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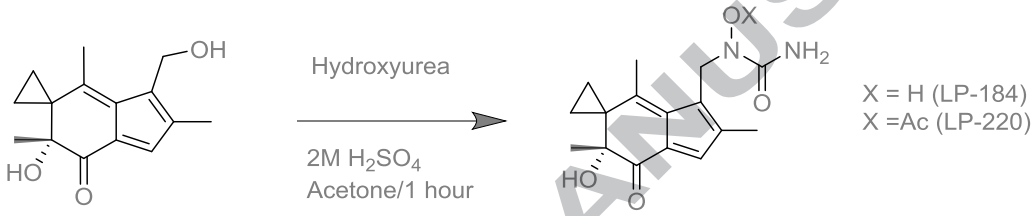

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Hydroxyurea derivatives of irofulven with improved antitumor efficacy

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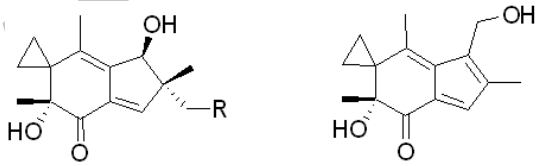
ABSTRACT

Irofulven is a semi-synthetic derivative of Illudin S, a toxic sesquiterpene isolated from the mushroom *Omphalotus illudens*. Irofulven has displayed significant antitumor activity in various clinical trials but displayed a limited therapeutic index. A new derivative of Irofulven was prepared by reacting hydroxyurea with irofulven under acidic conditions. Acetylation of this new compound with acetic anhydride produced a second derivative. Both of these new derivatives displayed significant antitumor activity *in vitro* and *in vivo* comparable to or exceeding that of Irofulven.

The toxic mushroom *Omphalotus illudins* is the source of the highly toxic sesquiterpenes illudin S **1** and illudin M **2** (Figure 1).[1] These compounds were tested by the National Cancer Institute and displayed significant antitumor activity but displayed a poor therapeutic index in xenograft studies.[2] Semisynthetic derivatives of these compounds were developed which displayed improved efficacy in xenograft studies including multidrug resistant tumor models.[3] One derivative, irofulven **3** [4] has been extensively investigated in numerous clinical trials and displayed significant activity against ovarian, prostate, and gastrointestinal cancers including hepatocellular tumors. [5,6]

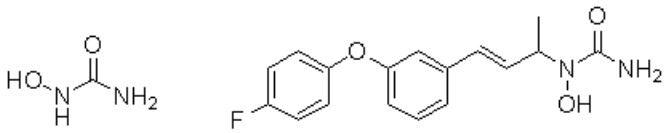


1. Illudin S (R = OH)
2. Illudin M (R = H)
3. Irofulven

Figure 1. Structures of Illudin S, Illudin M, and Irofulven

Irofulven has proven to be a potent antitumor agent, but its efficacy is limited by the hematologic side effects that reduce the maximum dose that can be administered to patients. Modifying the drug to increase its selectivity towards tumor cells versus normal cells should in theory reduce adverse side effects and allow administration of higher doses. We describe here the preparation of two new hydroxyurea derivatives of irofulven with improved *in vitro* antitumor activity.

The drug hydroxyurea **4** (Figure 2) is an inhibitor of DNA synthesis and has been used clinically to treat cancer.[7] Hydroxyurea, however, has limited efficacy as an anticancer drug as it displays a short half-life in humans and cancer cells readily develop resistance to the drug. The hydroxyurea derivative BWB70C **5** was developed and demonstrated superior inhibition of tumor growth *in vivo*.[8]



Hydroxyurea (**4**)
BWB70C (**5**)

Figure 2: Structures of hydroxyurea and BWB70C

Another hydroxyurea derivative, Zileuton[9] **7** has been prepared from benzo[b]thiophen-2-yl-ethanol **6** via an S_N1 displacement of the secondary hydroxyl by hydroxyurea under acidic conditions

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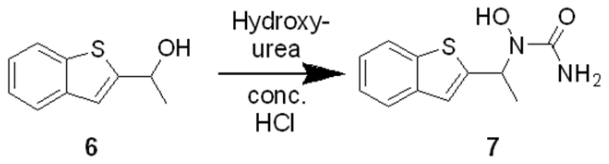
(Scheme 1).[10] Alkylation occurred specifically at the nitrogen bearing the hydroxyl group.

