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Synthesis and anticancer activity of side chain analogs of the crambescidin alkaloids

Zachary D. Aron,[a] Halina Pietraszkiewicz,[b] Larry E. Overman,[a,*] Fredrick Valeriote[b] and Carman Cuevas[c]

[a]*Department of Chemistry, 516 Rowland Hall, University of California, Irvine, CA 92697-2025, USA*
[b]*Josephine Ford Cancer Center, Detroit, MI 48202, USA*
[c]*Pharma Mar, S.A., Av. de los Reyes, 1, Polígono Industrial La Mina Norte, 28770-Colmenar Viejo, Madrid, Spain*

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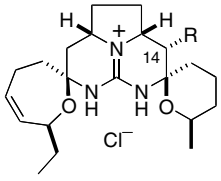
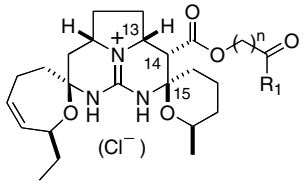
Abstract—Twenty three side chain analogs of the crambescidin alkaloids were prepared from the corresponding pentacyclic zwitterionic core acid. In the crambescidin 800 and 657 series, potency increased with increasing chain length. In addition, substantial variations in tumor selectivity with structure were seen. Crambescidin analogs having short, nonpolar side chains were identified for the first time as promising anticancer agents.

Members of the crambescidin family of marine sponge natural products are noteworthy both for their complex structures and potent biological activities.[1] Crambescidins are characterized by a unique pentacyclic guanidinium core, which is generally linked to various charged functional groups by a C14 ester side chain and a 16–18 carbon ω-hydroxyalkanoic acid spacer.[2] Crambescidins 431 (**4**),[2i] 657 (**16**),[2e,f] and 800 (**24**)[2c] are representative, varying only in their ester side chains (Fig. 1). The unique member of this natural products family is crambescidin 359 (**1**), which lacks an ester side chain.[2i] 13,14,15-Isocrambescidins 657 (**17**)[2e] and 800 (**25**)[2d] are the only members of this family having a different relative configuration of the pentacyclic guanidinium core (Fig. 1).

Varied biological activities are associated with the crambescidin alkaloids. They exhibit low to mid nanomolar cytotoxicities toward multiple human cancer tumor cell lines and are currently in early phase development as antineoplastic agents.[2a–g,3b] Antifungal activity toward *Candida albicans*[2a,b] antiviral activities toward herpes simplex virus type 1 (HSV-1)[2b–d,h] and human immunodeficiency virus (HIV),[2h] and inhibition of



1, R = H
2, R = CO_2^-
3, R = 14-*epi*-CO_2^-
4, R = CO_2Et
5, R = CO_2All
6, R = CO_2Bn
7, R = $CO_2CH_2CH(CH_2)_2$
8, R = $CO_2(CH_2)_2CH(CH_3)_2$
9, R = CO_2CH_2CH=CHPh
10, R = 14-*epi*-CO_2CH_2CH=CHPh



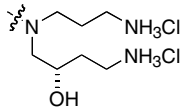
	$R_1 = O^-$	R_1 = 
n = 5	**11**	**19**
n = 7	**12**	**20**
n = 9	**13**	**21**
n = 11	**14**	**22**
n = 14	**15**	**23**
n = 15	**16**	**24**
n = 15	13,14,15-*epi*, **17**	13,14,15-*epi*, **25**
n = 15		13,15-*epi*, **26**
n = 17	**18**	**27**

Figure 1. Various crambescidin natural products and analogs.

* Corresponding author. Tel.: +1-949-824-7156; fax: +1-949-824-3866; e-mail: leoverman@uci.edu

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HIV-1 envelope mediated cell fusion have been described as well.[2j]

The striking architecture of the crambescidins and their biological potency has inspired numerous synthetic efforts,[1,3] with several total syntheses being recorded.[3a–d,f] Nonetheless, the structural basis of crambescidin biological activity remains poorly defined.[4,5] Biological data on a few crambescidin analogs with simplified core structures and spermidine-terminated side chains attached at positions other than C14 have been reported.[6] Despite the assortment of crambescidin natural products and analogs present in the literature, little comparative biological data has been described.

