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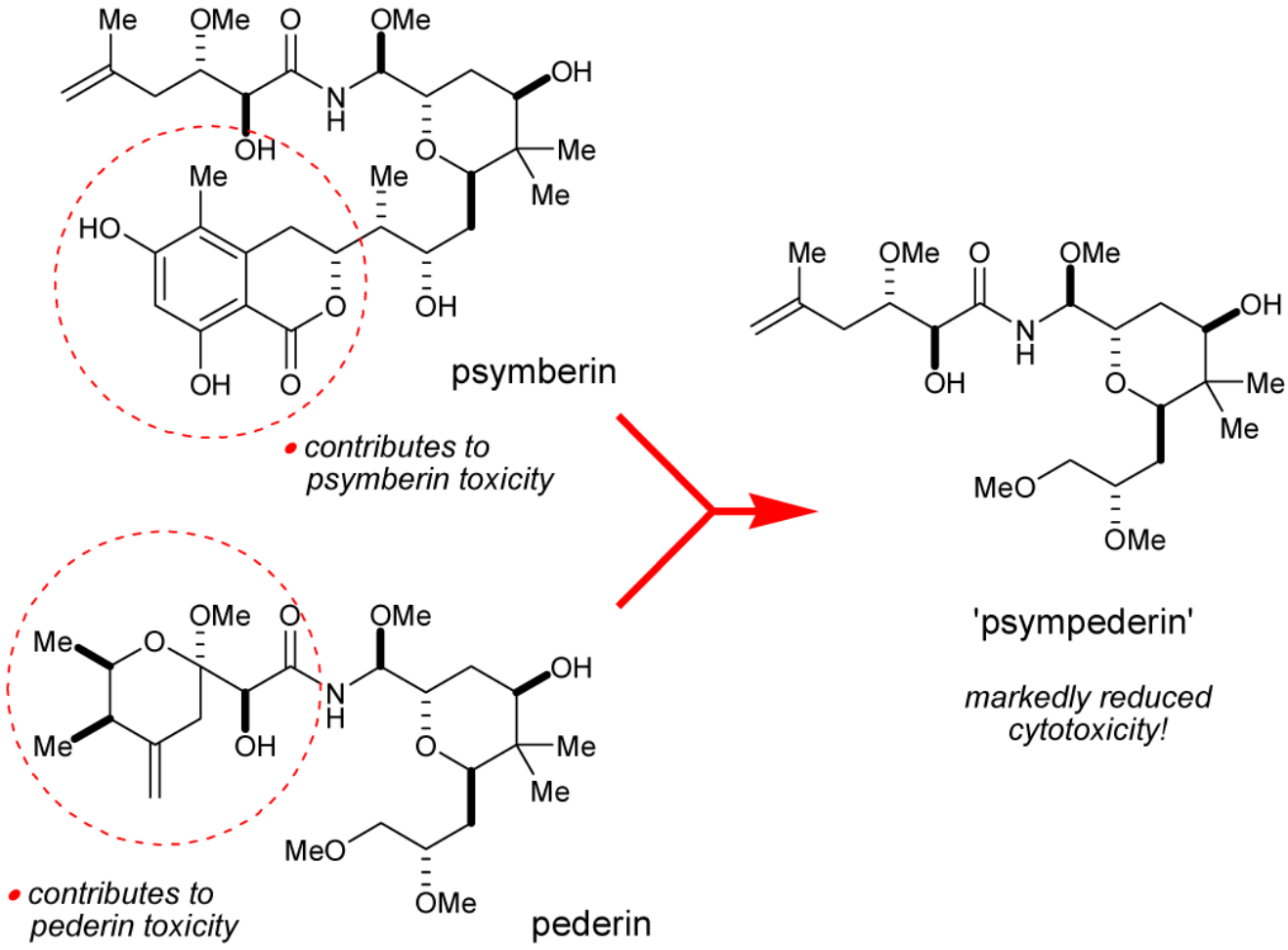
Synthesis of Psymberin Analogs:

Probing a Functional Correlation with the Pederin/Mycalamide Family of Natural Products

Xin Jiang, **Noelle Williams**, and **Jef K. De Brabander**
Department of Biochemistry, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75390-9038

Abstract



In this communication we describe an efficient synthesis of ‘psympederin’, a hybrid between the novel antitumor natural product psymberin and the blister beetle toxin pederin. Evaluation of antiproliferative activity reveals that the dihydroisocoumarin fragment is important for psymberin

Jef.debrabander@utsouthwestern.edu.

