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Chemical Synthesis and Biological Evaluation of Second-Generation Palmerolide A Analogues

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In this communication, second-generation analogues of palmerolide A, a recently reported cytotoxic agent against melanoma cancer cells, were rationally designed, synthesized, and biologically evaluated. Structural variations of the enamide side chain and the C1–C8 segment of palmerolide A revealed a narrow structure–activity relationship window of the newly synthesized compounds. In addition, mechanistic and pharmacological studies were performed to assess the therapeutic potential of palmerolide A.

Introduction

Palmerolide A (**1**) is a cytotoxic marine macrolide recently isolated from the circumpolar tunicate *Synoicum adareanum* around Anvers Island on the Antarctic Peninsula.[1] Reported by the Baker team in April 2006, it was found to exhibit potent and selective growth inhibitory properties against melanoma cancer cells UACC-62 (LC_{50} = 18 nM), and its mechanism of action correlates well with other enamide containing V-ATPase inhibitors.[1] Synthetic efforts in the palmerolide A (**1**) arena have culminated its total syntheses,[2] and approaches toward several key synthetic intermediates.[3] Furthermore, structure−activity relationship (SAR) studies[4] led to the identification of benzamide derivative **2** and deoxygenated analogue **3** with an eightfold increase in potency and comparable potency, respectively, relative to parent natural product **1**. Continuing our chemical–biology investigations in this area, here we report the synthesis and biological evaluation of palmerolide E (**10**),[5] and second-generation analogues including side-chain analogues **19**–**24** and C1–C8 analogues **49a**–**d**, against a selected panel of cancer and normal cell lines. Mechanistic and pharmacological studies of **1** and benzamide **2** were also carried out to assess the therapeutic potential of the palmerolide family of antiproliferative agents.

Results and Discussion

The synthetic strategy towards the designed analogues is in accordance to our previously described technology in the total synthesis of palmerolide A (**1**),[2b,2c] its stereochemical congeners and analogues,[4] as outlined in Figure 1. In view of the possible role of the enamide moiety involved in the reversible inhibition of the V-ATPase,[6] palmerolide E (**10**) and α,β-unsaturated methyl ester **11** could serve as negative controls in the biological studies against the enamide-containing compounds. As shown in Scheme 1, the preparation of **10** and α,β-unsaturated methyl ester **11** began with removal of the two MOM ether moieties from aldehyde **8**[2b,2c] (HCl, 52% yield) to afford dihydroxy aldehyde **9**, which underwent smooth ring-closing metathesis[7] in the presence of Grubbs II catalyst to furnish **10** in 62% yield. Treatment of **10** with $Ph_3P{=}CHCO_2Me$ smoothly delivered enoate **11** in 55% yield as a single geometric isomer.

On the basis of our previous studies,[4] the biologically inconsequential C7 hydroxy substituent of the palmerolide A core structure prompted us to employ structurally simplified 7-deoxyvinyl iodide **12**[4] to pursue further SAR investigations concerning the palmerolide enamide side chain. At the same time, we previously concluded that a lipophilic enamide side chain was necessary for retaining the potent biological activity, that is, benzamide **2**. Therefore, under our carefully optimized conditions, application of the Buchwald copper-catalyzed coupling (CuI, K_2CO_3 and *N*,*N*′-dimethylethylenediamine)[8] engaging macrocyclic iodide **12** and primary amides **13**–**18** afforded palmerolide A analogues **19**–**24** in moderate to average yields (26–51%) as summarized in Table 1.

Next, we returned our attention to the SAR exploration in the C1–C8 domain of palmerolide A. These second-generation analogues were designed to probe (i) the importance of the C2–C3 olefin in the α,β-unsaturated macrolactone (i.e., **49a**), (ii) the possibility of replacing the metabolically labile aliphatic side chain with a conformationally rigid aromatic moiety (i.e., **49b**), (iii) the effect of extending the C_1–

