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Multidrug resistance-selective antiproliferative activity of *Piper* amide alkaloids and synthetic analogues

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ABSTRACT

Twenty-five amide alkaloids (**1**–**25**) from *Piper boehmeriifolium* and 10 synthetic amide alkaloid derivatives (**39**–**48**) were evaluated for antiproliferative activity against eight human tumor cell lines, including chemosensitive and multidrug-resistant (MDR) cell lines. The results suggested tumor type-selectivity. 1-[7-(3,4,5-Trimethoxyphenyl)heptanoyl]piperidine (**46**) exhibited the best inhibitory activity (IC_{50} = 4.94 μM) against the P-glycoprotein (P-gp)-overexpressing KBvin MDR sub-line, while it and all other tested compounds, except **9**, were inactive (IC_{50} >40 μM) against MDA-MB-231 and SK-BR-3. Structure–activity relationships (SARs) indicated that (i) 3,4,5-trimethoxy phenyl substitution is critical for selectivity against KBvin, (ii) the 4-methoxy group in this pattern is crucial for antiproliferative activity, (iii) double bonds in the side chain are not needed for activity, and (iv), in arylalkenylacyl amide alkaloids, replacement of an isobutylamino group with pyrrolidin-1-yl or piperidin-1-yl significantly improved activity. Further study on *Piper* amides is warranted, particularly whether side chain length affects the ability to overcome the MDR cancer phenotype.

The genus *Piper* (Piperaceae) contains approximately 2000 plant species distributed mainly in tropical areas.[1] Among them, 10 *Piper* species have been used in traditional medicines to treat cancer or cancer-like symptoms.[2] Amide alkaloids, phenylpropanoids, lignans, neolignans, terpenes, steroids, kawapyrones, piperolides, flavonoids, and alkenylphenols have been isolated from *Piper* plants,[3–7] and amide alkaloids are the major cytotoxic constituents.[2] Especially, piplartine (piperlongumine, Fig. 1) exhibits the most promise showing broad-spectrum cytotoxicity against cancer cell lines in vitro. Furthermore, it also demonstrated excellent anticancer activity in vivo.[8,9] The amide alkaloids in *Piper* plants are usually constructed from acyl and amino moieties. The acyl moiety includes mainly alkylacyl, alkenylacyl, arylalkylacyl, and arylalkenylacyl groups. The amino moiety is generally either isobutylamine, pyrrolidine, piperidine, pyrrole, 5,6-dihydropyridin-2(1*H*)-one, or arylethylamine. In our previous study, several amides

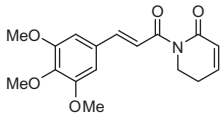

Figure 1. The structure of piplartine (piperlongumine).

(**3**–**7**, and **11**, Fig. 2) with cytotoxic activity against the human cervical carcinoma HeLa cell line were isolated from *Piper boehmeriifolium* Wall. The active amides belong to the arylalkenylacylpyrrolidine classification and 1-[(9*E*)-10-(3,4-methylenedioxyphenyl)-9-decenoyl]pyrrolidine (**7**) exhibited the best inhibitory activity with an IC_{50} of 7.78 μM.[10] However, the structure–activity relationships (SARs) of these amides against cancer cells remain unclear. In the present study, 25 amide alkaloids (**1**–**25**, Fig. 2) from *P. boehmeriifolium* and 10 synthetic amide alkaloid analogues (**39**–**48**) were evaluated for cytotoxicity against lung adenocarcinoma A549, nasopharyngeal carcinoma KB, P-glycoprotein (P-gp)-overexpressing MDR KB (KBvin), androgen insensitive prostate cancer DU145, and four breast cancer cell