Supporting Information Available: Experimental procedures, characterization data, and copies of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

toxicity and the cyclic pederate fragment is important for pederin/mycalamide toxicity. Based on preliminary results described herein, we speculate that, despite their structural resemblance, psymberin and pederin/mycalamide induce toxicity through different mechanisms.

Recently, the Pettit and Crews groups independently reported the isolation of two novel natural cytotoxins from the marine sponges *Ircinia ramose* (irciniastatin A)[1] and *Psammocinia* sp. (psymberin)[2] which appeared to be diastereomeric. Our total synthesis[3] has enabled a full stereochemical assignment, and firmly established that irciniastatin A and psymberin are in fact identical compounds with relative and absolute configuration proposed by the Crews group.[2, 4] In addition to these structural considerations, our synthetic efforts were fuelled by the impressive biological activity of these natural products. Irciniastatin inhibited the growth of human tumor cell lines with values ranging from 0.1-6.2 nM (GI_{50}, 50% growth inhibition), and exhibited antivascular activity (inhibition of human umbilical vein endothelial cells at 0.5 nM).[1] Psymberin on the other hand, was evaluated in the NCI Developmental Therapeutics in Vitro Screening Program where it exhibited an unprecedented differential cytotoxicity profile - three of the melanoma (MALME-3M, SK-MEL-5, UACC-62), one breast (MDA-MB-435) and one colon cancer cell line (HCT-116) were sensitive to psymberin concentrations below 2.5 nM (LC_{50}, 50% cell lethality), whereas most other cell lines did not respond to concentrations up to 25 μM.[2] Such data, supporting a $>10^4$ differential activity for **1**, are exceptional and might indicate a novel mode-of-action.

Psymberin (**1**) most closely resembles the pederin/mycalamide family of natural products.[5] They share pederin's 2,6-*trans*-substituted tetrahydropyranyl ring with axial *N*-acyl methoxyaminal substituent (**2**, Fig. 1). In mycalamides, this substructure is embedded within a conformationally locked trioxadecalin (Fig. 1, **3, 4**).[6, 7] Because of this partial structural resemblance to pederin-like natural products, psymberin could share the latter's well-documented pharmacological role as potent eukaryotic protein synthesis inhibitors.[8] However, the following observations indicate that psymberin could have a biological function that is at least partially distinct from the pederin/mycalamide family of protein synthesis inhibitors: (1) without exception, all ~33 members of the pederin/mycalamide family display an identical left-half pederate side chain,[5b] contrasting psymberin's lesscomplex acyclic side chain; (2) the dihydroisocoumarin bottom-half is unique to psymberin, indicating divergent biosynthetic machinery[9] not found in any of the other pederin-type producing organisms; and (3) psymberin displayed unprecedented differential cytotoxicity,[2] whereas pederin-like compounds display much more uniform cytotoxicity profiles.[8c-e, 10] Herein, we present the first structure-function data that reveal the central importance of psymberin's distinct dihydroisocoumarin fragment on cell proliferation.

