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Multidrug resistance reversal activity of permethyl ningalin B amide derivatives

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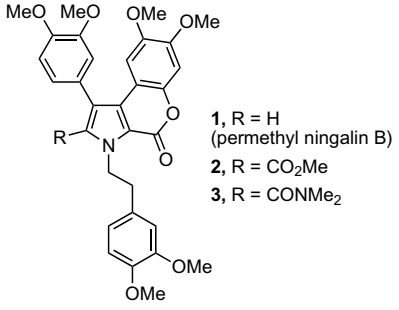
Abstract—A series of amide derivatives of permethyl ningalin B were prepared and examined as multidrug resistance (MDR) reversal agents illustrating that the C5 carboxylate is widely tolerant of such derivatization.

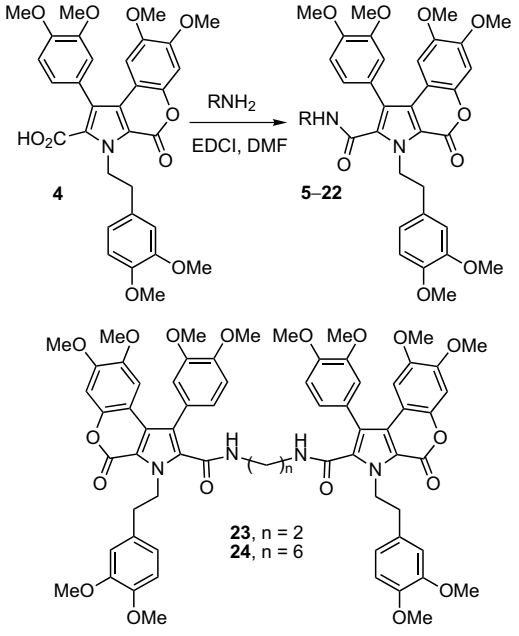
Multidrug resistance (MDR) is a serious problem limiting the effectiveness of a wide variety of anticancer drugs.[1–3] The human *MDR1* gene expression product, P-glycoprotein (P-gp), is a 170 kDa ATP-dependent plasma membrane glycoprotein, which confers resistance to cells by active extrusion of hydrophobic and amphiphatic drugs to below therapeutically useful concentrations. Expression of the *MDR1* gene has been found in cancers showing both intrinsic and acquired multidrug resistance. Most notably, increased expression of P-gp is commonly found in tumor relapses during or after chemotherapy treatment. Tumor cells may also become multidrug resistant by the overexpression of other ABC transporters, such as MDR-associated protein (MRP) and lung resistant protein (LRP).[1]

Table 1. Ningalin compounds



Compd (at 1 μM)	Vinblastine IC_{50} (μM)	% Reversion
None	0.07	—
1	0.002	100
2	0.002	100
3	0.002	100
3 (7.5 μM)	0.0002	1000
Verapamil	0.02	10

HCT116/VM46. For wild type HCT116: IC_{50} = 0.002 μM (vinblastine), % reversion = HCT116 $IC_{50}(-)$/HCT116/VM46 $IC_{50}(+) \times 100$, (−) = without added drug, (+) = with added drug.



Scheme 1.

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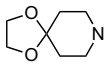
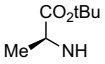
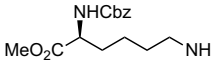
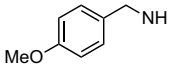
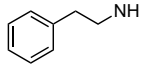
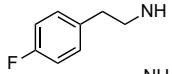
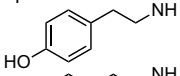
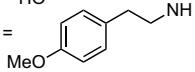
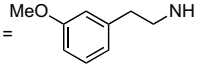
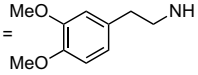
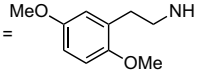
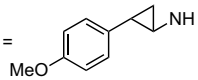
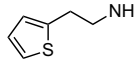
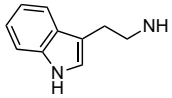
Although a number of agents have been developed that modify MDR, none are currently used in the clinic due, in part, to a lack of potency or observance of P-gp independent side effects at concentrations necessary for efficacy. To date, there is no single definition of the elements necessary for P-gp recognition of resistance modifiers.[2] Since the first definition of a MDR pharmacophore by Pearce,[4] numerous models reflecting multi-

Table 2.

Compd (at 1 μM)	Intrinsic cytotoxic activity		MDR reversal activity (HCT116/VM46)[a]			
	Compd, wild type HCT116 IC_{50} (μM)	Compd, MDR HCT116/VM46 IC_{50} (μM)	Vinblastine IC_{50} (μM)	% Reversion	Doxorubicin IC_{50} (μM)	% Reversion
None	—	—	0.07	—	1.0	—
1	30	40	0.002	100	—	—
2	6	40	0.002	100	—	—
3	25	20	0.002	100	0.1	100
3 (7.5 μM)	—	—	0.0002	1000	0.01	1000
Verapamil	—	—	0.02	10	0.5	20
5, R = H_2N	>100	>100	0.007	60	0.24	40
6, R = $(MeOCH_2CH_2)_2N$	25	15	0.009	45	0.15	50
7, R = 	15	10	0.006	67	0.10	100
8, R = 	7	4	0.008	50	0.18	55
9, R = 	45	40	0.005	80	0.10	100
10, R = 	40	30	0.004	100	0.12	80
11, R = Ph_2CHN	25	15	0.004	100	0.12	80
12, R = 	50	40	0.004	100	0.15	67
13, R = 	30	20	0.004	100	0.10	100
14, R = 	15	10	0.015	25	0.18	55
15, R = 	20	10	0.002	200	0.06	167
16, R = 	20	5	0.002	200	0.10	100
17, R = 	5	5	0.004	100	0.10	100
18, R = 	25	10	0.002	200	0.10	100
19, R = 	>100	>100	0.004	100	0.10	100
20, R = 	>100	>100	0.004	100	0.10	100
21, R = 	>100	>100	0.004	100	0.10	100
22, R = $AcS(CH_2)_6NH$	20	35	0.015	25	0.20	50
23	>100	>100	0.050	10	0.75	10
24	>100	>100	0.030	15	0.45	20

