Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1777–1781

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Multidrug Resistance Reversal Activity of Key Ningalin Analogues

Danielle R. Soenen, Inkyu Hwang, Michael P. Hedrick and Dale L. Boger*

The Scripps Research Institute and the Skaggs Institute for Chemical Biology, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

Received 17 December 2002; accepted 21 February 2003

Abstract—Key analogue derivatives of the ningalins, potent multidrug resistance (MDR) reversal compounds, were examined resulting in the discovery of a potent MDR reversal agent that hypersensitizes P-gp resistant tumor cell lines to front-line conventional therapeutic agents.

Multidrug resistance (MDR) is a persistent problem limiting the effectiveness of a wide variety of anticancer drugs, antibiotics, and protease inhibitors.[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 relevant concentrations. Expression of the *MDR1* gene has been found in cancers showing both intrinsic and acquired multidrug resistance.[3] P-gp is expressed in normal kidney, liver, colon, pancreas, and adrenal tissues and therefore is found in tumors derived from these tissues. Other untreated cancers stemming from tissues that do not express *MDR1* RNA at easily detected levels, such as certain leukemias and non-Hodgkin's lymphoma, frequently exhibit high levels of P-gp expression. Increased expression of P-gp is also 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]

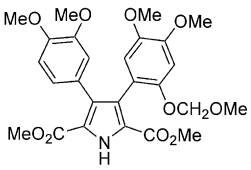
Although a number of agents have been developed that modify MDR, none are currently used in the clinic due in part to a lack of potency or observance of P-gp independent side effects at concentrations necessary for efficacy. To date, there is no single complete definition of the elements necessary for P-gp recognition of resistance modifiers.[2] Since the first definition of a MDR pharmacophore by Pearce[4] composed of two planar aromatic domains and the disposition of a basic nitrogen within an extended aliphatic chain, numerous models reflecting multiple 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 functionalities 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] (Table 1). Notably absent from the structural characteristics of **2**–**5** 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,[7] compounds **2**–**4** caused a pronounced resensitization of the multidrug resistant HCT116/VM46 cell line towards vinblastine and doxorubicin. At 1 μM concentrations, compound **3** and permethyl ningalin B (**4**) 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). Similar resensitization of HCT116/VM46 towards vinblastine and doxorubicin had been observed in our earlier

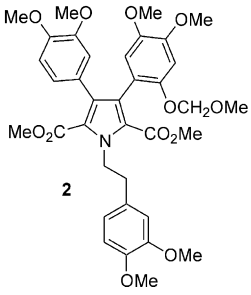
*Corresponding author. Tel.: +1-858-784-7522; fax: +1-858-784-7550; e-mail: boger@scripps.edu

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00294-4

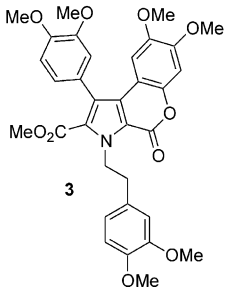
Table 1.



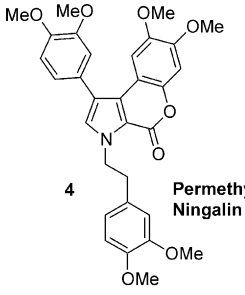
1



2



3



4 **Permethyl Ningalin B**

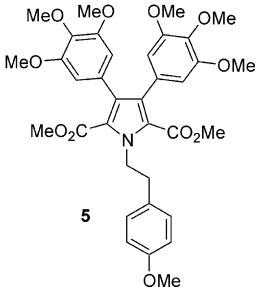
Compd at 1.0 μM	Vinblastine IC_{50}, (μM)[a]	% reversion[b] (gain in sensitivity)[c]	Doxorubicin IC_{50}, (μM)[a]	% reversion[b] (gain in sensitivity)[c]
1	0.02	10 (4)	0.1	0
2	0.004	50 (18)	0.05	20 (1)
3	0.002	100 (35)	0.02	50 (4)
4	0.002	100 (35)	0.02	50 (4)
Verapamil	0.02	10 (4)	0.13	

[a]IC_{50} (μM) of vinblastine or doxorubicin against the MDR resistant cell line HCT116/VM46 in the presence of 1 μM of the indicated compound. IC_{50} values in the absence of added compound are 0.07 μM (vinblastine) and 0.07 μM (doxorubicin). For the wild-type HCT116 cell line not subject to MDR, IC_{50} values are 0.002 μM (vinblastine) and 0.01 μM (doxorubicin).
[b]Percent reversion is measured as wild type $IC_{50}(-)$/MDR $IC_{50}(+) \times 100$.
[c]Gain in sensitivity is measured as MDR $IC_{50}(-)$/MDR $IC_{50}(+)$ [(−) = without added drug, (+) = with added drug].[16]

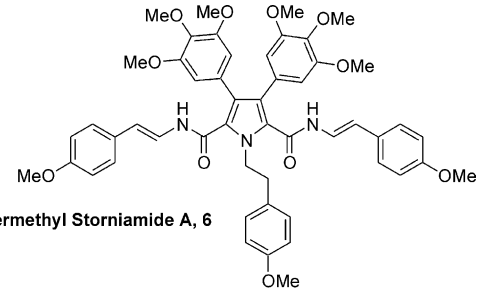
work[8] upon treatment of the resistant cell line with non-cytotoxic concentrations of permethyl storniamide A[10] (**6**) and its synthetic precursor **5** (Table 2). Moreover, we demonstrated that **5**[8] and related structures[7] inhibit the action of P-gp at concentrations equivalent to its MDR reversal activity. Interestingly, the corresponding ningalins, which bear free phenols versus the methyl ethers of **2–6** are more cytotoxic and lack potent MDR modifier properties,[7–9] and the derivatives **2–6** are among the most potent leads for MDR reversal disclosed to date.[1–3] Herein, we report a small series of related analogues prepared to establish some of the fundamental structural features of **2–6** responsible for their potent MDR reversal properties.