The first indication of a functional disconnect between psymberin and other members of the pederin/mycalamide family of natural products came from biological evaluation of psymberin and the epimeric variants that were obtained during our synthetic campaign to fully elucidate its stereostructure.[3] Psymberin (**1**) and the corresponding C8- and C4-epimers **5** and **6** (Figure 2) were evaluated against a selection of human tumor cell lines. As shown in Table 1, synthetic psymberin exhibited very potent antiproliferative activity against a selection of human tumor cell lines (KM12, PC3, SK-MEL-5, T98G) with IC_{50} values in the single digit to sub-nanomolar range (0.45 – 2.29 nM), about 2-fold more active than mycalamide A (0.95 – 3.79 nM).[11] Albeit less active, the 8-*epi*- and 4-*epi*-psymberin variants **5** and **6** were still able to prevent the proliferation of these cancer lines in the sub-micromolar range (37 – 763 nM). Similar structural changes (methoxyaminal epimer) in the pederin/mycalamide series resulted in >3 orders of magnitude reduction in cytotoxic activity.[5a, 8e]

In order to fully assess the importance of psymberin's unique dihydroisocoumarin moiety, we designed a truncated psymberin analog **18** (Scheme 1) that lacks this fragment, which is in

essence also an analog of pederin with the acyclic "psymberate" (C1-C6) side chain substituting the cyclic "pederate" fragment reminiscent of the pederin/mycalamide natural products.

For the synthesis of psymberin-pederin hybrid **18**, we required access to acetylpedamide **16**, followed by coupling with the C1-C6 "psymberate" side chain. Many synthetic approaches towards pederin have been reported,[5a, 7a-c] but we decided to exploit chemistry developed during our psymberin total synthesis campaign for the synthesis of acetylpedamide **16**.[3] As shown in Scheme 1, we started with the C_2-symmetrical diol 7[3] - a material that was efficiently monoacetylated via acid catalyzed cyclic orthoacetate formation followed by hydrolysis mediated by the addition of aqueous acetic acid (88% yield). Ozonolysis of monoacetate **8** followed by *in situ* reduction with triphenylphosphine yielded cleanly lactol **9** in 95% yield. Locking the lactol as the corresponding acetate allowed for the selective methylenation of the aldehyde, providing terminal olefin **10** in 60% yield over the two steps. Introduction of the axial nitrile (→ **11a**) was accomplished by ZnI_2 mediated acetate displacement with trimethylsilyl cyanide in 94% yield.

After some experimentation, we found that hydroxyquinine 9-phenanthtryl ether (HQP ether) [12] was the optimal ligand for the Sharpless asymmetric dihydroxylation (AD) of **11a**,[13] providing a ~3:1 mixture (**13**:**12**) favoring the desired C15-*S* configured diol **13** in 92% yield.[14, 15] Kocienski and coworkers had previously screened various ligands for the asymmetric dihydroxylation of the closely related substrate **11b** and found HQP ether also to be optimal, although selectivity for the desired diastereomer was lower (1.5:1) with this substrate.[7a] The epimeric mixture of α-diols **12** and **13** was methylated to yield the separable methyl ethers **14** and **15** in 91% yield.[16] Nitrile hydrolysis of the major methyl ether **15** using the Ghaffar-Parkins catalyst[17] provided acetylpedamide **16** in quantitative yield.[18]

The final introduction of the C1-C6 "psymberate" side chain was accomplished via a protocol consisting of our modified conditions for the formation of the imidate derivative of **16**, acylation with acid chloride **17**, *in situ* reduction with ethanolic $NaBH_4$, and final saponification of the protecting groups with LiOH in MeOH.[3] A 1:4 mixture of separable (silicagel, CH_2Cl_2/MeOH, 50:1 to 20:1) epimeric psymberin-pederin chimeras **18** and **19** was obtained in 60% yield from acetylpedamide **16**. This result is in sharp contrast with the corresponding psymberin result where the natural methoxyaminal epimer dominated (3:1),[3] and indicates that diastereoselectivity associated with the *N*-acylimidate reduction is highly dependent on the presence or absence of the dihydroisocoumarin fragment.[19]

The natural C8-(*S*) configuration of **18** was assigned based upon a detailed analysis of ^1H - ^1H coupling constants, 1D-, and 2D-NOE interactions - two possible conformations consistent with all the available data are shown in Figure 3.[20] The tetrahydropyranyl ring adopts a chair conformation with the C1-C8 side chain occupying the axial position, similar to psymberin and pederin. The methoxyaminal methine proton (H8) gave strong NOESY cross-peaks with the two axial tetrahydropyranyl protons H11 and H13 (see Scheme 1 for atom numbering). An anti-periplanar arrangement between H8/H9 and H8/NH was revealed by coupling constants (9.6 and 9.6 Hz) and further corroborated by a strong NH/H9 NOESY correlation. Finally, NOESY correlations between H5 and 15-OMe and 16-OMe, also observed as 1D enhancements upon irradiation of H5, unambiguously confirmed the assigned C8-configuration in **18**. No such correlations or enhancements were observed for **19**.[21]

