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The Discovery of Potent Antitumor Agent C11-Deoxypsymberin/irciniastatin A: Total Synthesis and Biology of Advanced Psymberin Analogs

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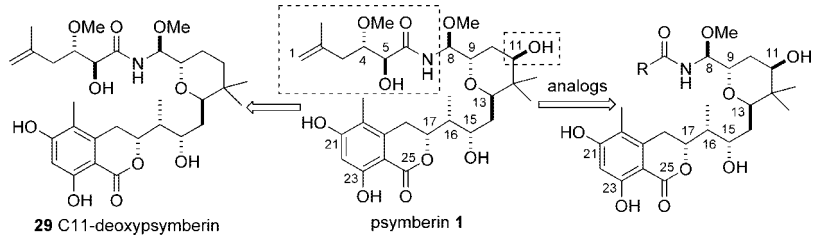
ABSTRACT



Structure−activity relationship (SAR) studies by modification of the unsaturated side chain of potent anticancer marine natural product psymberin/irciniastatin A (1) suggest that substitution at C4 and C5 is important for the cytotoxicity of psymberin, but the terminal double bond is not essential for activity. An aryl group is a good replacement for the olefin. The total synthesis of structurally simplified C11-deoxypsymberin (29) was completed, and its activity is consistently more potent than the natural product which provides a unique opportunity for further SAR studies in the psymberin and pederin family. Preliminary mechanism studies suggest the mode of action of psymberin is through cell apoptosis.

Cancer is a leading cause of death in the United States, and its incidence continues to rise. Although new anticancer agents continue to emerge, the pharmaceutical industry continues to seek more potent and selective drugs with fewer side effect liabilities. A large number of anticancer agents derived from marine natural products and their synthetic analogues are either already in clinical trials or advancing in preclinical development.[1] The discovery of potential anticancer drugs based on marine natural products will be very beneficial. Recently we completed the total synthesis[2] of the potent anticancer marine natural product psymberin/irciniastatin A (**1**)[3,4] which is a new member of the pederin family of natural products and an extremely potent and selective cytotoxin compared to other pederin natural products.[3a] Our convergent approach to psymberin provides us with a good opportunity and unique template to carry out rational structure−activity relationship (SAR) studies to discover potential lead anticancer agents. Because of its reported high potency and unprecedented selectivity, psymberin may have a different mode-of-action from that of

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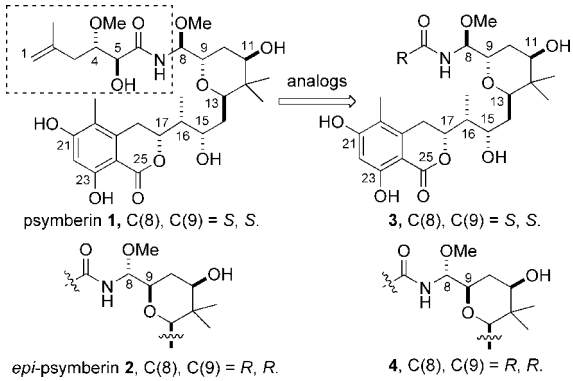
pederin/mycalamide.[5] Herein we report the synthesis of advanced psymberin analogs and the determination of their antitumor activity.

Before embarking on analog synthesis, we first tested the activity of synthetic psymberin **1** and its C(8)−C(9) epimer **2** in different human cancer cell lines to confirm the screening conditions for psymberin analogs as well as to potentially understand its mode of action. As expected, synthetic psymberin (**1**, C(8), C(9) = *S*,*S*, Scheme 1) displayed very

Scheme 1. Rational Design of Psymberin Analogs by Modification of the Unsaturated "Psymberate" Side Chain



potent cytotoxic activity against all tumor cell lines studied (Table 1) with IC_{50} values in the subnanomolar range.

