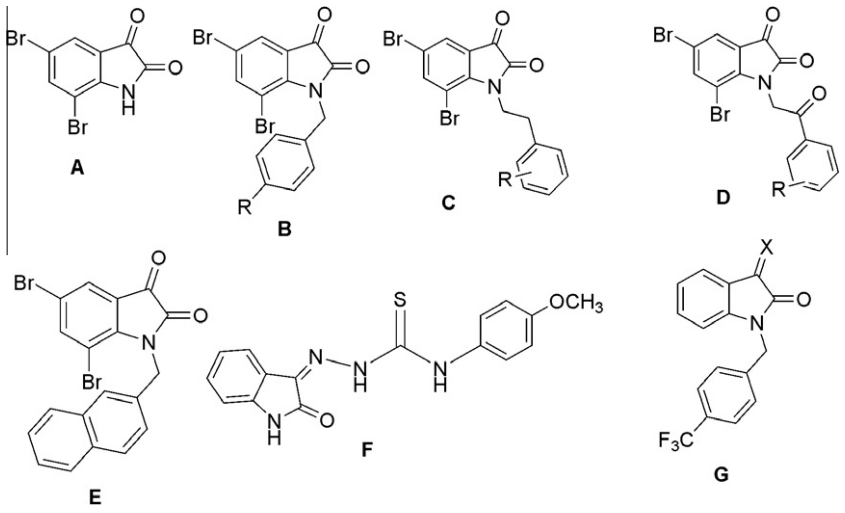
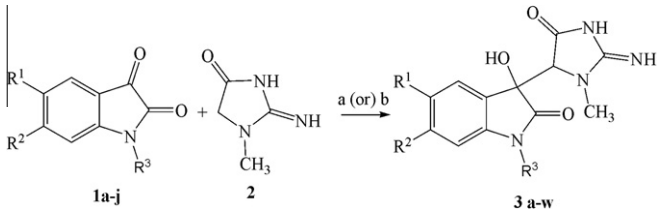


Fig 1. Structures of potent cytotoxic isatin derivatives.



Scheme 1. Reagents and conditions: (a) method A: sodium acetate in acetic acid, microwave irradiation, 20–40 s, 83–94% yield; (b) method B: sodium acetate in acetic acid, 115–120 °C, 6–8 h, 70–83% yield.

properties. The introduction of a chloro group at C-5 of the isatin moiety also afforded analogs with good growth inhibition properties.

The two most active compounds (**3n** and **3o**) from the preliminary 57 cell screen were subsequently evaluated in five dose–response studies for their in vitro cytotoxic effects on growth parameters against each of the 57 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}, TGI and LC_{50} values, which represent the molar drug concentration required to cause 50% growth inhibition, total growth inhibition, and the concentration that kills 50% of the cells, respectively. The results are presented in Table 2.

Compound **3o** exhibited growth inhibitory properties against 93% of all cancer cell lines in the panel, with GI_{50} values in the range of 0.19–30 μM (Table 2). Compound **3o** exhibited good growth inhibitory activity against A549/ATCC non-small cell lung cancer cell lines (GI_{50} = 193 nM; TGI = 1.96 μM, LC_{50} = 10.9 μM) and LOX IMVI melanoma cell lines (GI_{50} = 750 nM; TGI = 11.8 μM; LC_{50} = 60.0 μM), and showed moderate growth inhibitory activity against NCI-H522 lung cancer (GI_{50} = 1.86 μM), HT29 colon cancer (GI_{50} = 1.38 μM), UACC-257 melanoma (GI_{50} = 1.43 μM), and OVCAR-8 and NCI/ADR-RES ovarian cancer (GI_{50} = 1.35 and 1.88 μM, respectively), and CAKI-1 renal cancer (GI_{50} = 1.98 μM) cell lines.