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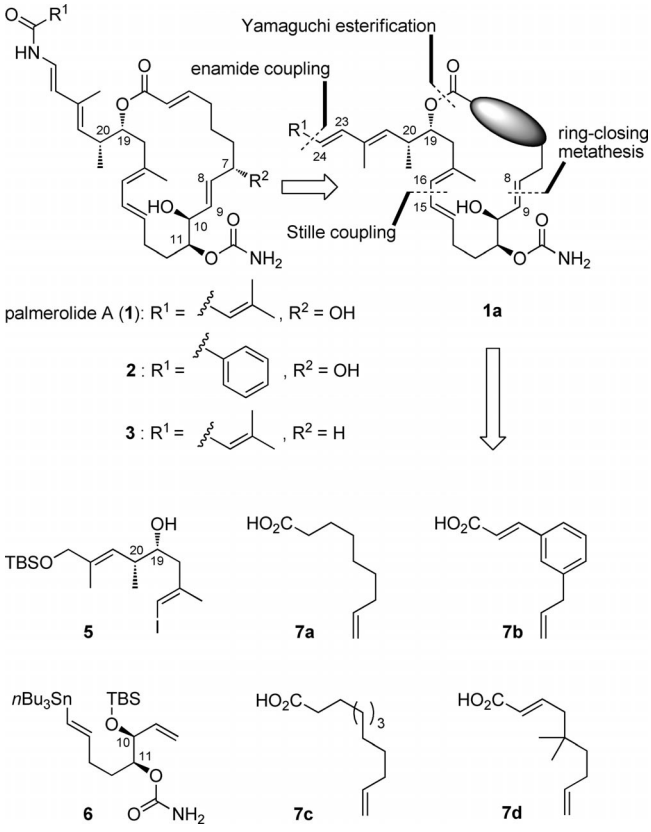
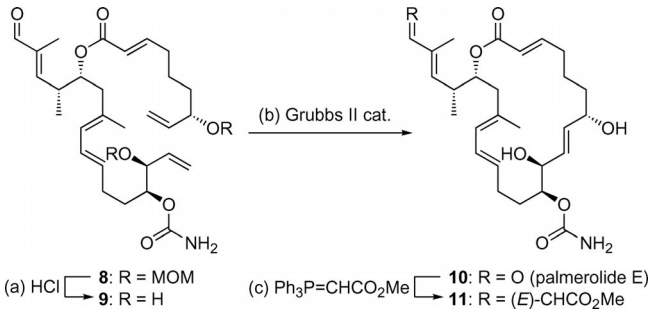


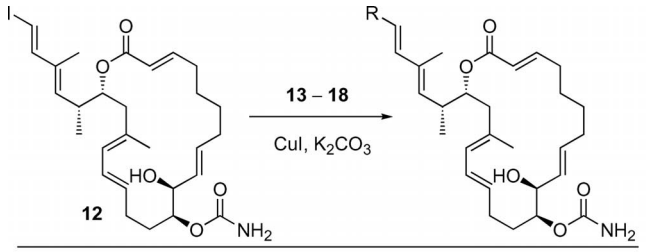
Figure 1. Structure of palmerolide A (**1**), benzamide (**2**), and 7-deoxypalmerolide A (**3**), and retrosynthetic analysis of palmerolide structure **1a** leading to building blocks **5**, **6**, **7a**, **7b**, **7c**, and **7d**. TBS = *tert*-butyldimethylsilyl.

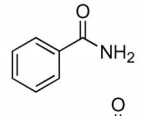
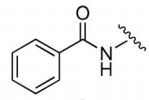
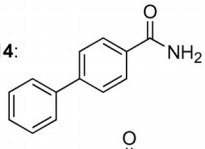
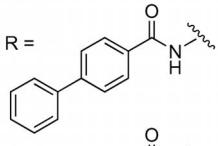
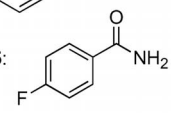
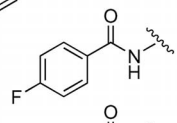
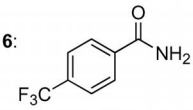
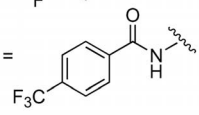
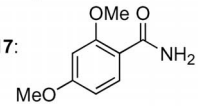
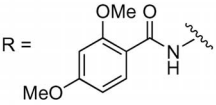
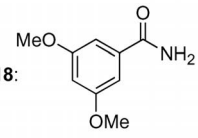
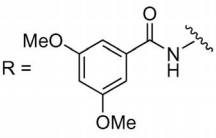


Scheme 1. Synthesis of palmerolide E (**10**) and α,β-unsaturated methyl ester analogue **11**. Reagents and conditions: (a) HCl (3.0 N aq., 20.0 equiv.), EtOH, 23 → 80 °C, 50 min, 52%; (b) Grubbs II cat. (0.05 equiv.), CH_2Cl_2, 23 °C, 1 h, 62%; (c) $Ph_3P{=}CHCO_2Me$ (2.5 equiv.), CH_2Cl_2, 23 °C, 2 h, 55%. MOM = methoxymethyl.

