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DESIGN, SYNTHESIS, AND ANTITUMOR ACTIVITY OF BICYCLIC AND ISOMERIC ANALOGUES OF ILLUDIN M

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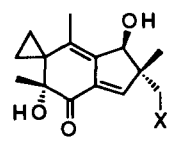
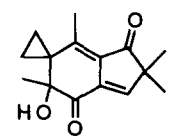
Abstract: Novel bicyclic and isomeric analogues of the cytotoxic sesquiterpine illudin M were prepared using 1,3-dipolar cycloaddition reactions. Nearly every analogue investigated demonstrated low μM IC_{50} values when tested in a panel of four human tumor cell lines.

The illudins comprise a class of cytotoxic sesquiterpenes produced by the fungus *Omphalotus illudens*.[1-4] Illudins M (**1a**) and S (**1b**) were reported to be preferentially cytotoxic *in vitro* to a variety of human tumor cell lines. Selective toxicity was attributed to rapid uptake of the illudins by cells in an energy dependent process.[5] Once inside tumor cells illudins are activated metabolically to a reactive intermediate that binds to DNA.[6-8] While **1a** and **1b** lack an *in vivo* therapeutic window, the semisynthetic illudin derivatives dehydroilludin M (**2**) and acylfulvene (**3**) possess greatly improved *in vivo* efficacy against a number of adenocarcinomas.[9]



1a X = H
1b X = OH



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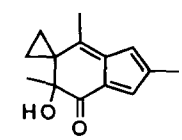
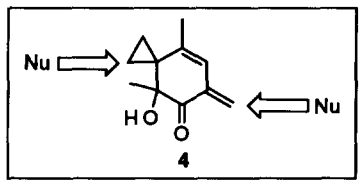


3

Existing illudin analogue structure-activity relationship (SAR) studies have been limited to the native illudins and some semisynthetic derivatives. The spirocyclopropane and unsaturated ketone most likely constitute a bis-electophile that either directly or indirectly causes DNA damage. Previous SAR efforts on existing semisynthetic illudin analogues have shown that the array of functional groups that comprise **4** is essential for antitumor activity.[6] Herein, SAR studies of the first totally synthetic illudin analogues are reported.



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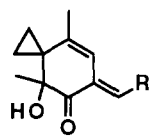
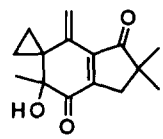
Bicyclic illudin analogues **5-11** were chosen to determine whether antitumor activity is maintained in the absence of the fused cyclopentane ring. Furthermore, the control of lipophilicity and chemical reactivity was investigated through substituents (R) that varied in size, shape, and electron delocalizing ability. In addition to the bicyclic illudins, isodehydroilludin M (**12**) was chosen to elucidate the spatial requirement of the two electrophilic centers for antitumor activity. Compound **12** is unlike any natural illudin or illudin analogue in that the two putative sites for bionucleophillic attack are adjacent to one another.



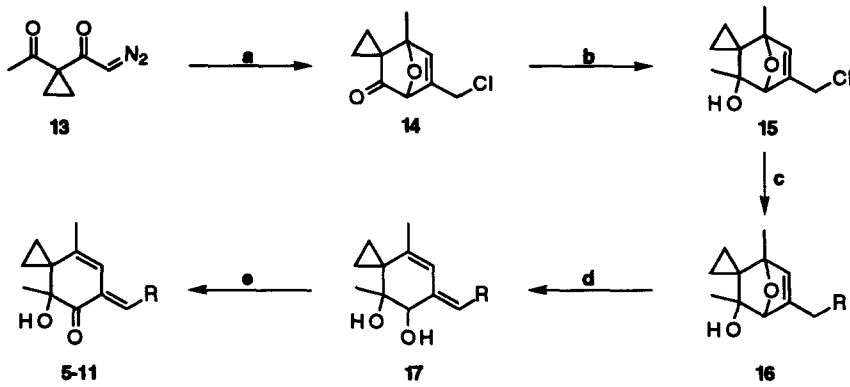
5 R = Cl
6 R = Me
7 R = i-Pr
8 R = vinyl
9 R = Ph
10 R = thienyl
11 R = $\equiv$—CH_2CH_2OH



