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Synthesis and antitumor activity of amine analogs of irofulven

Trevor C. McMorris,[a,*] Ramesh Chimmani,[a] Mahender Gurram,[a]
Michael D. Staake[a] and Michael J. Kelner[b]

[a]*Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093-0358, USA*
[b]*Department of Pathology, UCSD Medical Center, San Diego, CA 92103-8320, USA*

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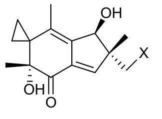
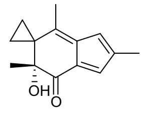
Abstract—Acylfulvenes, a class of semisynthetic analogs of Illudin S, show high toxicity toward prostate cancer cells. Here we probe the effect of changes in hydrophilic character of the analogs.

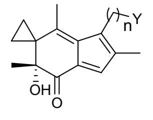
Illudin S and M (**1** and **2**)[1] (Fig. 1), two sesquiterpenes derived from the toxic mushroom *Omphalotus illudens*, were tested many years ago by the National Cancer Institute and were found to possess antitumor activity but with a poor therapeutic index.[2] The toxicity and antitumor activity of illudins can be attributed to their ability to undergo Michael-type addition of thiols or NADPH to the α,β-unsaturated ketone to give a cyclohexadiene intermediate. This is an extremely reactive alkylating agent, which is converted rapidly into a stable aromatic product alkylating macromolecules such as DNA[3] (Fig. 2). Acylfulvene (**3**) (Fig. 1), formed by treatment of illudin S with dilute sulfuric acid, showed similar reactions to illudin S but at a much slower rate. Toxicity of acylfulvene (**3**) to HL60 cells was more than two orders of magnitude lower than that of illudin S. However, this reduced toxicity was accompanied by greatly improved selectivity in toxicity to malignant cells versus normal cells.[4] Reaction of illudin S (**1**) with formaldehyde and sulfuric acid gives hydroxymethylacylfulvene (irofulven, NSC 683863; **4**). Although this compound **4**, like acylfulvene (**3**), reacts slowly with thiols and with NADPH, it is substantially more toxic than **3**.



1 X=OH, Illudin S
2 X=H, Illudin M

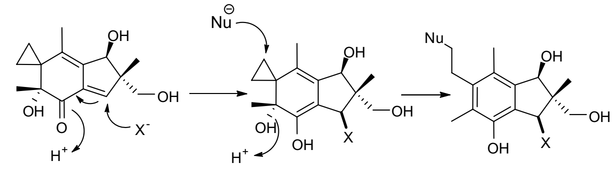


3 Acylfulvene



4 n=1, Y=OH
5 n=3, Y=OH
6 n=1, Y=NH_2
7 n=3, Y=NH_2

Figure 1.



X = SR, H (from NADPH) Nu = protein, DNA

Figure 2.

Irofulven (**4**) was tested in the MV522 metastatic lung carcinoma xenograft model in comparison with well-known anticancer drugs[5] and it caused early (2–5 h) translocation of the proapoptotic Bax from cytosol to mitochondria followed by the dissipation of mitochondrial membrane potential and cytochrome c release at 4–12 h.[6]

Considerable recent interest in acylfulvenes and irofulven has led to their total syntheses.[7] The high therapeutic index of **4** was thought to result from hydrophilicity of the allylic hydroxyl.[8] To make it more water soluble, one would envisage replacement of this primary hydroxyl with an amine[9] group. This also allows the possibility for synthesizing peptide conjugate 'prodrugs' via amide bond. An advantage to using an amide bond

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Keywords: Irofulven; Analogs synthesis; Staudinger ligation; Reductive amination; Antitumor activity.