Table 1
Reaction times and yields of 3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)indolin-2-ones (**3a–w**)

Compd	R^1	R^2	R^3	Method A		Method B	
				Yield (%)	Time (s)	Yield (%)	Time (h)
3a	H	H	H	92	20	80	6
3b	F	H	H	94	30	81	6
3c	Cl	H	H	91	40	78	6
3d	Br	H	H	87	40	75	7
3e	Br	Br	H	89	40	79	8
3f	NO_2	H	H	92	40	83	8
3g	H	H	$-CH_3$	88	30	78	7
3h	F	H	$-CH_3$	90	40	76	7
3i	Cl	H	$-CH_3$	86	40	77	8
3j	Br	H	$-CH_3$	85	40	80	8
3k	H	H	–Bz	87	30	72	7
3l	Cl	H	–Bz	91	40	76	7
3m	Br	H	–Bz	88	40	75	8
3n	H	H	4-OCH_3 Bz	84	30	70	6
3o	Cl	H	4-OCH_3 Bz	85	30	74	8
3p	Br	H	4-OCH_3 Bz	83	40	79	8
3q	H	H	4-Cl Bz	92	40	77	7
3r	H	H	4-$COOCH_3$Bz	87	20	75	6
3s	H	H	4-CN Bz	86	40	79	8
3t	H	H	$-C_6H_5$	83	30	80	7
3u	H	H	$-COCH_3$	87	30	73	7
3v	Cl	H	$-COCH_3$	85	40	75	8
3w	H	H	$-SO_2C_6H_5$	89	30	78	6

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 57 cell lines screen

Panel/cell line	Compound **3o**		Compound **3n**	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}
Leukemia cancer				
CCRF-CEM	2.06	>100	2.18	>100
HL-60(TB)	3.22	>100	4.91	>100
K-562	5.04	>100	3.73	>100
MOLT-4	2.94	>100	4.79	>100
RPMI-8226	2.62	>100	2.87	>100
Non-small cell lung cancer				
A549/ATCC	0.19	10.9	43.6	>100
EKVX	28.5	>100	71.7	>100
HOP-62	5.12	>100	4.31	>100
NCI-H226	26.8	>100	38.4	>100
NCI-H23	4.74	>100	13.9	>100
NCI-H322M	14.3	>100	41.1	>100
NCI-H460	26.8	>100	29.0	>100
NCI-H522	1.86	20.5	3.65	>100
Colon cancer				
COLO 205	14.3	>100	19.9	>100
HCC-2998	18.7	73.9	12.8	63.9
HCT-116	3.00	34.5	2.40	>100
HCT-15	12.6	>100	11.8	>100
HT29	1.38	44.1	4.06	>100
KM12	2.05	30.0	2.87	80.4
SW-620	2.59	49.6	4.12	>100
CNS cancer				
SF-268	3.31	64.9	5.00	>100
SF-295	3.71	>100	26.0	>100
SF-539	3.12	79.1	3.73	>100
SNB-19	17.2	>100	30.6	>100
SNB-75	3.39	>100	28.8	>100
Melanoma cancer				
LOX IMVI	0.75	60.0	8.09	>100
MALME-3M	29.6	>100	27.4	>100
M14	3.59	61.4	4.93	>100
MDA-MB-435	6.12	93.1	10.6	>100
SK-MEL-2	10.4	96.0	95.2	>100
SK-MEL-28	50.3	>100	36.9	>100
SK-MEL-5	22.6	>100	37.3	>100
UACC-257	1.43	19.4	6.28	>100
UACC62	23.4	>100	27.7	>100
Ovarian cancer				
IGR-OV1	2.59	>100	3.26	>100
OVCAR-3	2.08	11.5	2.29	30.1
OVCAR-4	5.52	>100	7.13	>100
OVCAR-5	34.9	>100	42.1	>100
OVCAR-8	1.35	17.2	3.52	>100
NCI/ADR-RES	1.88	36.5	1.99	>100
SK-OV-3	30.1	>100	45.5	>100
Renal cancer				
786-0	3.44	43.0	23.0	>100
A498	34.5	>100	>100	>100
ACHN	20.4	>100	>100	>100
CAKI-1	1.98	>100	53.9	>100
RXF 393	6.29	>100	7.40	>100
SN12C	5.94	>100	>100	>100
TK-10	4.11	>100	>100	>100
UO-31	4.15	>100	83.2	>100
Prostate cancer				
PC-3	4.27	>100	46.9	>100
DU-145	3.76	74.6	21.4	>100
Breast cancer				
MCF7	3.22	>100	6.93	>100
MDA-MB-231/ATCC	8.55	>100	19.4	>100
HS 578T	2.04	>100	60.3	>100
BT-549	2.98	54.9	8.45	97.0
T-47D	13.5	>100	32.0	>100
MDA-MB-468	2.29	>100	11.6	>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 **3n** exhibited growth inhibitory properties against 89% of all cancer cell lines in the panel, with GI_{50} values in the range of 2–60 μM (Table 2). Moderate growth inhibitory activity was observed against CCRF-CEM leukemia (GI_{50} = 2.18 μM), HCT-116 colon (GI_{50} = 2.40 μM), and OVCAR-3 and NCI/ADR-RES ovarian (GI_{50} = 2.29 and 1.99 μM, respectively) cell lines.