C8 aliphatic backbone (i.e., **49c**), and (iv) the effect of the sterically demanding C5 *gem*-dimethyl substituents (i.e., **49d**). In accordance to our generalized retrosynthetic blueprint for the preparation of the palmerolide family of compounds (Figure 1), the synthesis of the required building blocks and their unifications leading to analogues **49a**–**d** are outlined in Schemes 2–4. In our previously reported syntheses of palmerolide structures,[2b,2c] the C10 hydroxy group was protected as its MOM ether throughout the synthetic sequence and removed prior to the macrocyclic ring closure

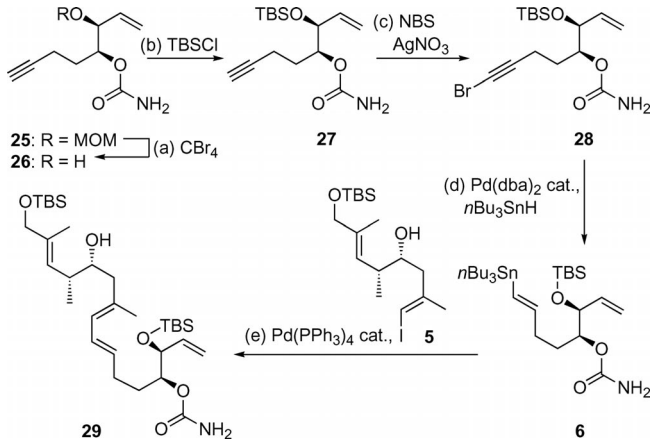
Table 1. Preparation of palmerolide A analogues **19**–**24** through copper-mediated coupling reactions.[a]



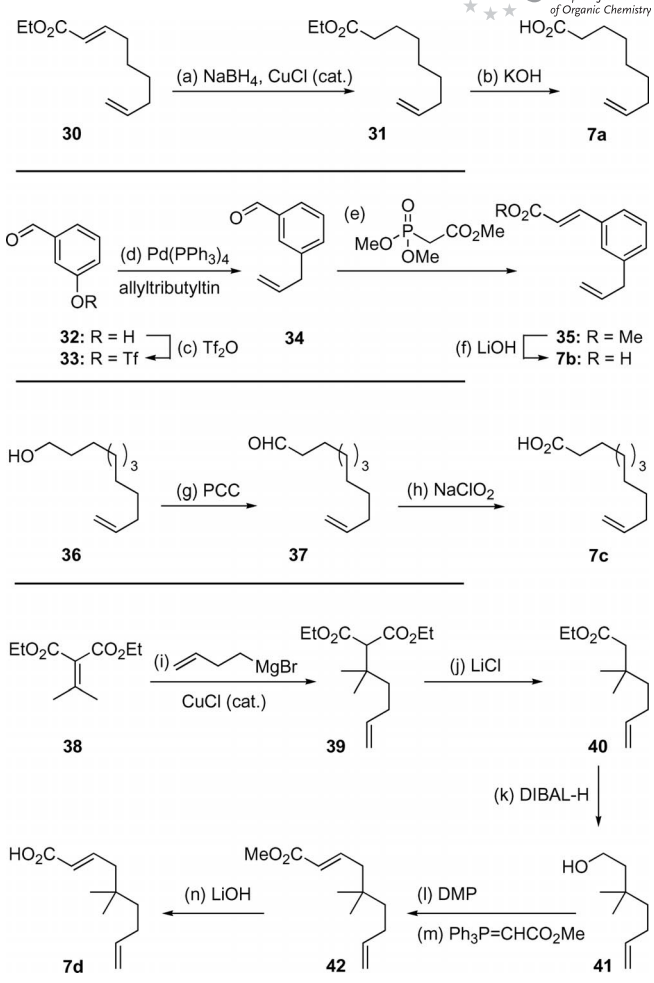
Entry	Amide	Yield [%]	Product
1	**13**: 	45	**19**: R = 
2	**14**: 	26	**20**: R = 
3	**15**: 	34	**21**: R = 
4	**16**: 	37	**22**: R = 
5	**17**: 	51	**23**: R = 
6	**18**: 	45	**24**: R = 