[*] Corresponding author. Tel.: +1 858 534 4178; fax: +1 858 534 5383; e-mail: mcm@chem.ucsd.edu

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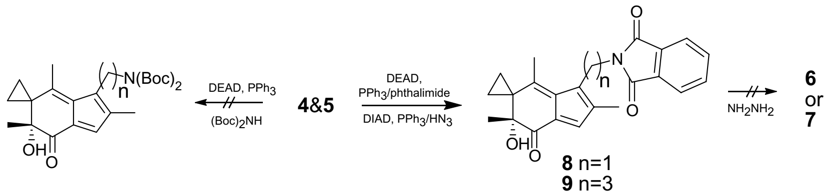


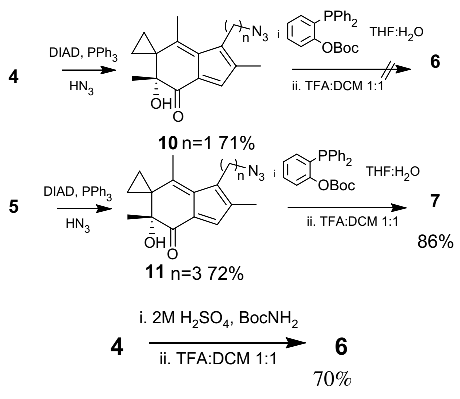
Scheme 1. Failed syntheses of **6** and **7**.

instead of an ester bond is that the former would not be vulnerable to hydrolysis by nonspecific esterase(s) in serum as is possible with the latter. In this paper we wish to report the synthesis of irofulven analogs and their antitumor activity.

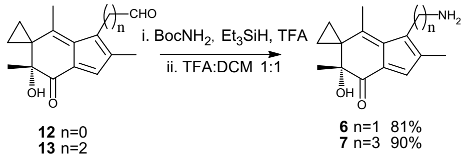
First, compounds **4** and **5** were proposed to be converted to the (Boc)$_2$N-derivative under Mitsunobu conditions as applied by Sharpless et al.[10] The Boc groups could then be cleaved to form an ammonium salt under acidic conditions. Attempts to form derivatives of **4** and **5** (Scheme 1), however, were unsuccessful. In another approach **4** and **5** were converted to their phthalimide derivatives **8** and **9**, under Mitsunobu conditions, which on treatment of either with hydrazine caused only decomposition. In Staudinger conditions, decomposition resulted azides **10** and **11** were subjected sequentially to PPh$_3$ and water or in one-pot. In fact, decomposition accompanied by much lower yields of azide product resulted if any PPh$_3$ remained after completion of the Mitsunobu reaction.

It was then realized that direct amide formation would be the choice and the Staudinger ligation[11] method was found to be effective for the synthesis of **7** but failed for **6** (Scheme 2). The method was applied to synthesize several peptide derivatives in one-pot.[12] However, preparation of **6** was easily achieved via a direct nucleophilic displacement of allylic hydroxyl of **4** under acidic conditions (Scheme 2).

Finally, a common and direct method involving a reductive amination approach[13] starting from aldehydes[14] (Scheme 3) was found to avoid the intermediates encountered during the synthesis of amino derivatives. The method was applicable for the synthesis of both amines **6** and **7** in excellent yields and was also scalable.



Scheme 2. Preparation methods for **6** and **7**.



Scheme 3. Method for the preparation of **6** and **7**.

Antitumor activity. For cytotoxicity studies, the compounds were dissolved in cell culture grade DMSO (1 mg/mL stock solution), and the solutions were diluted in sterile 20% DMSO/phosphate-buffered saline just prior to addition to cultures of the MV522 cells. Control cells received equal amounts of the DMSO/phosphate-buffered saline. As inhibition of DNA synthesis by irofulven analogs occurs rapidly (<2 h), DNA synthesis rate was determined after a 2-h exposure by pulsing cells in culture with tritiated thymidine for 30 min, as previously described in detail.[15] Analogs of irofulven selectively target and kill tumor cells by apoptosis at 24 to 48 h after drug exposure.[16] Cell survival was determined at 48 h by use of trypan blue exclusion studies as previously described in detail.[17] Results represent the means of 3–4 experiments ($N = 3$ or 4) (see Table 1).

The results seem to indicate that the hydroxymethyl (in **4**) is more effective than the hydroxypropyl (**5**) in enhancing toxicity but, surprisingly, the aminomethyl group (in **6**) is less so than aminopropyl (**7**). Most surprising is the toxicity of analog **13** which is capable of inducing cell death at nanomolar concentrations.

In conclusion, we have synthesized a new class of irofulven analogs which possess important antitumor activity.