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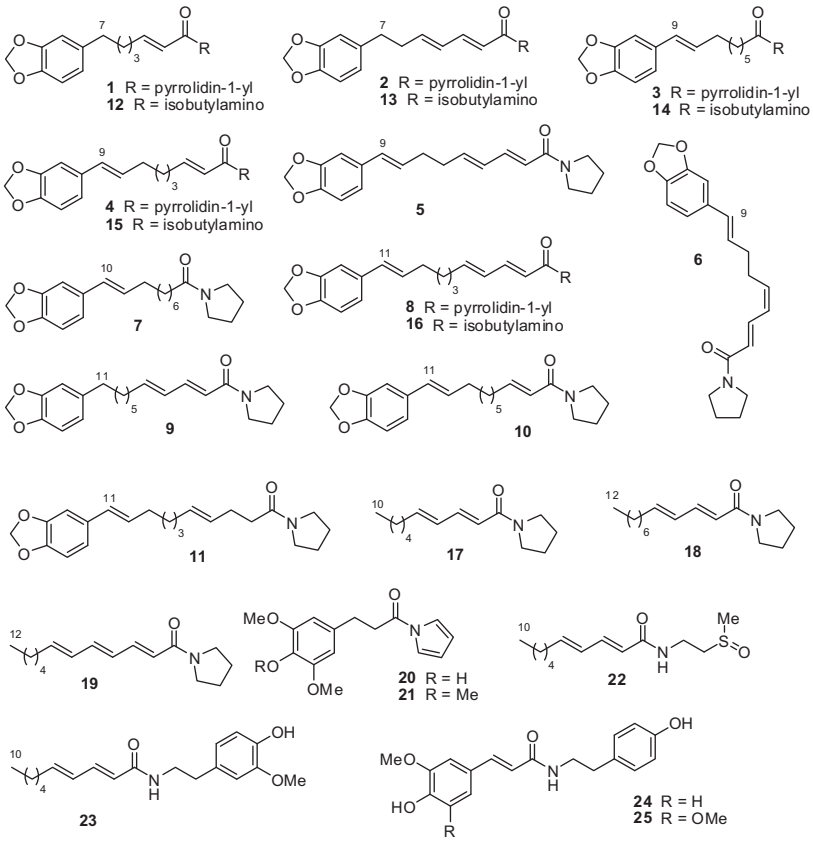


Figure 2. The structures of amide alkaloids (**1**–**25**) from *Piper boehmeriifolium*.

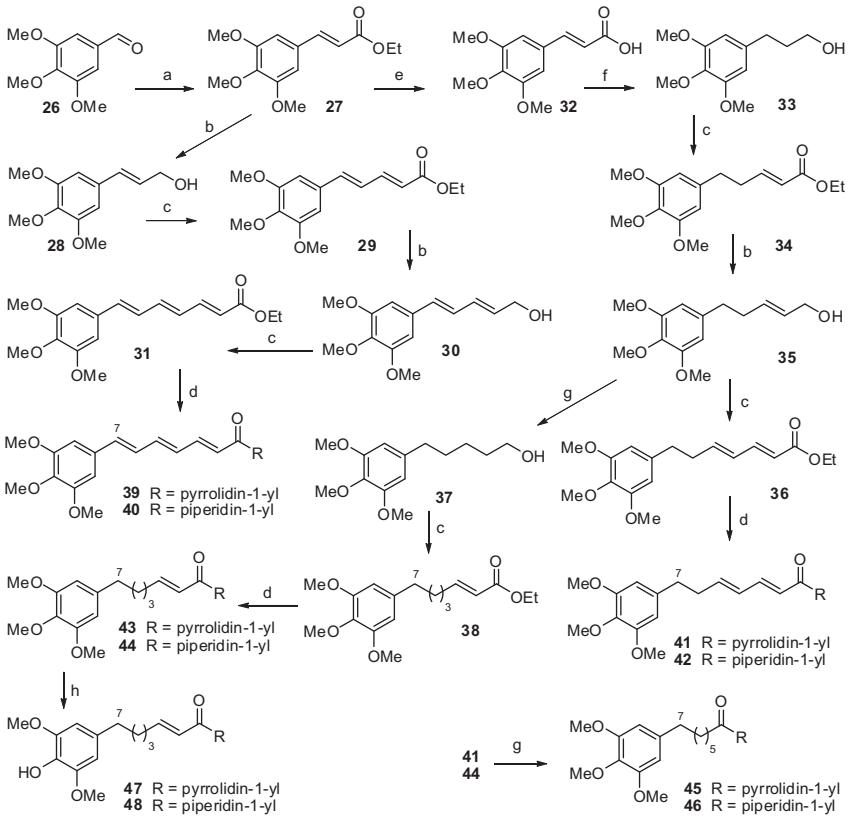
Table 1
Cytotoxicity of natural (**1**–**25**) and synthetic (**39**–**48**) amide alkaloids[a]