[a] Reagents and conditions: **13**–**18** (2.0 equiv.), CuI (1.5 equiv.), K_2CO_3 (5.0 equiv.), *N*,*N*′-dimethylethylenediamine (3.0 equiv.), DMF, 23 °C, 1 h. DMF = *N*,*N*′-dimethylformamide.

under the olefin metathesis conditions. The MOM ether could be removed at a later stage under mild acid conditions (HCl/MeOH or TMSCl/MeOH); however, this transformation was routinely complicated with the formation of the C10–C11 cyclic carbonate as a byproduct.[2b,2c] Therefore, we thought to circumvent this late-stage counterproductive event by exchanging the MOM ether to a TBS ether early in the synthetic sequence (Scheme 2). In this instance, starting from MOM ether **25**,[2b,2c] a two-step protecting group interconversion (CBr_4; TBSCl) smoothly delivered TBS ether **27** in 58% yield over the two steps. Further elaboration of the alkyne terminus in **27** to key building block vinyl stannane **6** was carried out by following our previously reported palladium-catalyzed hydrostannation procedure,[9] through intermediate bromide **28**, in 69% overall yield as a single geometric isomer. Stille[10] coupling between vinyl stannane **6** and vinyl iodide **5**[2b,2c] afforded tetraene **29** in 79% yield, which serves as a common intermediate for all ensuing synthetic investigations concerning the variation of the C1–C8 segment of palmerolide A.



Scheme 2. Synthesis of tetraene **29**. Reagents and conditions: (a) CBr_4 (0.1 equiv.), *i*PrOH, 23 → 80 °C, 2 h, 62%; (b) TBSCl (1.3 equiv.), imidazole (2.5 equiv.), 4-DMAP (cat.), DMF, 23 °C, 5 h, 94%; (c) NBS (1.1 equiv.), $AgNO_3$ (0.1 equiv.), acetone, 23 °C, 1 h, 98%; (d) $Pd(dba)_2$ (0.05 equiv.), PPh_3 (0.22 equiv.), nBu_3SnH (2.2 equiv.), THF, 23 °C, 1 h, 70%; (e) $Pd(PPh_3)_4$ (0.1 equiv.), CuCl (5.0 equiv.), LiCl (6.0 equiv.), DMSO, 23 °C, 8 h, 79%. 4-DMAP = *N*,*N*′-dimethylaminopyridine, NBS = *N*-bromosuccinimide; dba = dibenzylideneacetone.