Table 1. Antitumor Activity of Synthetic Psymberin and Its C(8)−C(9) Epimer[a]

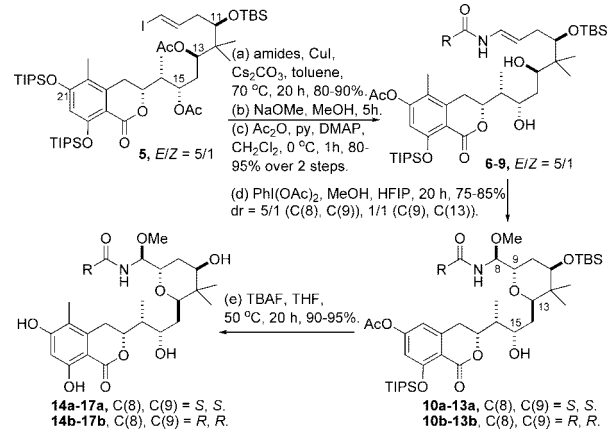
1 (IC_{50} nM)	**2** (IC_{50} nM)	cell line	human tissue type
0.76 ± 0.07	6800 ± 244	ACHN	kidney
0.30 ± 0.03	3800 ± 301	DU145	prostate
0.18 ± 0.02	2400 ± 431	H226	lung
0.81 ± 0.14	4900 ± 187	HCT116	colon
0.42 ± 0.02	4600 ± 68	HOP62	lung
0.27 ± 0.01	4200 ± 174	MB231	breast
0.28 ± 0.03	3600 ± 155	MB435	melanoma
0.28 ± 0.02	5200 ± 195	MKN45	gastric
0.19 ± 0.02	3100 ± 341	PC3	prostate
0.82 ± 0.04	4800 ± 177	SW620	colon
0.84 ± 0.08	n.d.	NHDF	normal

[a] The CellTiter-Glo Luminescent Cell Viability Assay (Promega, Technical bulletin 288) was employed in this study. IC_{50} data are the mean value of six experiments with statistical significance calculated.

However, the C(8)−C(9) epimer (**2**, C(8), C(9) = *R*,*R*) consistently showed dramatically decreased cytotoxicity. This data strongly suggested that the C(8)−C(9) stereochemistry could be important for the cytotoxicity of natural pysmberin. In addition, we performed some biological studies to understand the mode of action of the natural product. Preliminary results supported the idea that psymberin's mode of action is through cell apoptosis (see Supporting Information). With the assay conditions established, we decided to use the readily available HOP62 cell line for testing of analogs.

On the basis of a recent report from the De Brabander group,[6] the dihydroisocoumarin fragment is vitally important for pysmberin cytotoxicity. Therefore, we decided to focus our initial analog synthesis on the modification of the unsaturated "psymberate" side chain (**3** and **4**, Scheme 1) since this is a unique feature of psymberin in its class.[3a] These compounds can be synthesized using our recently developed methodology[7] to quickly assemble the tetrahydropyran ring. The synthesis of analogs is described in Scheme 2 (**14a**−**17a** and their epimers **14b**−**17b**). Com-

Scheme 2. Synthesis of "Psymberate" Side Chain Modified Psymberins



pound **5**[2] was coupled with amides using CuI[8] to give the protected *N*-acyl enamine, in which the C13, C15 acetate, and O21 TIPS groups were removed with NaOMe/MeOH followed by selective acetylation of O21 to give compounds **6**−**9**. Enamides **6**−**9** cyclized smoothly using the $PhI(OAc)_2$ mediated cyclization reaction[7] to give the corresponding cyclization products **10a**−**13a** and their epimers **10b**−**13b**. The major two diastereomers (C(8)−C(9) = *S*,*S* and C(8)−C(9) = *R*,*R*)[9] were separately treated with TBAF at 50 °C, and a global deprotection was realized to give the final products **14a**−**17a** and their epimers **14b**−**17b**.