Compd	IC_{50} (μM)								SI[b]
	A549	MCF-7	ZR-75-1	SK-BR-3	MDA-MB-231	DU145	KB	KBvin	
1	21.20 ± 1.76	>40	>40	>40	>40	36.15 ± 0.03	31.08 ± 2.03	28.25 ± 4.68	1.1
2	24.65 ± 2.37	>40	>40	>40	>40	>33	>33	31.00 ± 4.85	>1.1
3	19.57 ± 2.40	30.17 ± 1.64	40.99 ± 0.71	>40	>40	24.90 ± 3.98	25.57 ± 2.64	21.95 ± 2.80	1.2
7	25.74 ± 4.96	27.68 ± 1.04	40.93 ± 0.61	>40	>40	23.68 ± 4.78	23.74 ± 1.34	20.70 ± 3.73	1.1
8	18.30 ± 1.30	30.13 ± 0.58	40.60 ± 0.12	>40	>40	20.43 ± 1.10	21.20 ± 3.82	18.93 ± 3.71	1.1
9	14.42 ± 1.18	22.01 ± 0.31	26.71 ± 0.35	30.11 ± 0.86	31.58 ± 0.59	16.02 ± 3.10	16.92 ± 2.45	15.42 ± 3.07	1.1
10	27.55 ± 4.90	30.68 ± 0.75	36.89 ± 2.24	>40	>40	23.18 ± 2.54	27.32 ± 0.79	20.87 ± 3.83	1.3
43	9.48 ± 0.43	41.27 ± 3.24	>40	>40	>40	ND[c]	35.51 ± 0.83	13.03 ± 2.72	2.7
44	23.40 ± 1.47	29.60 ± 1.20	41.01 ± 3.16	>40	>40	ND	29.79 ± 0.51	7.76 ± 0.45	3.8
45	20.39 ± 2.07	26.85 ± 0.27	>40	>40	>40	ND	27.59 ± 1.77	8.86 ± 0.74	3.1
46	16.28 ± 5.33	11.78 ± 2.86	33.10 ± 2.39	>40	>40	ND	13.62 ± 2.48	4.94 ± 0.91	2.8
PXL (nM)[d]	2.40 ± 0.16	>100	>100	>100	7.80 ± 0.02	4.88 ± 0.21	3.70 ± 0.11	1580 ± 40	0.002

[a] Compounds **4**–**6**, **11**–**25**, **39**–**42**, **47** and **48** were inactive against 8 human tumor cell lines used in this study (IC_{50} >40 μM).
[b] SI: selective index = (IC_{50}-KB)/(IC_{50}-KBvin).
[c] ND: not determined.
[d] IC_{50} of paclitaxel (PXL) is represented in nM.

lines, including triple-negative [ER^-/PgR^-/erbB2 ($HER2)^-$] MDA-MB-231, triple-positive ZR-75-1, double-positive expressing multidrug resistance-associated protein (MRP) 1 MCF-7, and HER2-overexpressing SK-BR-3 cell lines. The SARs and selective antiproliferative activity against KBvin are discussed.

Natural amide alkaloids (**1**–**25**) were obtained from the whole plant of *P. boehmeriifolium* in our recent study.[10] From results of their cytotoxic evaluation (Table 1), both an arylalkenylacyl side chain and pyrrolidin-1-yl moiety were necessary for antiproliferative activity. To determine whether double bonds in the arylalkenylacyl side chain affected activity, ten new amide alkaloids (**39**–**48**) were synthesized (Scheme 1). 3,4,5-Trimethoxybenzaldehyde (**26**) was selected as the starting material to synthesize promising amide piplartine analogues. Ethyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**27**) was obtained from **26** by 2C-Wittig homologation with (carboethoxymethylene)triphenylphosphorane in benzene at reflux.[11] The esters **27**, **29**, and **34** were reduced with diisobutylaluminum hydride to afford alcohols **28**, **30**, and **35**, respectively,[12,13] while alcohol **33** was obtained by reduction of acid **32** using lithium aluminum hydride.[14] Acid **32** was produced by hydrolysis of ester **27** with lithium hydroxide in a tetrahydrofuran (THF) and water (2:1) mixture.[15] The alcohols **28**, **30**, **33**, **35**,



Scheme 1. Synthesis of amide alkaloids (**39**–**48**). Reagents and conditions: (a) $Ph_3P{=}CHCO_2Et$, benzene, refluxed, 4 h; (b) DIBAL-H (1.0 M in THF), CH_2Cl_2, −50 °C to rt, 2 h; (c) (i) Py·SO_3, DMSO, Et_3N, CH_2Cl_2, 0 °C to rt, 2 h; (ii) $Ph_3P{=}CHCO_2Et$, benzene, refluxed, 4 h; (d) (i) LiOH·H_2O, THF–H_2O (2:1), rt, 36 h; (ii) $SOCl_2$, CH_2Cl_2, refluxed, 1 h; (iii) pyrrolidine (for **39**, **41**, and **43**) or piperidine (for **40**, **42**, and **44**), CH_2Cl_2, rt, 30 min; (e) LiOH·H_2O, THF–H_2O (2:1), rt, 36 h; (f) $LiAlH_4$, THF, 0 °C to rt, overnight; (g) 10% Pd/C, EtOAc, H_2, 50 psi, 24 h; (h) $AlCl_3$, CH_2Cl_2, rt, 4 h.