Table 1. IC$_{50}$ value (nM) for irofulven and derivatives when tested in MV522 adenocarcinoma cells

Compound	2 h	48 h
4	110 ± 40	70 ± 10
5	500 ± 30	850 ± 180
6	8000 ± 1500	1300 ±100
7	470 ± 120	430 ± 100
12	4900 ± 900	1200 ($N = 2$)[a]
13	8900 ± 1500	170 ± 60

[a] Only two experiments were run.

Acknowledgments

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References and notes

1. McMorris, T. C.; Anchel, M. *J. Am. Chem. Soc.* **1965**, *87*, 1594.
2. Kelner, M. J.; McMorris, T. C.; Taetle, R. *J. Natl. Cancer Inst.* **1990**, *82*, 1562.
3. (a) McMorris, T. C.; Kelner, M. J.; Wang, W.; Moon, S.; Taetle, R. *Chem. Res. Toxicol.* **1990**, *3*, 574; (b) Gong, J.; Vaidyanathan, V. G.; Yu, X.; Kensler, T. W.; Peterson, L. A.; Sturla, S. J. *J. Am. Chem. Soc.* **2007**, *129*, 2101.
4. Kelner, M. J.; McMorris, T. C.; Estes, L.; Starr, R. J.; Rutherford, M.; Montoya, M.; Samson, K. M.; Taetle, R. Cells. *Cancer Res.* **1995**, *55*, 4936.
5. Kelner, M. J.; McMorris, T. C.; Estes, L. A.; Oval, M. Y.; Rafael, J. R.; Lynn, J. R.; Lanham, K. A.; Samson, K. M. *Anti-Cancer Drugs* **2000**, *11*, 217.
6. Huiyun, L.; Richard, A. S.; Belinda, Z. L.; Teresa, K. C.; Christopher, J. M.; Stephen, J. W.; Terence, S. H.; Jan, M. W.; Barbara, A. W. *Mol. Cancer Ther.* **2004**, *3*, 1385.
7. (a) Movassaghi, M.; Piizzi, G.; Siegel, D. S.; Piersanti, G. *Angew. Chem. Int. Ed.* **2006**, *45*, 5859; (b) McMorris, T. C.; Staake, M. D.; Kelner, M. J. *J. Org. Chem* **2004**, *69*, 619; (c) Jiachang, G.; James, F. N.; Xiang, Y.; Thomas, W. K.; Lisa, A. P.; Shana, J. S. *J. Med. Chem.* **2006**, *49*, 2593.
8. McMorris, T. C. *Bioorg. Med. Chem.* **1999**, *7*, 881.
9. Salt form of amine is ideal drug choice due to its water solubility, see: Carstensen, J. T.; Rhodes, C. T. *Drug Stability: Principles and Practices*, 3rd ed.; 2000, p 241.
10. Walsh, P. J.; Bennani, Y. L.; Sharpless, K. B. *Tetrahedron Lett.* **1993**, *34*, 5545.
11. Saxon, E.; Armstrong, J. L.; Bertozzi, C. R. A. *Org. Lett.* **2000**, *2*, 2141.
12. Staake, M. D. Ph.D. Thesis, University of California at San Diego, 2004.
13. Daniel, D.; Andrew, A. S. *Tetrahedron Lett.* **1999**, *40*, 2295.
14. (a) Hu, Y. Ph.D. Thesis, University of California at San Diego, 1998; (b) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155; (c) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277; (d) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899; (e) McMorris, T. C.; Yu, J.; Lira, R.; Dawe, R.; MacDonald, J. R.; Waters, S. J.; Estes, L. A.; Kelner, M. J. *J. Org. Chem.* **2001**, *66*, 6158.
15. Kelner, M. J.; McMorris, T. C.; Beck, W. T.; Zamora, J. M.; Taetle, R. *Cancer Res.* **1987**, *47*, 3186.
16. Woynarowska, B. A.; Woynarowski, J. M.; Herzig, M. C.; Roberts, K.; Higdon, A. L.; MacDonald, J. R. *Biochem. Pharm.* **2000**, *59*, 1217.
17. Kelner, M. J.; McMorris, T. C.; Estes, L.; Wang, W.; Samson, K. M.; Taetle, R. *Invest. New Drugs* **1996**, *14*, 161.