Analysis of the antiproliferative activity of psymberinpederin hybrids **18** and **19** was particularly informative (Table 1). The dihydroisocoumarin-truncated psymberin analog **18** with natural C8-configuration displayed dramatically reduced cytotoxicity (~1,000-fold) compared to psymberin (**1**). Furthermore, the unnaturally configured C8-epimer **19** was devoid of cytotoxic activity against three out of four tested human cancer cell lines (at 1 μM) whereas

the corresponding dihydroisocoumarincontaining psymberin epimer **5** was active at concentrations between 37 and 352 nM.[22] *These results strongly support the notion that the dihydroisocoumarin fragment is vitally important for psymberin cytotoxicity*.

Considering hybrid **18** as a pederin/mycalamide analog reveals the *vital importance of the cyclic pederate side chain for pederin/mycalamide cytotoxicity*. Indeed, compound **18** also displayed markedly reduced antiproliferative activity (>300-fold) compared to mycalamide A (**3**, Table 1). Detailed structure - activity relationships in the pederin/mycalamide series have revealed the importance of a free C5-alcohol with (*S*)-configuration, a free NH, a correctly configured C8-(*S*) alkoxyaminal, and the cyclic C4-acetal for potent cytotoxic activity (P388 and/or HeLa cell line).[5a] Analog **18** retains these features save for the cyclic C4-acetal. We therefore conclude that the C4-acetal functionality significantly contributes to pederin/mycalamide cytotoxicity although is not required for psymberin cytotoxicity. Interestingly, the acid lability and vesicant (blistering) effects of pederin/mycalamide has been attributed to the reactive homoallylic acetal functionality.[5a] Unfortunately, previously reported studies do not illuminate on the structural determinants for specific versus non-specific toxicity, and correlation with protein synthesis inhibition.

In conclusion, we have initiated the first structure-function analysis of the novel antitumor natural product psymberin. Although psymberin is structurally partially related to the pederin/mycalamide family of antitumor compounds, we demonstrated that psymberin's mode of cytotoxicity is distinct to that induced by the pederin/mycalamide natural products. We synthesized a synthetic psymberin-pederin chimera **18** - a compound that is at the same time a psymberin analog lacking its characteristic dihydroisocoumarin fragment, and a pederin analog with an acyclic "psymberate" (C1-C6) side chain substituting for the "pederate" cyclic acetal fragment. We will continue these structure-function studies, including evaluation for protein synthesis inhibition, to further illuminate the mode-of-action of psymberin as well as mycalamide.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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(14). The ratio was determined by ^{1}H NMR.

(15). Asymmetric dihydroxylation with $(DHQ)_2PYR$ or $(DHQ)_2PHAL$ was non-selective (1:1 ratio). Dihydroxylation using the UpJohn Process (cat. OsO_4, *N*-methylmorpholine) revealed an intrinsic facial bias slightly favoring the undesired diastereomer **12** (1:1.4).

(16). The stereochemistry was determined by chemical correlation of acetate **15** to the corresponding known *tert*-butyldimethylsilyl ether,[7a] see the Supporting Information for details.

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(18). Our synthesis of acetylpedamide **16**, accomplished in 13 steps from isobutyraldehyde, compares favorable to Nakata's[7c] and Kocienski's[7a] 15 step syntheses of benzoylpedamide (from *S*-malic acid) and *tert*-butyldimethylsilylpedamide (from ethyl isobutyrate), respectively.

(19). A similar coupling between benzoylpedamide and the pederin "pederate" fragment in Nakata's pederin total synthesis also yielded a 1:3 mixture of pederin and *epi*-pederin (38% yield), see ref. 7c.