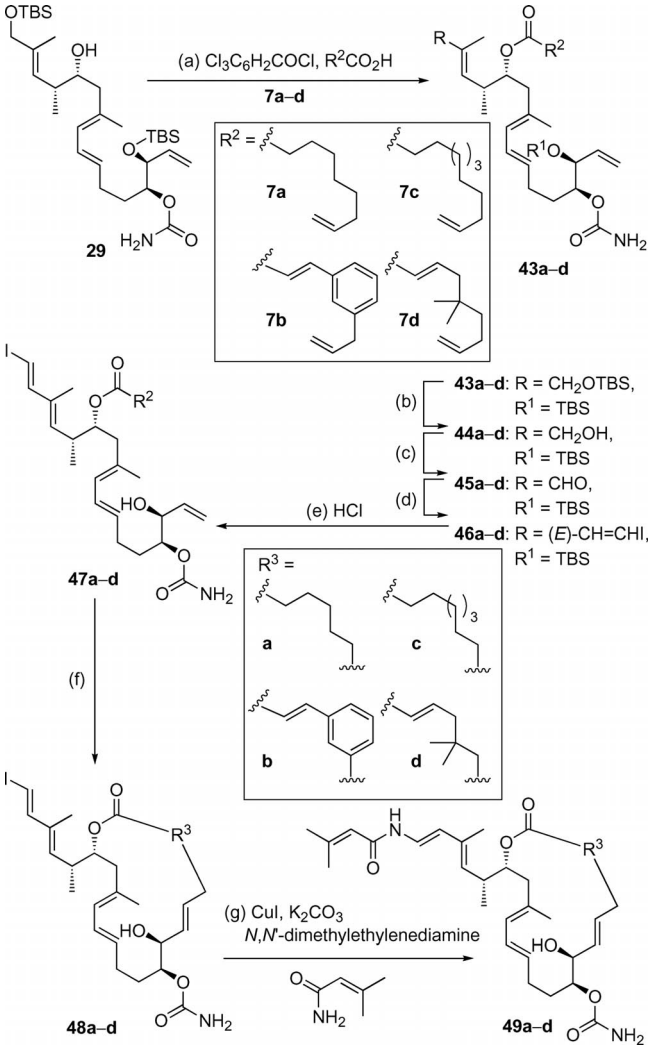


Scheme 3. Synthesis of carboxylic acids **7a**–**d**. Reagents and conditions: (a) $NaBH_4$ (10.0 equiv.), CuCl (0.75 equiv.), MeOH/THF (4:7), 0 °C, 6 h, 91%; (b) KOH (5.0 equiv.), dioxane/H_2O, (4:1), 23 °C, 12 h, 82%; (c) Tf_2O (1.2 equiv.), pyridine (2.0 equiv.), CH_2Cl_2, 0 → 23 °C, 3 h, 46%; (d) $Pd(PPh_3)_4$ (0.1 equiv.), LiCl (3.0 equiv.), allyltributyltin (1.1 equiv.), 1,4-dioxane, 100 °C, 16 h, 99%; (e) trimethyl phosphonoacetate (1.2 equiv.), LiBr (4.0 equiv.), Et_3N (2.0 equiv.), THF, 23 °C, 16 h, 35%; (f) LiOH (3.0 equiv.), THF/MeOH/H_2O (2:1:1), 23 °C, 4 h, 95%; (g) PCC (1.6 equiv.), CH_2Cl_2, 23 °C, 16 h, 88%; (h) $NaClO_2$ (3.0 equiv.), 2-methyl-2-butene (10 equiv.), NaH_2PO_4 (5.0 equiv.), *t*BuOH/H_2O (1:1), 0 °C, 2 h, 73%; (i) 3-butenylmagnesium bromide (2.0 equiv.), CuCl (0.05 equiv.), THF, –30 → 23 °C, 1.5 h, 97%; (j) LiCl (2.0 equiv.), H_2O (1.0 equiv.), DMSO, reflux, 24 h, 95%; (k) DIBAL-H (1.4 M in CH_2Cl_2, 2.5 equiv.), CH_2Cl_2, –78 °C, 1.5 h, 90%; (l) DMP, (1.1 equiv.), $NaHCO_3$ (5.0 equiv.), CH_2Cl_2, 23 °C, 0.5 h; (m) trimethyl phosphonoacetate, (1.1 equiv.), LiBr (4.0 equiv.), Et_3N (2.0 equiv.), 23 °C, 12 h, 78% over two steps; (n) LiOH (3.0 equiv.), THF/MeOH/H_2O (2:1:1), 23 °C, 4 h, 95%. PCC = pyridinium chlorochromate, Tf = trifluoromethanesulfonyl.

Variation of the C1–C8 segment of palmerolide A also called for the preparation of carboxylic acids **7a**–**d**, and their syntheses are shown in Scheme 3. Acid fragment **7a** was prepared through conjugate reduction of enoate **30** ($NaBH_4$, CuCl), followed by saponification of resulting ethyl ester **31** (KOH, 75% yield over the two steps). Aromatic acid **7b** was synthesized from phenolic benzaldehyde **32** through intermediate triflate **33** (Tf_2O, 46% yield), followed by palladium-mediated cross-coupling with allyltributyltin (**34**, 99% yield), homologation to enoate **35** (35% yield), and saponification (LiOH, 95% yield). Two-carbon homologated acid **7c** was synthesized through sequential oxidation of alcohol **36** (PCC; then $NaClO_2$) in 64% yield over the two steps. Finally, preparation of *gem*-dimethyl acid **7d** began with conjugate addition of 3-butenylmagnesium bromide to α,β-unsaturated diester **38** followed by thermal decarboxylation to give ethyl ester **40** in 92% yield over the two steps. Two-carbon homologation of ethyl ester **40** involved a reduction/oxidation/Wittig olefination sequence (DIBAL-H, DMP, $Ph_3P{=}CHCO_2Me$), through the intermediacy of alcohol **41**, to furnish α,β-unsaturated methyl ester **42** (70% overall yield). Finally, saponification of methyl ester **42** (LiOH) afforded desired acid **7d** in 95% yield.