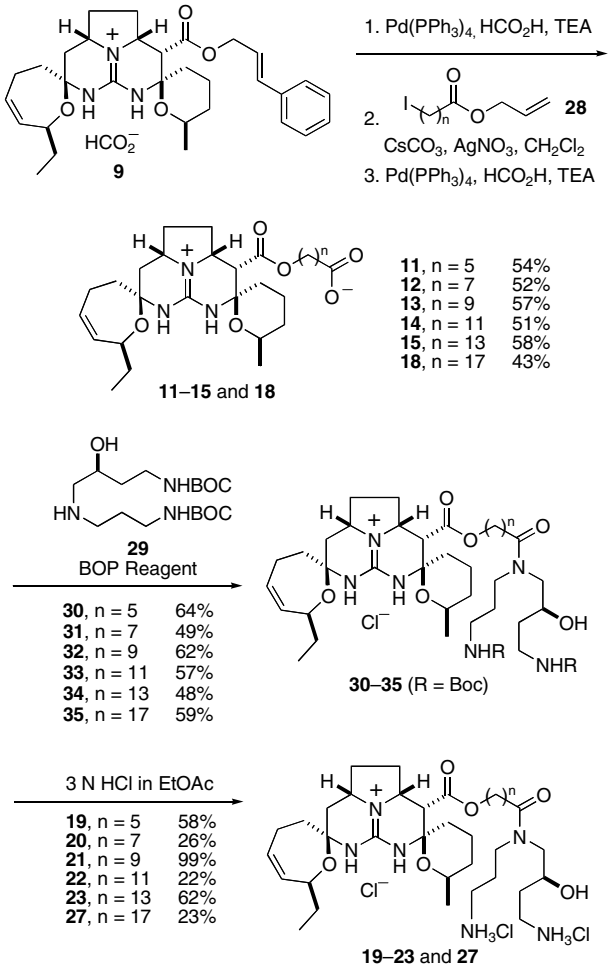
During our total synthesis studies,[3a–c,f] we generated an array of crambescidin natural products and analogs. A practical synthesis of the crambescidin core acid **2** was also developed,[3f] which allows the preparation of crambescidin analogs in which the C14 side chain is systematically varied. The synthesis and biological evaluation of such crambescidin analogs is the topic of this communication.

At the outset of this work, we aimed to generate analogs of crambescidins 657 (**16**) and 800 (**24**) that varied in the length of the side chain tether. As both of these natural products can be prepared from a precursor having a 15-(alloxycarbonyl)pentadecylcarboxyl C14 side chain,[3a,b] we desired to generate a range of congeneric allyl esters that varied in chain length. The starting point for this endeavor was cinnamyl ester **9** (Scheme 1), a known precursor of the crambescidin zwitterionic core acid **2**.[3f] Palladium mediated deprotection of cinnamyl ester **9** generated core acid **2**, which was used without purification. Alkylation of this intermediate with iodides **28** in the presence of $AgNO_3$ and $CsCO_3$, followed by removal of the terminal allyl unit, generated analogs **11**–**15** and **18** in 43–58% yield over the three steps.[7]

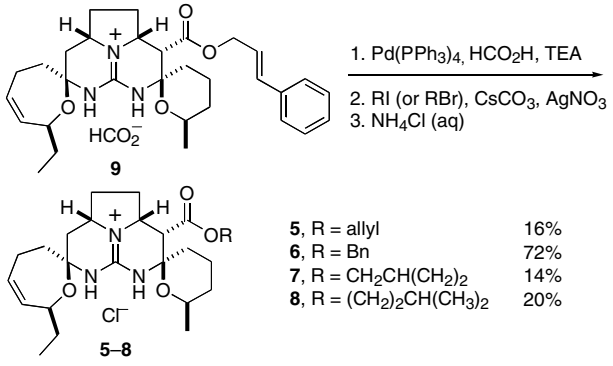
Analogs of crambescidin 800 were generated by BOP-mediated coupling of guanidino acids **11**–**15** and **18** with di-Boc-protected hydroxy spermidine (**29**),[3b] followed by acidic deprotection of the resulting products to generate crambescidin analogs **19**–**23** and **27**.[7]

Early results from biological evaluations of crambescidin 431 (**4**) and cinnamyl ester **9** suggested that analogs with short, nonpolar side chains had substantial antitumor activity. To further explore these observations, ester analogs **5**–**8** were prepared (Scheme 2). Allyl and benzyl bromide were competent electrophiles for the preparation of **5** and **6**, whereas the corresponding iodides were employed to form **7** and **8**. The low yields realized in the synthesis of these analogs resulted from difficulties in purification[7] rather than poor yields in the alkylation reaction.