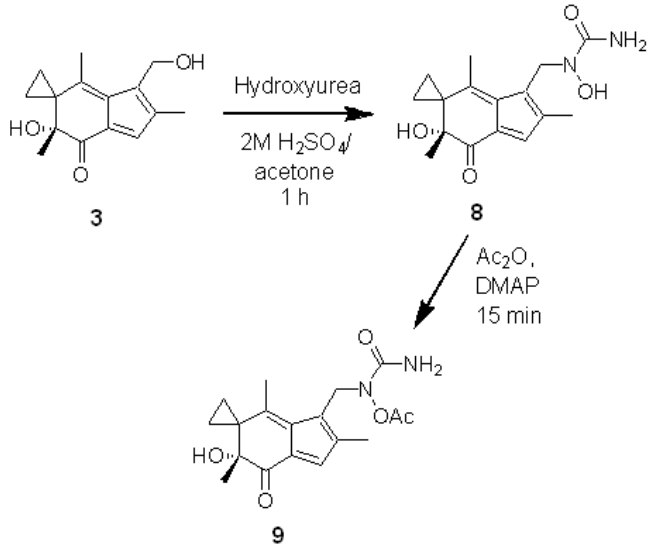


Scheme 1

We followed an analogous approach to obtain the hydroxyurea derivative of irofulven. The primary hydroxyl group of irofulven was previously demonstrated to undergo S_N1 displacement by a variety of nucleophiles under acidic conditions. [11,12] In a 1:1 mixture of 2M H_2SO_4 and acetone the primary hydroxyl group of irofulven was rapidly replaced by hydroxyurea to yield **8** in 83% yield (Scheme 2). Alkylation was also observed to occur exclusively at the nitrogen bearing the hydroxy group. An acetylated derivative **9** was obtained in quantitative yield by treating **8** with acetic anhydride and catalytic DMAP.

Scheme 2

The antitumor activity of compound **8** was compared to previous



irofulven results in the NCI DTP 60 cell line screening assay. Results indicated that anticancer activity of compound **8** equaled or exceeded that of irofulven (Table 1) in 43 of 52 comparable cell lines (cell lines used in the screening assay have varied with time so that only 52 of the solid tumor lines were tested with both irofulven and compound **8**). Compound **8** retained activity against multidrug resistant cell lines, such as Ovarian NCI/ADR-RES, consistent with previous reports on the activity of acylfulvenes against multidrug resistant phenotypes.[3]

The antitumor activities of compounds **8** and **9** were then compared against that of irofulven using the MV522 adenocarcinoma cell line as the target cell line because these cells have previously been demonstrated to be resistant to a wide variety of conventional chemotherapeutic agents.[13] The megakaryocytic cell line CHRF 288-11 was chosen as the non-target cell line as the primary toxicity of irofulven is thrombocytopenia.[14] The *in vitro* cytotoxicity assay has been previously described.[15] While the hydroxyurea derivatives retained cytotoxicity towards the target MV522 adenocarcinoma cell line, the analogs were markedly less toxic to the megakaryocytic CHRF 288-11 cell line (Table 2).

Table 1: Solid tumor IC_{50} values (nM) for irofulven and hydroxyurea derivative **8** from the NCI DTP 60 cell line analysis. * designates equal or increased cytotoxicity by derivative **8**.

	Log_{10} IC_{50}	
Panel/Cell Line	Irofulven	Compound **8**
Non-small cell lung		
A549	-6.9	< -8.0 *
EKVX	-6.7	-6.5
HOP-62	-6.8	-7.6 *
HOP-92	-6.5	-7.4 *
NCI-H226	-6.7	-6.7 *
NCI-H23	-7.1	-7.8 *
NCI-H322M	-6.6	-7.0 *
NCI-H460	-7.3	< -8.0 *
NCI-H522	-6.8	-6.0
Colon		
COLO 205	-6.8	-7.6 *
HCC-2998	-6.8	-7.9 *
HCT-116	-6.7	-6.8 *
HCT-15	-6.5	-6.9 *
HT29	-6.6	-6.9 *
KM12	-6.2	-6.4 *
SW-620	-6.2	-4.8
CNS		
SF-268	-6.6	-7.7 *
SF-295	-6.5	-7.3*
SF-539	-6.8	-7.7 *
SNB-19	-6.5	-6.9 *
SNB-75	-6.7	-7.5 *
U251	-6.5	-6.9 *
Melanoma		
LOX IMVI	-6.2	-6.5 *
MALME-3M	-6.7	-6.9 *
M14	-6.6	-6.7*
MDA-MB-435	-5.8	-6.4 *
SK-MEL-2	-6.7	-6.4
SK-MEL-28	-6.2	-6.9 *
SK-MEL-5	-6.8	-7.2 *
UACC-257	-6.6	-6.6 *
Ovarian		
IGROV1	-6.8	-7.6 *
OVCAR-3	-6.6	-7.3 *
OVCAR-4	-6.6	-7.0 *
OVCAR-5	-6.8	-7.6 *
OVCAR-8	-6.7	-6.5
NCI/ADR-RES	-6.6	-6.8 *
SK-OV-3	-6.6	-6.5
Renal		
786-O	-6.7	-6.9 *
A498	-6.7	-7.5 *
ACHN	-6.6	-7.5 *
CAKI-1	-6.6	-7.3 *
RXF 393	-7.3	-7.8 *
SN12C	-6.4	-6.5 *
TK-10	-6.6	-7.5 *
UO-31	-6.7	-7.6 *
Prostate		
PC-3	-6.6	-6.4
DU-145	-7.0	< -8.0 *
Breast		
MCF-7	-6.6	-5.9
MDA-MB-231	-6.5	-6.6 *
HS 578T	-6.5	-6.3
BT-549	-6.5	-6.8 *
T-47D	-6.7	-7.2 *
Mean	-6.38	-7.02 *