With building blocks **29** and **7a**–**d** in hand, the next task became their unification and further elaboration to targeted analogues **49a**–**d**, as summarized in Scheme 4. Thus, Yamaguchi esterification[11] (2,4,6-trichlorobenzoyl chloride, Et_3N, 4-DMAP) between alcohol **29** and acid fragments **7a**–**d** afforded esters **43a** (69%), **43b** (70%), **43c** (72%), and **43d** (60%), respectively. Selective removal of the primary TBS ether in **43a**–**d** under the mild action of TFA, followed by oxidation (DMP) of resulting alcohols **44a**–**d** gave corresponding aldehydes **45a** (77%), **45b** (68%), **45c** (72%), and **45d** (50%). Further elaboration of aldehydes **45a**–**d** to macrocyclic ring-closing metathesis precursors **47a**–**d** involved Takai olefination[12] ($CrCl_2$, CHI_3; **46a**–**d**) and TBS ether removal (HCl aq.; **47a**: 76%, **47b**: 40%, **47c**: 42%, and **47d**: 44% yield over the two steps). With allylic alcohols **47a**–**d** in hand, their ring-closing-metatheses[7] under the action of Grubbs II catalyst (CH_2Cl_2, 0.005 M,

25 °C) proceeded smoothly to afford macrocycles **48a** (61%), **48b** (60%), **48c** (60%), and **48d** (60%), respectively. Finally, installation of the enamide moiety onto vinyl iodides **48a**–**d** through the established copper-catalyzed cross-coupling protocol[8] provided C1–C8 palmerolide A analogues **49a** (56%), **49b** (28%), **49c** (26%), and **49d** (31%), respectively.



Scheme 4. Synthesis of palmerolide A analogues **49a**–**d** employing acid fragments **7a**–**d**, respectively. Reagents and conditions: (a) 2,4,6-trichlorobenzoyl chloride (1.1 equiv.), Et_3N (2.0 equiv.), **7a** or **7b** or **7c** or **7d** (1.2 equiv.), 4-DMAP (1.0 equiv.), toluene, 23 °C, 12 h, **43a**: 69%; **43b**: 70%; **43c**: 72%; **43d**: 60%; (b) TFA (10% aq., 1.0 equiv.), THF, 0 °C, 12 h, **44a**: 91%; **44b**: 90%; **44c**: 90%; **44d**: 65%; (c) Dess–Martin periodinane (1.1 equiv.), $NaHCO_3$ (5.0 equiv.), CH_2Cl_2, 23 °C, 20 min, **45a**: 85%; **45b**: 75%; **45c**: 80%; **45d**: 75%; (d) $CrCl_2$ (10.0 equiv.), CHI_3 (3.0 equiv.), THF/dioxane (1:6), 23 °C, 2 h, **46a**: 87% (>95:5 *E*/*Z*); **46b**: 55% (>95:5 *E*/*Z*); **46c**: 60% (>95:5 *E*/*Z*); **46d**: 60% (>95:5 *E*/*Z*); (e) HCl (3.0 N aq., 2.0 equiv.), EtOH, 23 °C, 12 h, **47a**: 87%; **47b**: 73%; **47c**: 70%; **47d**: 74%; (f) Grubbs II cat. (0.05 equiv.), CH_2Cl_2, 23 °C, 1 h, **48a**: 61%, **48b**: 60%; **48c**: 60%; **48d**: 60%; (g) 3-methyl-2-butenamide (2.0 equiv.), CuI (1.5 equiv.), K_2CO_3 (6.0 equiv.), *N*,*N*′-dimethylethylenediamine (3.0 equiv.), DMF, 23 °C, 1 h, **49a**: 56%; **49b**: 28%; **49c**: 26%; **49d**: 31%. TFA = trifluoroacetic acid.