Initial studies focused on a small portion of the compound library: compounds **2**, **4**, **9**, **10**, **11**, **13**, **14**, **15**, **19**, **21**, **22**, and **23**. These analogs were assayed for both cytotoxicity (not shown) and growth inhibition against 14 different tumor cell lines (DU-145, LN-caP, IGROV,



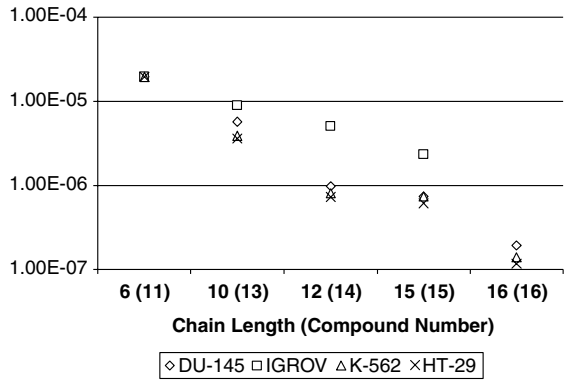
Scheme 1. Synthesis of crambescidin chain length analogs.



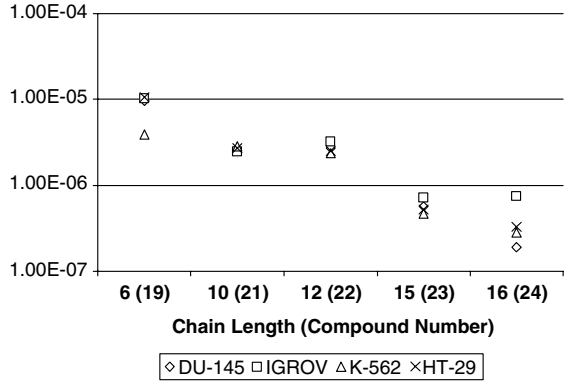
Scheme 2. Synthesis of short chain crambescidin analogs.

IGROV-ET, SK-BR-3, Mel-28, A549, K562, PANC-1, HT-29, LOVO, LOVO-DOX, HELA, and HELA, APL). The results for select cell lines are summarized in Graphs 1–3.[8,9] Additionally, crambescidins 657 (**16**), 800 (**24**), and 13,14,15-isocrambescidin 800 (**25**) were examined in the NCIs 60 cancer cell line panel.[10]

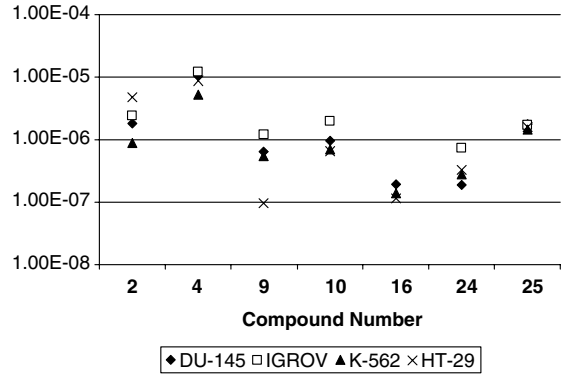
For the sake of brevity, only GI_{50} data for select cell lines will be discussed. Structure–activity relationships observed with these cell lines are representative of trends



Graph 1. GI_{50} of crambescidin 657 side chain analogs.



Graph 2. GI_{50} of crambescidin 800 side chain analogs.



Graph 3. GI_{50} of various natural crambescidins and analogs.

observed with all the cell lines assayed. Graphs 1 and 2 summarize the results of evaluation of crambescidin 657 and crambescidin 800 side chain analogs. The most striking observation in both series is the clear trend of increasing cytotoxicity with increasing chain length.

Graph 3 describes the GI_{50} data for a variety of crambescidin analogs, including analogs having: short, nonpolar ester side chains (**4** and **9**), an analog of **9** that is epimeric at C14 (**10**), the crambescidin core acid **2**, 13,14,15-isocrambescidin 800 (**25**), and crambescidins 657 (**16**) and 800 (**24**).[10] It is apparent in this data that crambescidins with short, nonpolar side chains can exhibit significant biological activity. This conclusion is most striking for cinnamyl ester **9**, the most potent compound assayed against the colon adenocarcinoma cell line HT-29. In contrast, the polar crambescidin core acid **2** shows low activity, suggesting that polar substituents are poorly tolerated in short side chain analogs.