and **37** were oxidized using the Parikh–Doering reaction with sulfur trioxide pyridine complex, dimethyl sulphoxide, and triethylamine and then subjected to Wittig reaction to yield esters **29**, **31**, **34**, **36**, and **38**, respectively.[16] Esters **31**, **36**, and **38** were hydrolyzed with lithium hydroxide in THF/water (2:1) to yield the corresponding acids. Reaction of these acids with pyrrolidine and thionyl chloride gave amides **39**, **41**, and **43**, and with piperidine gave amides **40**, **42**, and **44**, respectively.[17] Hydrogenation of **35**, **41**, and **44** with hydrogen gas at 50 psi yielded **37**, **45** and **46**, respectively,[18] and selective de-4′-*O*-methylation of **43** and **44** using aluminum trichloride gave **47** and **48**, respectively.[9]

The cytotoxic activity was determined by the sulforhodamine B (SRB) colorimetric assay as previously described.[19] As shown in Table 1, the natural (**1**–**3**, and **7**–**10**) and synthetic (**43**–**46**) alkaloids showed cytotoxic activity against at least one of the eight human tumor cell lines (Table 1). Within the natural products (**1**–**25**), compounds **3** and **7**–**10** exhibited weak and broad spectrum activity against all tumor cell lines tested except MDA-MB-231 and SK-BR3. Compounds **1** and **2** were inactive (IC_{50} >40 μM) against four types of breast cancer cell lines. Interestingly, none of the compounds except **9** were active against MDA-MB-231 and SK-BR-3, suggesting tumor type-selective antiproliferative activity. Compared with the natural products, the synthetic amides **43**–**46** showed better cytotoxic activity against KBvin cells. 1-[7-(3,4,5-Trimethoxyphenyl)heptanoyl]piperidine (**46**) was the most active analog against KBvin cells (IC_{50} = 4.94 μM). P-glycoprotein (P-gp)-overexpressing KBvin was sensitive to the synthetic analogs (**43**–**46**), while chemosensitive cell lines, including parental cell line KB, were tolerant. Compound **44** showed the best selective index (3.8-fold selective) against MDR sub-line KBvin compared with parental KB. It is obvious that the 3,4,5-trimethoxy groups in the phenyl ring of the synthetic compounds increase the selectivity. Multidrug resistance-associated protein 1 (MRP1)-expressing MCF-7 was also tolerant to amides **43**–**46**, suggesting that these analogs may be selectively cytotoxic to MDR cells overexpressing P-gp.

Double bonds in the side chain might decrease the cytotoxic activity, because compounds **39**–**42** were inactive against the tested cell lines. Compounds **47** and **48** were inactive, implying that a 4-methoxy group, rather than 4-hydroxy group, in the 3,4,5-trisubstituted phenyl ring is necessary for activity. Compared with amides **1** and **2**, amides **12** and **13** were inactive indicating that pyrrolidin-1-yl derivatives are more potent than isobutylamino derivatives, while both pyrrolidin-1-yl (**43** and **45**) and piperidin-1-yl (**44** and **46**) groups are acceptable for activity.

In conclusion, arylalkenylacylpyrrolidines (**1**–**3**, **7**–**10**, **43**), an arylalkylacylpyrrolidine (**45**), an arylalkenylacylpiperidine (**44**), and an arylalkylacylpiperidine (**46**) showed inhibitory activity against at least one of eight tested human tumor cell lines. Double bonds in the side chain were not needed for activity. 3,4,5-Trimethoxy substitution in the phenyl ring of the amide alkaloids increased selectivity against MDR sub-line KBvin, while the 4-methoxy group in this pattern is required for cytotoxic activity. These results demonstrate that newly synthesized amide alkaloids show tumor type-selective antiproliferative activity, especially against MDR cells. These synthesized analogues may target a unique protein, that is, highly responsible for the proliferation of KBvin, while dispensable in chemosensitive cell lines, such as SK-BR-3 and MDA-MD-231. Compounds, such as verapamil and desmosdumotin B analogues,[19] that show at least a two-fold

selective cytotoxicity against MDR cells are called collateral sensitivity (CS) agents.[20] Although their mechanisms of action are largely unclear, CS agents are expected to be a new class of chemopreventive adjuvant for cancer chemotherapy to suppress development of MDR phenotype.[21] Thus, it is worthwhile to conduct further SAR study of *Piper* amides, including the effect of the side chain length on the activity of these amide alkaloids against cancer cells showing the MDR phenotype.

Acknowledgments

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Supplementary data

Supplementary data (experimental details and compound characterization for all synthesized compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.08.063.

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