The synthesized compounds were tested against a panel of cancer cells, including breast (MCF-7), melanoma (UACC-62), CNS (SF268), lung (NCI-H460), ovarian (1A9), Taxol®-resistant ovarian (PTX22),[13] epothilone-resistant ovarian (A8),[14] and human melanocyte (HM2) cells using doxorubicin, Taxol®, and natural palmerolide A (**1**) as standards; the results are summarized in Table 2. As expected and on the basis of the hypothesized reversible V-ATPase inhibition involving the enamide side chain,[6] both palmerolide E (**10**) and methyl ester **11** showed complete lost of activity up to 10 μM against all cells tested. Side-chain analogues **19**–**24** revealed some interesting findings. Benzamide **19** exhibited the most potent activity, an observation similar to that previously reported for its C7 oxygenated relative (compound **2**). In stark contrast, biphenyl **20** and 2,4-dimethoxy **23** showed significantly lower activities, whereas 4-fluoro **21**, 4-trifluoromethyl **22**, and 3,5-dimethoxy **24** displayed modest activities. This seems to suggest a very narrow SAR window concerning the steric environment of the aromatic side chain, even for 4-fluoro analogue **21**. It is interesting to note that 2,4-dimethoxy and 3,5-dimethoxy analogues **23** and **24** exhibited drastically different cytotoxic activities, possibly attributed to the electron-donating groups at the *ortho* and *para* positions of analogue **23**, which shift the equilibrium towards the protonated enamide rather than the enamide/V-ATPase complex.[6] The biological activities of C1–C8 analogues **49a**–**d** also revealed little SAR flexibility concerning this domain of the palmerolide A core structure. In this instance, whereas C2–C3 saturated analogue **49a** displayed an approximately 4–10-fold reduction in activity across all cell lines examined, aromatic analogue **49b**, two-carbon homologated analogue **49c**, and *gem*-dimethyl analogue **49d** all exhibited a dramatic reduction in their biological activities. Once again, this indicates that a subtle increase in the steric environment around the C1–C8 domain of palmerolide A cannot be tolerated at the active site of the enzyme. The therapeutic indexes of the palmerolide family of compounds were also determined by comparing their cytotoxicities against the melanoma cancer cell line (UACC-62) and normal human melanocyte (HM-2). Synthetic palmerolide A (**1**) and analogues **2**, **3**, **19**, **21**, and **24** all exhibited excellent selectivity towards UACC-62, with benzamide analogue **2** being the most potent and most selective compound prepared to date.

Finally, mechanistic and pharmacological investigations of palmerolide A (**1**) and benzamide analogue **2** were carried out to further assess the therapeutic potential of the palmerolide family of antitumor agents.[15] In the Apo-BrdU TUNEL assay (to detect the 3′-hydroxy ends of fragmented DNA in apoptotic cells), palmerolide A (**1**) displayed cell killing effect resembling a late-apoptotic pathway. Furthermore, direct evidence in support of the V-ATPase inhibitory properties of palmerolide A (**1**) was obtained through LysoSensor studies (monitor pH level changes of the lysosomes and acidic organelles), in comparison with concanamycin, a known V-ATPase inhibitor. Palmerolide A (**1**) and benzamide analogue **2** were also found to inhibit CYP3A4 metabolic activity (IC_{50} = 1.182

Table 2. Cytotoxicity of natural and synthetic palmerolides against selected cancer and normal cell lines.