[a] For **1**–**3** and verapamil: wild type HCT116: IC_{50} = 0.002 μM (vinblastine); % reversion = HCT116 IC_{50}(−)/HCT116/VM46 IC_{50}(+) × 100, (−) = w/o and (+) = with added drug; for **5**–**14**: wild type HCT116: IC_{50} = 0.004 μM (vinblastine), 0.1 μM (doxorubicin); % reversion = HCT116 IC_{50} (−)/HCT116/VM46 IC_{50} (+) × 100, (−) = w/o and (+) = with added drug at 1 μM.

ple or overlapping sites and substrates have been disclosed.[5] The hydrophobicity of the modifier is an important, but not the sole, requirement for recognition and inhibition of P-gp. Some common features found in resistance modifiers include a planar hydrophobic aromatic domain and a basic nitrogen atom present within an extended side chain off the aromatic ring system.[2,4,5]

In the course of recent synthetic efforts on the ningalin family[6] of marine natural products, we identified several compounds that showed remarkable multidrug resistance modifier activity[7,8] (Table 1). Notably absent from the structural characteristics of **1**–**3** is this putative basic nitrogen making them an especially interesting, as well as potent, new class of MDR modifiers. The resistant human colon carcinoma cell line HCT116/VM46 derives its resistance from the overexpression of P-gp. Although these compounds exhibited little or no cytotoxic activity, compounds **1**–**3** caused a pronounced resensitization of the multidrug resistant HCT116/VM46 cell line towards vinblastine and doxorubicin. At 1 μM concentrations, all three compounds effected a 100% reversion of sensitivity of the MDR cell line, compared to 10% reversion effected by the prototypical MDR modifier verapamil (at 1 μM). Moreover, we demonstrated that these and related structures inhibit the action of P-gp at concentrations equivalent to the MDR reversal activity.[7,8] Interestingly, the corresponding naturally occurring ningalins, which bear free phenols versus the methyl ethers of **1**–**3**, are more cytotoxic and lack potent MDR modifier properties,[7–9] and the derivatives **1**–**3** are among the most potent leads for MDR reversal disclosed to date.[1–3] In a recent study to define the key features of the permethyl ningalins that contribute to their MDR reversal activity, we observed an enhanced potency and hypersensitization (at 7.5 μM) with the dimethyl amide derivative **3**.[10] The activity of this derivative, prepared to address a potential in vivo lability of the corresponding methyl ester **2**, and the comparable activity of permethyl ningalin B (**1**) suggested that considerable modification of C5 carboxylate may be possible and that it may serve as a site to further enhance activity, selectivity, or for attachment of an affinity label. Herein we reported a series of C5 carboxylate amide derivatives of **2** and **3** that explore this opportunity.

The amide derivatives **5**–**24** were prepared by coupling the carboxylic acid **4**, which is inactive as an MDR reversal compound, with a series of amines (EDCI, DMAP) (Scheme 1). Included in this group are two dimers **23** and **24** prepared by coupling **4** with ethylenediamine and 1,6-diaminohexane.

The MDR reversal assessments reported herein were run simultaneously in triplicate alongside internal standards.[10] Like **1**–**3** and their derivatives,[7,8,10] each of the amide derivatives exhibited MDR reversal properties, Table 2. The exceptions to this generalization were the two dimer derivatives **23** and **24**, which proved essentially inactive at 1 μM. Although the series is not extensive, a few interesting trends emerge from the study. First, a wide range of amide derivatives may be incorporated without seriously impacting or abrogating the MDR reversal activity. The primary carboxamide derivative **5** was not quite as effective as **1**–**3**, but it also no longer exhibits the modest cytotoxic activity of **1**–**3**. Derivatization of **4** with amines that lack an aromatic ring (**6**–**8**, **22**) typically exhibited less MDR reversal activity than **1**–**3** or those that incorporate an aryl ring (**10**–**21**). Substitution of the aryl ring with methoxy substituents analogous to the peripheral aryl rings found in the ningalin B core enhance the activity (**15**–**19** vs **12**) although incorporation of a hydrophilic substituent (**14**, phenol) diminishes the activity. This is consistent with the comparisons with the parent ningalins themselves and expectations of P-gp binding. Significantly, this methoxy substitution leads to a slight hypersensitization of the HCT116/VM46 cell line to treatment with vinblastine or doxorubicin (e.g., **15**) even with the treated concentration of 1 μM (compare to **3**). Although the origin of this hypersensitization is not known and is unique to ningalin P-gp inhibitors, the synergistic effect is observed with **15**, **16**, and **18** at concentrations $\leqslant$5–10-fold lower than their modest cytotoxic activity. Most interesting and significant, the derivatives **19**–**21** exhibited the MDR reversal potency of **1**–**3**, but lack the intrinsic, modest cytotoxic activity ($IC_{50} > 100\,\mu M$) providing a more than 100-fold therapeutic window.

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