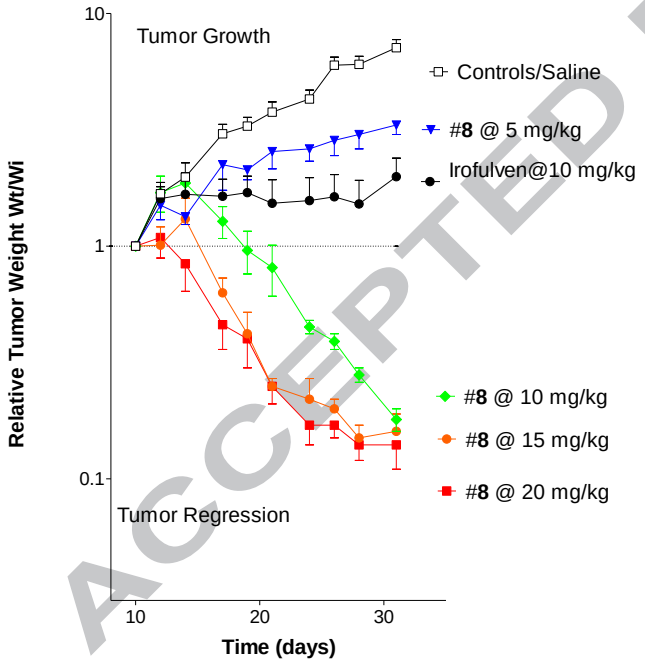
Table 2: IC_{50} values (nM) for irofulven and hydroxyurea derivatives when tested against MV522 adenocarcinoma cells and CHRF 288-11 megakaryocytic cells.[a]

Compound	MV522	CHRF 288-11	Ratio [b]
Irofulven **3**	70 ± 10	290 ± 20	4.1
8	210 ± 20	8,800 ± 1,700	41.9
9	130 ± 10	1,900 ± 100	14.6

[a] IC_{50} is the concentration of the irofulven derivative at which 50% inhibition of growth occurs as measured by the trypan blue exclusion assay for a 48 hour exposure.[15]

[b] The ratio is defined as the CHRF IC_{50} value/MV522 IC_{50} value

Based on the screening results with MV522 and CHRF 288-11 cells, compound **8** was then tested in the MV522 xenograft model. As a comparator irofulven was tested at 10 mg/kg, the maximum tolerated dose when administered intraperitoneally at 3 doses per week for 3 weeks. Compound **8** was less toxic than irofulven, in agreement with the screening results (Table 2) and could be administered at higher doses (Figure 3). The *in vivo* antitumor activity of compound **8** was superior to irofulven when administered at an equivalent dose of 10 mg/kg (the maximum tolerated dose for irofulven), and compound **8** could be administered at a twofold higher dose than irofulven.



All drugs administered i.p.; 3 times per week, for 3 weeks

Figure 3: Activity of compound **8** in the drug-resistant MV522 Balb/c nu/nu mouse xenograft model[13] as compared to Irofulven. All drugs were administered intraperitoneally, 3 times a week, for 3 consecutive weeks as previously described (N = 8). [13]

It is not entirely clear why the hydroxyurea derivatives **8** and **9** of irofulven display an improved therapeutic index towards solid tumor cells and not cells of a megakaryocytic lineage. One could argue that these derivatives are simply functioning as an equimolar mixture of irofulven and hydroxyurea, however, hydroxyurea is nontoxic to MV522 cells (IC50 > 650 μM). Further biological studies will be required to clarify the mechanism of increased therapeutic ratio for the hydroxyurea derivatives of irofulven.

Acknowledgements

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Supplementary data

Experimental details for the synthesis and ^{1}H-NMR and ^{13}C-NMR spectra of compounds **8** and **9** can be found in the online version. Compound **8** has been selected for phase I clinical trials and now carries the drug designation LP-184 for these trials.

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