Entry	Cell line/ Compound	GI_{50}[a] [μM] UACC-62[b]	MCF-7[b]	SF268[b]	NCI-H460[b]	IA9[c]	PTX22[c]	A8[c]	HM-2[d]	SI[e]
1	**1**	0.061 ±0.011	0.068 ±0.006	0.040 ±0.012	0.014 ±0.004	0.070 ±0.009	0.067 ±0.004	0.059 ±0.015	6.177 ±1.272	101.3
2	**2**	0.009 ±0.000	0.008 ±0.001	0.007 ±0.001	0.007 ±0.001	0.008 ±0.001	0.029 ±0.011	0.007 ±0.001	2. 309 ±1.269	256.6
3	**3**	0.076 ±0.010	0.153 ±0.087	0.069 ±0.004	0.069 ±0.004	0.087 ±0.006	0.194 ±0.078	0.091 ±0.004	14.006 ±4.589	184. 3
4	**10**	>10	>10	>10	>10	>10	>10	>10	>10	
5	**11**	>10	9.061 ±0.174	>10	>10	>10	>10	>10	>10	
6	**19**	0.055 ±0.011	0.061 ±0.004	0.045 ±0.008	0.027 ±0.017	0.072 ±0.004	0.075 ±0.003	0.065 ±0.003	8.256 ±2.222	150.1
7	**20**	5.823 ±0.625	6.320 ±0.978	8.181 ±0.134	0.701 ±0.028	7.309 ±1.172	7.035 ±0.858	6.217 ±0.472	56.641 ±0.989	9.7
8	**21**	0.227 ±0.048	0.339 ±0.093	0.282 ±0.074	0.069 ±0.005	0.646 ±0.098	0.631 ±0.060	0.486 ±0.122	27.186 ±4.734	119.8
9	**22**	0.882 ±0.041	0.864 ±0.086	0.936 ±0.041	0.582 ±0.072	3.004 ±0.764	2.232 ±0.491	0.785 ±0.029	23.701 ±1.541	26.9
10	**23**	9.026 ±0.423	5.452 ±0.659	>10	5.093 ±0.018	>10	9.206 ±0.240	8.317 ±0.299	42.201 ±8.480	4.7
11	**24**	0.753 ±0.113	0.855 ±0.006	0.789 ±0.111	0.517 ±0.048	0.869 ±0.077	0.863 ±0.161	0.710 ±0.013	>100	>132.8
12	**49a**	0.750 ±0.123	0.849 ±0.083	0.687 ±0.121	0.465 ±0.086	0.820 ±0.066	0.723 ±0.056	0.807 ±0.075	8.020 ±1.243	10.7
13	**49b**	4.958	2.357	2.686	0.845	7.084	5.489	4.269	7.787 ±0.889	3.8
14	**49c**	6.857 ±0.071	5.708	6.793	5.41	8.102	6.66	5.626	8.619 ±0.361	1.2
15	**49d**	>10	>10	>10	>10	–	–	–	>10	–
16	Taxol	0.015 ±0.007	0.006 ±0.000	0.024 ±0.008	0.006 ±0.001	0.006 ±0.000	0.083 ±0.007	0.035 ±0.016	0.061 ±0.010	4.1
17	Doxorubicin	0.207 ±0.091	0.057 ±0.007	0.160 ±0.053	0.008 ±0.001	0.035 ±0.010	0.159 ±0.051	0.063 ±0.013	0.172 ±0.083	0.8

[a] Antiproliferative effects of tested compounds against human tumor cell lines and drug-resistant cell lines in a 48 h growth inhibition assay using the sulforhodamine B staining methods. Human cancer cell lines: melanoma (UACC-62); breast (MCF-7), CNS (SF268), lung (NCI-H460), ovarian (IA9) and its drug resistant mutant PTX22 (taxol-resistant) and A8 (epithilone resistant), normal melanocyte (HM-2). Growth inhibition of 50% (GI_{50}) is calculated as the drug concentration, which caused a 50% reduction in the net protein increase in control cells during the drug incubation. GI_{50} values for each compound represent mean of 2–5 independent experiments ± SE. [b] These cell lines were provided by the National Cancer Institute (NCI), Division of Cancer Treatment and Diagnosis (DCTD). [c] These cell lines were provided by Professor Paraskevi Giannakakou, Weill Medical College of Cornell University. [d] These cells were purchased from Cell Research Corporation (Singapore). [e] Selectivity index, SI (GI_{50} HM-2/GI_{50} UACC-62).

and 0.956 μM, respectively) and verapamil-stimulated Pgp-ATPase activity (IC_{50} = 1.220 and 1.384 μM, respectively) and inhibited hERG channel at 3.5 μM (with E4031 as the positive control).

Conclusions

In conclusion, the design and synthesis of second-generation palmerolide A analogues with structural variations in the enamide side chain and the C1–C8 aliphatic backbone were accomplished. The synthesized compounds were evaluated against a panel of cancer and normal cell lines, revealing a narrow SAR window concerning both of these structural domains. The beneficial effect of the benzamide group was once again confirmed through C7 deoxygenated analogue **19**. The most active members of the palmerolide analogues also exhibited promising therapeutic indexes when compared between cancerous (UACC-62) and normal (HM-2) cell lines. In conjunction with the preliminary mechanistic and pharmacological findings of palmerolide A (**1**) and benzamide **2**, in vivo biological evaluations of selected candidate(s) will be carried out, and the results of these studies will be reported in due course.

Supporting Information (see footnote on the first page of this article): Experimental section; details of the cytotoxicity assay, APO-BrdU TUNEL assay, pH-lysosensor assay, verapamil-stimulated Pgp-ATPase assay, Vivid®CYP3A4 assay, and Predictor™ hERG fluorescence polarization assay; ^1H and ^{13}C NMR spectra of compounds.

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[15] For methods and results of the mechanistic and pharmacological studies of palmerolide A (**1**) and benzamide analogue **2**, see the Supporting Information.

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