(20). There is conformational flexibility in the acyclic C1-C6 and C14-C16 fragments. The conformations in Fig. 3 are meant to illustrate the proximity of these two fragments as observed by NOE interactions between H5 and C15/C16-OMe protons. For a complete listing of chemical shifts, coupling constants, and NOE correlations, and copies of corresponding spectra, see the Supporting Information.

(21). The ^{1}H chemical shifts and coupling constants in the C1-C13 and the C6-C16 portion of **18** are very similar to the corresponding ones in psymberin[2, 3] and pederin[7b] respectively. For the C8-epimer **19**, dissimilarities were observed mainly for the NH, H8, and H9 protons.

(22). Interestingly, analog **19** is equipotent to C8-*epi*-psymberin **5** and about three-fold more potent than **18** against the PC3 pancreatic tumor cell line.

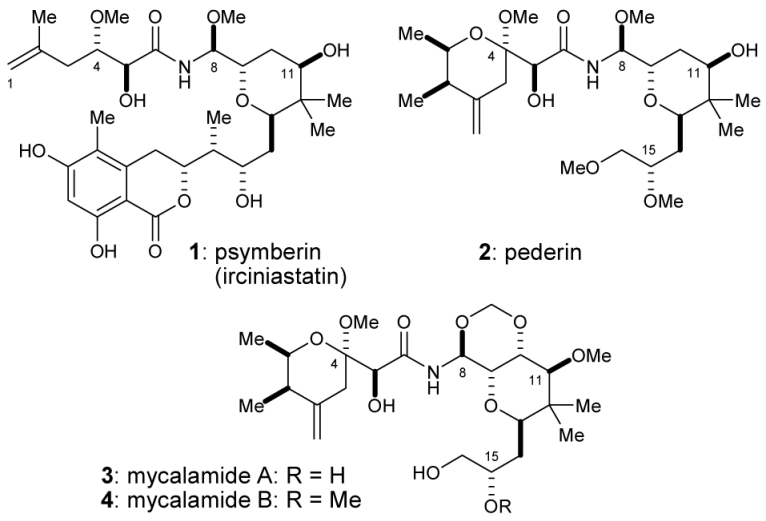


Figure 1.
Structures of psymberin, pederin, and mycalamides.

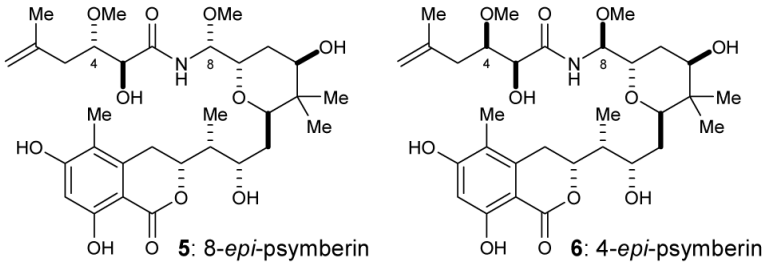
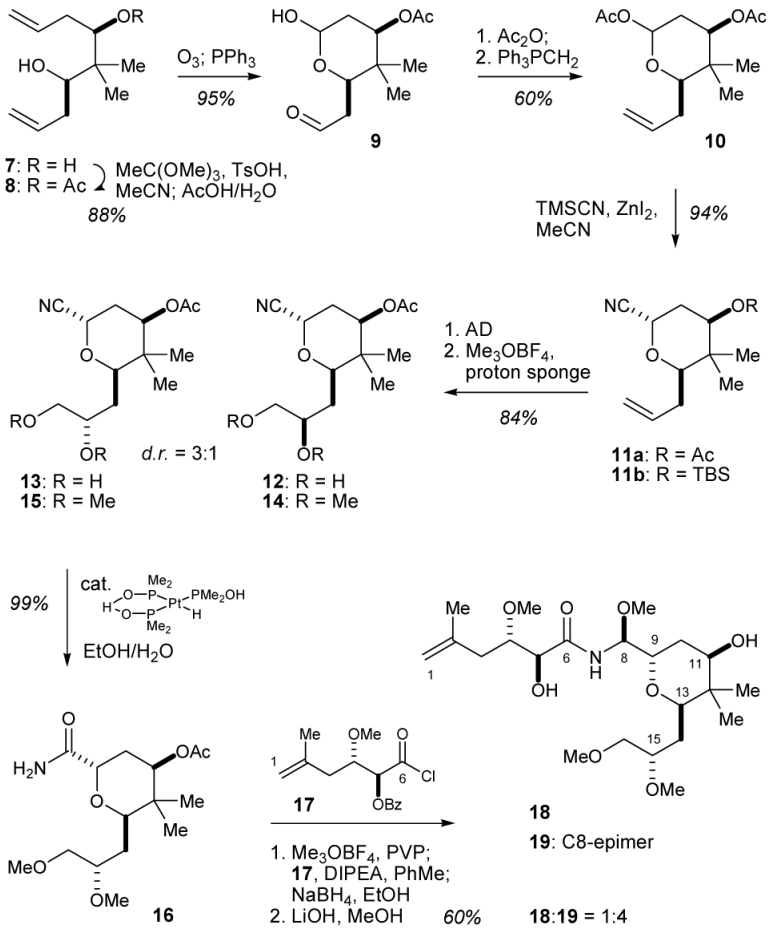