With compounds **14a**−**17a** and their epimers **14b**−**17b** in hand, we tested them in the human lung cancer cell line

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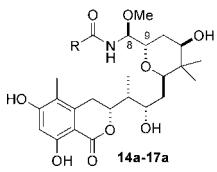
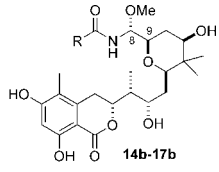
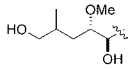
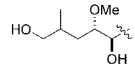
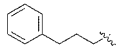
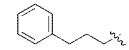
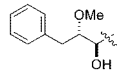
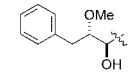
(9) The C(8)-R and C(9)-R stereochemistry was assigned by comparison with ^1H spectra from the psymberin synthesis which were determined by 2D NOE NMR and proton−proton coupling between N7, C8, and C9.

(HOP62), and the results are summarized in Table 2. We first chose to examine the role of the side chain. When the side chain was truncated to a methyl group, both **14a** and its epimer **14b** showed much reduced cytotoxic activity. We then looked into the importance of the terminal olefin. When the double bond was converted to a terminal hydroxy group, compound **15a** retained a good level of cytotoxicity (260 nM) but was 650-fold weaker compared to psymberin, while its epimer at C(8)−C(9) (**15b**) was not as active (>10 000 nM). This suggests that the double bond plays an important role in psymberin's activity. We then used a phenyl ring to mimic the electronic effect of the double bond with no substituents at C4 and C5; however, compound **16a** and its epimer **16b** showed dramatically decreased activity (>10 000 nM). On the other hand, when we installed substituents on the phenyl-substituted side chain, compound **17a** regained its cytotoxicity (32 nM), and interestingly, its epimer **17b** also displayed moderate activity (615 nM). This result further confirms that some π-electron character is favorable for the cytotoxicity of psymberin and suggests the substitution on the side chain (C4 and C5) plays an important role in psymberin activity.

Table 2. Antitumor Activity of "Psymberate" Side Chain Modified Psymberin Analogs Against Human Lung Cancer Cell Line (HOP62)[a]





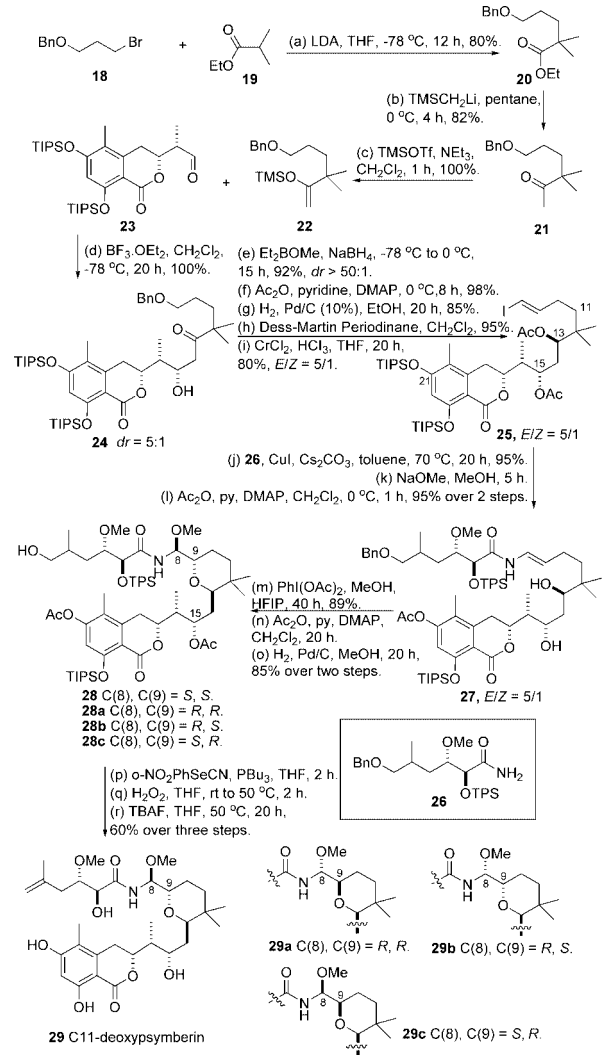
compound	R	IC_{50} (nM)	compound	R	IC_{50} (nM)
14a		>10000	**14b**		>10000
15a		260 ± 36	**15b**		>10000
16a		>10000	**16b**		>10000
17a		32 ± 1	**17b**		615 ± 15