In some cases significant variations in cytotoxicity profiles were seen with only minor variations in structure. For example, cinnamyl ester **9** inhibits the growth of HT-29 cells at a concentration five-fold lower than that required for inhibition of the other three cell lines. In contrast, the ethyl ester, crambescidin 431 (**4**), is the least effective analog surveyed against HT-29. Although the trends observed in this data are not large, they encouraged us to investigate tumor selectivity further.

As the growth inhibition studies suggested that the selectivity of crambescidin alkaloids varies with structure, the entire library (Fig. 1) was screened for selectivity toward both solid tumors and leukemia (Table 1).[11] In this disc diffusion assay, a compound is recognized as selective for one of two tumor cell lines (or a tumor and a normal cell line) if the difference in zone units is equal to or greater than 250.[11]

The most significant observation of this study is the substantial variation in selectivity sometimes observed with minor changes in structure. This variation is seen in comparing the selectivity profile of allyl ester **5** with that of crambescidin 359 (**1**), compounds that differ only by the presence of an allyl ester side chain at C14 of the former. Whereas allyl ester analog **5** is highly selective toward solid tumors in the murine assays, crambescidin 359 (**1**) is highly selective toward leukemia cell lines. Even more striking, simply changing the C14 side chain from an allyl ester (**5**) to an ethyl ester (**4**), results in a significant loss in selectivity. Additional examples are seen in both the crambescidin 657 and crambescidin 800 analog series in assays against murine cell lines. Whereas short chain analogs **11** and **19** are leukemia selective, longer chain analogs show a substantial drop (and in some cases a reversal) in selectivity.

Although this method of assay does not allow definitive comparisons of overall potency between compounds, examination of the concentration required for comparable results does give some indication of activity. The most interesting compound identified in this survey is allyl ester **5**, which is both highly selective toward solid tumors in the mouse model and highly active, requiring a 64-fold dilution relative to crambescidin 800 (**24**) to obtain comparable results.

In conclusion, an extensive library of crambescidin natural products and analogs was assembled and examined for trends in both antitumor potency and selectivity. Analogs of crambescidins 657 (**16**) and 800 (**24**) show increasing potency with increasing length of the side chain. Substantial variations in tumor selectivity with structure were also seen, suggesting the potential for further tuning the therapeutic targets of these

Table 1. Zone unit differentials in the disk diffusion soft agar colony formation assay[a]

Compd	Concd (μg/disk)	Murine tumor selectivity[b]			Human tumor selectivity[b]	
		$_{C38}\Delta s_{L1210}$	$_{C38}\Delta s_{CFU}$	$_{L1210}\Delta s_{CFU}$	$_{H116}\Delta s_{CEM}$	$_{H125}\Delta s_{CEM}$
Short Chain analogs						
1[c]	19	−350	0	350	0	150
2	75	−100	200	300	50	50
3	19	−250	−50	200	50	100
4[c]	5	−50	150	200	0	100
5	1	350	450	100	0	0
6	5	50	100	50	50	150
7	5	200	300	100	100	150
8	5	50	0	−50	50	150
9	19	150	100	−50	200	200
10	5	50	100	50	100	200
Isocrambescidin analogs						
17[c]	75	100	50	−50	100	150
25[c]	75	−50	0	50	50	100
26	75	0	50	50	50	50
Crambescidin 657 analogs						
11	75	−200	0	200	200	150
12	75	100	200	100	−50	−50
13	75	300	100	−200	100	50
14	75	200	250	50	100	200
15	75	200	100	−100	100	100
16[c]	75	150	200	50	50	100
18	75	100	100	0	100	150
Crambescidin 800 analogs						
19	75	−300	0	300	50	−50
20	75	−200	100	300	200	200
21	75	0	100	100	100	100
22	75	50	100	50	50	100
23	75	50	150	100	150	400
24[c]	75	−200	−50	150	−50	50
27	75	50	150	100	100	150

[a] Measured in zone units where $_{a}\Delta s_{b}$ refers to the zone differential a–b.[11] Murine cell lines: L1210 (lymphocytic leukemia), C38 (colon adenocarcinoma), CFU-GM (normal bone marrow), human cell lines: H116 (colon tumor), H125 (lung small cell carcinoma), CEM (lymphocytic leukemia).
[b] Significant selectivity is defined by a difference of $\geqslant$250 zone units.
[c] Natural product.

compounds by minor variations in structure. One of the most significant observations of this work is the substantial antitumor potential of crambescidin analogs having short, nonpolar side chains, with allyl ester **5** serving as an intriguing lead compound. This study reveals a valuable opportunity to substantially simplify the structures of the crambescidin alkaloids whilst maintaining substantial biological activity.

Acknowledgements

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