12

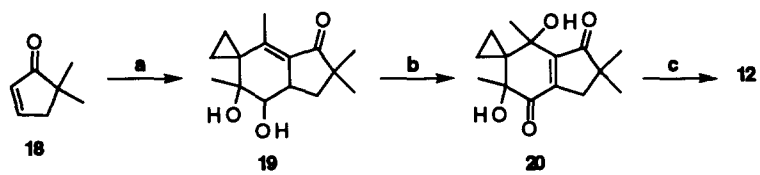
The synthesis of **5-11** follows the same strategy that was employed in the total synthesis of (±)-illudin M.[10] Oxabicyclo[2.2.1]heptane **14** was prepared in 80% yield from diazoketone **13** and propargyl chloride. The reaction proceeds through a $Rh_2(OAc)_4$-catalyzed carbonyl ylide 1,3-dipolar cycloaddition reaction with complete regioselectivity.[11] Treatment of cycloadduct **14** with methylmagnesium chloride gave tertiary alcohol **15** in 75% yield. Vinylogous acid chloride **5** was prepared in two steps from **15**. LDA-mediated allylic proton abstraction and subsequent oxo-bridge opening of **15** gave vicinol diol **17** (R = Cl) in 67% yield. Oxidation of **17** with $(PPh_3)_3Ru(II)Cl$ (50 mole %) and *N*-methylmorpholine-*N*-oxide (NMO) (5 equiv) gave **5** in 44% yield. Analogues **6-11** were prepared in three or four steps from **15**. Treatment of **15** with the corresponding alkyl- or arylmagnesium bromide (RMgBr) gave **16** in 70-90% yield. Reaction of n-BuLi (2 equiv) and **16** resulted in allylic proton abstraction and oxo-bridge opening which gave diols **17** in good yield. All exocyclic alkenes **17**

were determined by nmr to be the E configuration. Oxidation as described above furnished the desired hydroxyketones in 30-50% yield. In the case of homopropargyllic alcohol **11**, where RMgBr = $TMDMSOCH_2CH_2C{\equiv}CMgBr$, the TBDMS protecting group was removed with HF/CH_3CN following oxidation.[12]



Scheme 1: (a) $Rh_2(OAc)_4$ (cat.), propargyl chloride, rt, 80%; (b) MeMgCl, THF, 0 °C-rt, 75%; (c) RMgBr, THF, 0 °C-rt, 70-90%; (d) for R = Cl: LDA, -78 °C-rt, 67%; for R = alkyl or aryl: n-BuLi, -78 °C-rt, 70-90%; (e) $(PPh_3)_3Ru(II)Cl_2$ (50 mole %), NMO, rt, 30-50%.

The synthesis of **12** is described in Scheme 2. Illudane **19** was prepared in three steps from cyclopentenone **18** in the same manner that was reported earlier.[10] PCC oxidation of **19** furnished illudin derivative **20** in 44% yield as a mixture of diastereomers. Treatment of **20** with acetyl chloride/pyridine produced isodehydroilludin M (**12**) in 65% yield.[13] The details of this synthesis will be published elsewhere.



Scheme 2: (a)1. **13**, $Rh_2(OAc)_4$ (cat.), CH_2Cl_2, rt, 55%; 2. MeMgCl, THF, 0 °C-rt; then 10% KOH/MeOH, reflux, 63%; (b) PCC, CH_2Cl_2, rt, 44%; (c) AcCl, pyridine, 0 °C-rt, 65%.

The *in vitro* antitumor activity of analogues **2-12** and Adriamycin is presented in the table below. The melanoma, lung, breast, and colon human solid tumor cell lines were selected because they are among the most difficult

tumors to treat in the clinic. Six of the seven bicyclic illudin analogues possessed low micromolar IC_{50} values in the cell lines tested. The fused cyclopentane ring is not required for antitumor activity. The most cytotoxic bicyclic illudins were those where the substituent was small (R = Me, vinyl). The alkyne and aryl substituents rendered the analogues less potent.

Table: IC50 (μM) Values for Illudin Analogues in Human Tumor Cell Lines[14]

compound	A375 (melanoma)	A549 (lung)	MB-231 (breast)	SW480 (colon)
2	0.55	0.2	0.45	0.3
5	1.0	0.2	0.3	0.03
6	0.004	0.15	0.11	0.12
7	0.13	0.11	0.3	0.03
8	0.018	0.002	0.028	0.18
9	>10	1.5	4.0	2.5
10	>10	>10	>10	>10
11	2.0	3.0	2.0	4.0
12	0.032	0.25	2.0	0.06
adriamycin	0.07	0.12	0.15	0.06

The most dramatic SAR discovery was that the orientation of electrophilic centers common to all antitumor illudins is not a requirement for antitumor activity. In fact, isodehydroilludin M (**12**) was nearly an order of magnitude more potent in the melanoma and colon lines than dehydroilludin M (**2**). In summary, it has been shown that simplified analogues of the antitumor illudins can be easily prepared and are generally equal or greater in potency to dehydroilludin M. Additional results will be reported at a later date.

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12. Compound **5**: ^{1}H NMR (300MHz, $CDCl_3$) δ6.96 (s, 1H), 6.42 (s, 1H), 3.57 (s, 1H), 1.68 (s, 3H), 1.37 (s, 3H), 1.15 (m, 1H), 0.96 (m, 1H), 0.83 (m, 1H), 0.44 (m, 1H). ^{13}C NMR (75MHz, $CDCl_3$) δ200.9, 145.3, 132.4, 124.4, 117.7, 75.1, 31.4, 24.6, 20.0, 9.5, 6.6.