[a] The CellTiter-Glo Luminescent Cell Viability Assay (Promega, Technical bulletin 288) was employed in this study. IC_{50} data are the mean value of three experiments with statistical significance calculated. The value for psymberin in this assay is 0.42 nM.

We next turned our attention to the psymberin core tetrahydropyran ring modification. We noticed[3b] that Irciniastatin B (C11 substituted with =O) consistently showed 10 times stronger cell growth inhibition than Irciniastatin A (psymberin, C11 substituted with −OH) in most of the cancer cell lines tested. On the basis of this observation, we decided to synthesize the C11-deoxypsymberin. By doing this, we could evaluate the role of the oxygen atom in the activity of psymberin and its congeners since the C11 oxygen substitution is present in each of the pederin family members, and more importantly the synthesis of those natural products can be simplified by eliminating a heteroatom (O) and a stereogenic center. The same synthetic sequence to natural psymberin[2] was employed for the synthesis of C11-deoxypsymberin.

Our synthesis started with the preparation of the central linker **22** (Scheme 3) which was quickly synthesized in 66% overall yield in three steps from the commercially available bromide **18** (Scheme 3). A high yield alkylation reaction between **18** and **19** gave ester **20**. Treatment of **20** with $TMSCH_2Li$ in pentane[10] gave ketone **21** in a single operation and was converted to enol ether **22** by treatment with TMSOTf/Et_3N. A substrate-controlled aldol reaction[11] between **22** and **23**[2] gave ketone **24** in good yield (75%, dr > 20:1). Chelation-controlled reduction[12] of ketone **24** provided a secondary alcohol at C13 with excellent diastereoselectivity (dr = 15:1)[13] which was transferred into **25** (*E*/*Z* = 5/1) via bisacetylation at C13 and C15, debenzylation, Dess-Martin oxidation, and Takai vinyl iodide formation.[14] Enamide **27**

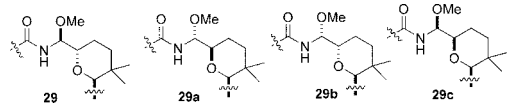
Scheme 3. Total Synthesis of C11-Deoxypsymberin



(E/Z = 5/1) was synthesized from **25** (E/Z = 5/1) in three operations: (1) coupling with **26**[2] with CuI to give protected *N*-acyl enamine, (2) removal of C13, C15 acetate and O21 TIPS groups with NaOMe/MeOH, (3) acetylation of O21. Enamide **27** cyclized smoothly under the $PhI(OAc)_2$-mediated cyclization reaction to give the cyclized product in a total of 89% yield as a mixture of diastereomers. The mixture of four pairs of diastereomers (C(8)−C(9) = *S*,*S*; C(8)−C(9) = *R*,*R*; C(8)−C(9) = *R*,*S*; C(8)−C(9) = *S*,*R*)[15] was acetylated at C15 and debenzylated to give alcohols **28** and epimers **28a**−**c** which were carefully isolated through extensive silica gel column chromatography and preparative thin-layer chromatography (with a dr of 5:1 at C(8) and 1:1 at C(9)). The C1 terminal double bond was revealed by converting **28** and **28a**−**c** to the *o*-nitrophenyl selenides followed by treatment with H_2O_2 at 50 °C.[16] Upon treatment with TBAF at 50 °C, a global deprotection was realized to give the final products **29** and epimers **29a**−**c**.