Of particular significance was the observation that both **3o** and **3n** were effective in inhibiting the growth of all five cell lines in the sub-panel of leukemia cancer cells, with GI_{50} values in the narrow range of 2–5 μM.

In conclusion, a series of 3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)indolin-2-one analogs have been synthesized and evaluated for anticancer activity against a panel of 57 human tumor cell lines. Compounds **3n** and **3o** were identified as molecules of interest from a single dose assay, and were then evaluated for dose-dependent growth inhibition and cytotoxicity in all 57 human cancer cell lines. Compound **3o** had GI_{50} values of 190 nM and 750 nM against A549/ATTC non-small cell lung cancer and LOX IMVI melanoma cell lines, respectively, and both **3n** and **3o** exhibited GI_{50} values ranging from 2 to 5 μM against CCRF-CEM, HL-60(TB), K-562, MOLT-4, and RPMI-8226 leukemia cell lines. These results indicate that *N*-4-methoxybenzyl-3-hydroxy-(2-imino-3-methyl-5-oxo-4-yl)indolin-2-one analogs may be useful leads for anticancer drug development.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.042.

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16. Analytical data for compound **3n**. MF: $C_{20}H_{20}N_4O_4$, mp: 213–215 °C, ^{1}H NMR (DMSO-d_6): δ 3.14 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 4.20 (s, 1H, CH), 4.65–4.84 (Abq, 2H, CH_2), 6.56 (s, 1H, OH), 6.65–6.68 (d, *J* = 8.1 Hz, 1H, C_7H), 6.85–6.94 (m, 4H, Ar-H), 7.10–7.16 (m, 2H, C_5H, C_6H), 7.33–7.40 (d, *J* = 8.4 Hz, 1H, C_4H), 7.57 (br s, 2H, NH_2) ppm; ^{13}C NMR (DMSO-d_6): δ 32.64 (CH_3), 42.30 (CH_2), 54.97 (OCH_3), 69.46 (CH), 75.99 (C–OH), 109.05, 111.00, 113.62, 121.79, 123.69, 127.43, 127.81, 128.55, 129.30, 137.74, 143.15, 158.20, 171.98 (C=N),

174.31 (isatin C=O), 182.29 (creatinine C=O) ppm.
Analytical data for compound **3o**. MF: $C_{20}H_{19}ClN_4O_4$, mp: 188–190 °C, ^{1}H NMR (DMSO-d_6): δ 3.18 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 4.21 (s, 1H, CH), 4.66–4.85 (Abq, 2H, CH_2), 6.67 (s, 1H, OH), 6.70–6.72 (d, J = 5.1 Hz, 1H, C_7H), 6.85–6.88 (d, J = 8.7 Hz, 2H, Ar-H), 7.06–7.07 (d, J = 2.4 Hz, 1H, C_4H), 7.24–7.27 (dd, J = 8.1 Hz, 2.4 Hz, 1H, C_6H), 7.37–7.40 (d, J = 8.4 Hz, 2H, Ar-H), 7.57 (br s, 1H, NH), 7.82 (br s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 32.94 (CH_3), 42.42 (CH_2), 55.03 (OCH_3), 69.62 (CH), 76.05 (C–OH), 110.63, 113.72 (2C) 123.73, 125.88, 127.43, 128.62 (2C), 129.16, 129.45, 142.10, 158.31 (C–Cl), 172.21 (C=N), 174.00 (isatin C=O), 182.08 (creatinine C=O) ppm.