Compound **6**: ^{1}H NMR (300MHz, $CDCl_3$) δ6.55 (q, J = 9Hz, 1H), 6.30 (s, 1H), 3.72 (s, 1H), 1.87 (d, J = 9Hz, 3H), 1.63 (s, 3H), 1.35 (s, 3H), 1.10 (m, 1H), 0.95 (m, 1H), 0.83 (m, 1H), 0.40 (m, 1H). ^{13}C NMR (75MHz, $CDCl_3$) δ202.5, 140.3, 131.6, 130.7, 118.3, 74.5, 30.6, 24.3, 9.6, 13.3, 8.6, 6.0.

Compound **7**: ^{1}H NMR (300MHz, C_6D_6) δ6.55 (d, J = 11Hz, 1H), 6.21 (s, 1H), 4.04 (s, 1H), 2.52 (m, 1H),1.44 (s, 3H), 1.35 (s, 3H), 1.30 (m, 1H), 1.03 (m, 1H), 0.84 (d, J = 7.5 Hz, 6H), 0.75 (m, 1H), 0.24 (m, 1H). ^{13}C NMR (75MHz, $CDCl_3$) δ203.2, 142.7, 140.4, 128.7, 118.6, 74.7, 30.7, 26.9, 24.5, 22.4, 22.1, 19.7, 8.7, 6.1.

Compound **8**: ^{1}H NMR (300MHz, C_6D_6) δ7.04 (d, J = 12.5Hz, 1H), 6.56 (m, 1H), 6.22 (s, 1H), 5.24 (m, 2H), 3.96 (s, 1H), 1.39 (s, 3H), 1.30 (m, 1H), 1.26 (s, 1H), 0.98 (m, 1H), 0.70 (m, 1H), 0.21 (m, 1H). ^{13}C NMR (75MHz, C_6D_6) δ202.8, 142.4, 131.3, 130.7, 130.0, 125.2, 119.2, 75.0, 31.3, 24.8, 19.6, 9.2, 6.5.

Compound **9**: ^{1}H NMR (300MHz, $CDCl_3$) δ7.40 (m, 5H), 6.73 (s, 1H), 3.78 (s, 1H), 1.67 (s, 3H), 1.41 (s, 1H), 1.22 (m, 1H), 0.98 (m, 1H), 0.87 (m, 1H), 0.25 (m, 1H). ^{13}C NMR (75MHz, $CDCl_3$) δ204.1, 143.7, 135.8, 131.5, 130.3, 130.2, 128.8, 128.7, 119.8, 75.2, 31.4, 24.9, 20.2, 9.4, 6.6.

Compound **10**: ^{1}H NMR (300MHz, $CDCl_3$) δ7.47 (d, J = 7.5Hz, 1H), 7.42 (s, 1H), 7.33 (d, J = 5.5Hz, 1H), 7.06 (dd, J = 7.5 and 5.5Hz, 1H), 6.92 (s, 1H), 3.80 (s, 1H), 1.75 (s, 3H), 1.37 (s, 3H), 1.22 (m, 1H), 1.02 (m, 1H), 0.88 (m, 1H), 0.49 (m, 1H). ^{13}C NMR (75MHz, $CDCl_3$) δ203.3, 143.9, 138.9, 133.4, 129.9, 127.6, 126.5, 123.8, 120.1, 74.9, 31.2, 24.9, 20.4, 9.4, 6.6.

Compound **11**: ^{1}H NMR (300MHz, $CDCl_3$) δ6.59 (s, 1H), 6.22 (d, J = 2Hz, 1H), 3.82 (q, J = 8Hz, 2H), 3.63 (s, 1H), 2.79 (dt, J = 8 and 2Hz, 2H), 1.75 (t, J = 8Hz, 1H), 1.71 (s, 3H), 1.35 (s, 3H), 1.17 (m, 1H), 0.97 (m, 1H), 0.84 (m, 1H), 0.44 (m, 1H). ^{13}C NMR (75MHz, $CDCl_3$) δ202.1, 144.9, 138.6, 121.0, 110.7, 101.0, 79.6, 74.9, 60.9, 31.5, 24.6, 24.5, 19.7, 9.4, 6.5.

13. Compound **12**: ^{1}H NMR (500MHz, $CDCl_3$) δ6.40 (s, 1H), 5.30 (s, 1H), 3.25 (s, 1H), 2.74 (d, J = 19Hz, 1H), 2.53 (d, J = 19Hz, 1H), 1.35 (s, 3H), 1.22 (s, 3H), 1.21 (s, 3H), 1.05 (m, 2H), 0.97 (m, 1H), 0.24 (m, 1H). ^{13}C NMR (75MHz, $CDCl_3$) δ212.0, 202.5, 151.8, 144.6, 138.7, 115.8, 75.5, 45.8, 38.8, 31.8, 25.4, 25.1, 24.9, 12.6, 4.2.

14. Ethanol solutions of illudin analogues were added to ATCC A375, A549, MDA-MB-231, and SW480 cells at day 1 after plating of cells. Three days after illudin analogue addition, growth inhibition was determined through the measurement of cell density using MTS (see Promega Technical bulletin) mixture. See Mossman, T. *J. Immunol. Meth.* **1983**, *65*, 55 and Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. *Cancer Commun.* **1991**, *3*, 207.

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