Figure 2.
Two epimeric psymberins.



Scheme 1.

Synthesis of psymberin-pederin hybrids **18** and **19**.[a] [a]Detailed experimental procedures and characterization data are provided in the supporting information.

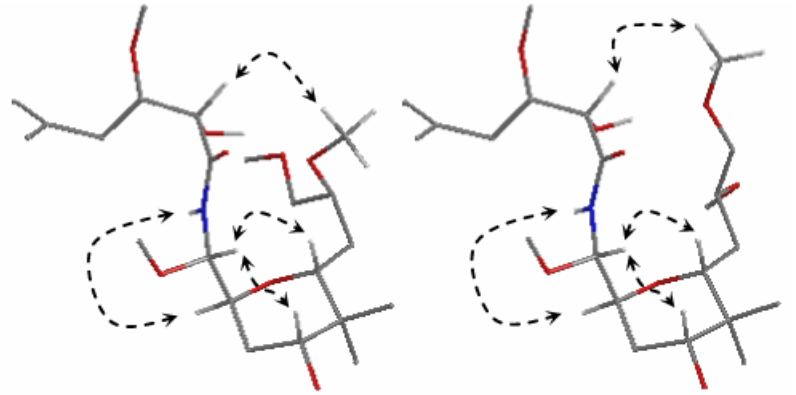


Figure 3.
Model of two proposed conformations of **18** consistent with NOE interactions (selected correlations indicated by dashed arrows) and ^{1}H-^{1}H coupling constants.[20]

Table 1

Cytotoxicities of psymberin, mycalamide A, and analogs against various human tumor cell lines.[a]

cmpd	IC_{50}[nm]			
	KM12	**PC3**	**SK-MEL-5**	**T98G**
1	0.45 ± 0.01	0.98 ± 0.12	2.29 ± 0.13	1.37 ± 0.06
3	0.95 ± 0.02	2.5 ± 0.2	3.79 ± 0.04	2.87 ± 0.07
5	37.1 ± 5.5	200.2 ± 27.6	352.0 ± 12.1	85.8 ± 48.4
6	126.08 ± 8.6	346.5 ± 102.8	762.8 ± 70.0	186.7 ± 51.3
18	710.9 ± 35.8	821.8 ± 89.1	>1,000	>1,000
19	>1,000	255.5 ± 11.4	>1,000	>1,000

[a]The Promega CellTiter Glo™ assay was utilized to measure cell viability after cells were exposed to compounds for 48 h. IC_{50} values represent the mean of triplicate experiments ± standard error of the mean. KM12: colon tumor; PC3: prostate tumor; SK-MEL-5: melanoma; T98G: glioblastoma.