When we subjected compounds **29** and **29a**−**c** to cell proliferation studies in the HOP62 human lung cancer line, compound **29** displayed extremely potent activity, and gratifyingly, its epimers (**29a**−**29c**) showed improved activity compared to its corresponding psymberin epimers. Compound **29** was consistently 3−10-fold more potent than psymberin across all cancer cell lines tested with IC_{50} values in the subnanomolar ranges with epimer **29c** showing excellent activity against all cell lines tested, with IC_{50} values in single digit nanomolar ranges (Table 3). These findings should facilitate further SAR studies with psymberin since it suggests that the C11-oxygen is not essential for good potency. This finding may apply to all members of the pederin family of natural products.

Table 3. Anticancer Activity of C11-Deoxypsymberin and Its Epimers[a]



29 (IC_{50} nM)	**29a** (IC_{50} nM)	**29b** (IC_{50} nM)	**29c** (IC_{50} nM)	cell line	human tissue type
0.265 ± 0.008	n.d.	n.d.	8.7 ± 0.18	ACHN	kidney
0.149 ± 0.005	n.d.	n.d.	5.9 ± 0.18	DU145	prostate
0.034 ± 0.004	n.d.	n.d.	1.6 ± 0.27	H226	lung
0.055 ± 0.002	177 ± 6	46 ± 7	3.0 ± 0.12	HOP62	lung
0.142 ± 0.007	n.d.	n.d.	5.3 ± 0.15	MB231	breast
0.076 ± 0.004	n.d.	n.d.	3.9 ± 0.48	MKN45	gastric
0.073 ± 0.006	n.d.	n.d.	2.9 ± 0.21	PC3	prostate
0.160 ± 0.015	n.d.	n.d.	6.1 ± 0.22	SW620	colon
0.066 ± 0.004	n.d.	n.d.	3.8 ± 0.10	NHDF	normal

[a] The CellTiter-Glo Luminescent Cell Viability Assay (Promega, Technical bulletin 288) was employed in this study. IC_{50} data are the mean value of three experiments with statistical significance calculated.

In conclusion, we have prepared a series of advanced analogs of the antitumor natural product psymberin by modification of the "psymberate" unsaturated side chain using our novel $PhI(OAc)_2$-mediated oxidative cyclization method[7] as the key step and tested them in various human cancer cell lines. Our results suggest that substitution at C4 and C5 is important for the cytotoxicity of psymberin, but the terminal double bond is not essential for activity. An aryl group is a good replacement for the olefin. Diastereomers at C(8) and C(9) constantly showed decreased activity compared to their natural isomer. We finished the total synthesis of C11-deoxypsymberin and discovered that this compound is consistently more potent than the natural product psymberin which may be true for the other members of the psymberin/pederin family; this actually provides an opportunity for further SAR studies in the psymberin and pederin family. The activity of our synthetic psymberin is consistent with that reported in the literature. This natural product is extremely potent against all human cancer cell lines tested but showed no selectivity between cell lines, and its diastereomer (**2**) at C(8) and C(9) showed much decreased activity. Preliminary mechanism studies suggest the mode of action of psymberin is through cell apoptosis which is therapeutically important. Further SAR studies of psymberin, C11-deoxypsymberin, and the deoxy-pederin family of natural products are in progress, and the results will be reported in due course.

Acknowledgment. We thank Drs. Ismail Kola and John Piwinski at SPRI for their strong support of the SPRI postdoctoral program.

Supporting Information Available: Biological assay protocol, apoptosis studies, experimental details, and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The C(8)- and C(9)-stereochemistry of final products were determined by ^1H spectra comparison with synthetic psymberin and its epimers which was assigned by 2D NOE NMR and proton−proton coupling between N7, C8, and C9.
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