It is clear upon comparison of the MDR modifier activity of **1** with the related compounds **2–4** that the presence of the *N*-alkylaryl substituent is necessary for activity. Thus, we examined the effect of alkyl tether length on the activity with a series of ningalin-type analogues in which the permethyl storniamide A pyrrole core **7** was functionalized with the ningalin 3,4-dimethoxyphenyl group through a systematically varied alkyl linker (Fig. 1). A copper-mediated boronic acid coupling[11] provided the *N*-aryl homologue **8** (21%, 4 days, 65 °C, DMF). Homologues **9**, **11**, and **12** bearing one, three, and four carbon linkers, respectively, were formed upon alkylation of **7** with the requisite alcohols under Mitsunobu conditions.[12] Alkylation (89%,

Table 2.



5



Permethyl Storniamide A, 6

Compd at 1.0 μM	Vinblastine IC_{50}, (M)[a]	% reversion[b] (gain in sensitivity)[c]	Doxorubicin IC_{50}, (M)[a]	% reversion[b] (gain in sensitivity)[c]
5	0.009	33 (22)	0.6	33 (4)
6	0.003	100 (67)	0.1	200 (22)
Verapamil	0.02	15 (10)		

[a]IC_{50} (μM) of vinblastine or doxorubicin against the MDR resistant cell line HCT116/VM46 in the presence of 1 μM of the indicated compound. IC_{50} values in the absence of added compound are 0.2 μM (vinblastine) and 2.2 μM (doxorubicin). For the wild-type HCT116 cell line not subject to MDR, IC_{50} values are 0.003 μM (vinblastine) and 0.2 μM (doxorubicin).
[b]Percent reversion is measured as wild type $IC_{50}(-)$/MDR $IC_{50}(+) \times 100$.
[c]Gain in sensitivity is measured as MDR $IC_{50}(-)$/MDR $IC_{50}(+)$ [(−) = without added drug, (+) = with added drug].

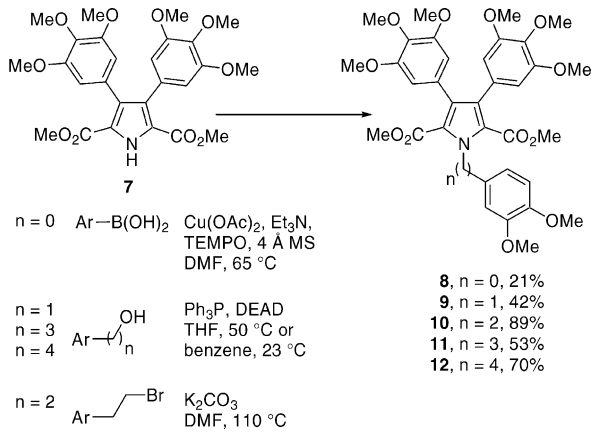


Figure 1.

K_2CO_3, DMF, 110 °C) of pyrrole **7** with the requisite phenethyl bromide derivative[7] provided homologue **10** bearing a two-carbon tether.

The MDR reversal assessments reported herein were conducted in four groupings (Tables 1–4) at various intervals over a 4-year period. The results recorded in each table were run simultaneously in triplicate and the internal comparisons reflect accurate relative and quantitative distinctions. The comparisons from table to table are best assessed by comparisons with the internal controls (e.g., verapamil or **4**). The HCT116 and HCT116/VM46 sensitivity to vinblastine remained relatively invariant throughout these studies (IC_{50} = 0.001–0.004 and 0.07–0.2 μM, respectively) whereas the doxorubicin sensitivity was much more variable (IC_{50} = 0.01–0.2 and 0.07–6.7 μM, respectively). The consequences are that the studies with vinblastine exhibit greater correlations from table to table, that the gain in sensitivity can exhibit large variations from table to table while still reflecting accurate trends within a table, and that the % reversion represents the best means to make a table to table comparison (vs IC_{50} or gain in sensitivity).

Like **1**–**6**, compounds **8**–**12** showed little cytotoxic activity against either the wild type (HCT116) or MDR (HCT116/VM46) cell line (Table 5). When tested in combination with vinblastine or doxorubicin against HCT116/VM46 at the modest concentration of 1 μM, the series exhibited a well-defined trend in MDR reversal activity: **12** > **11** > **10** ≈ **9** > **8** (Table 3). Whereas the *N*-aryl homologue **8** showed only minor activity as a resistance modifier, **9** and **10** achieved 45 and 30% reversion of the resistant cell line to doxorubicin, similar to the activity effected by verapamil. Analogues **11** and **12** (1 μM) achieved 44 and 67% reversion, respectively, of the MDR cell line towards vinblastine. Derivative **12**, bearing a four-carbon alkyl chain, displayed modifier activity identical to that of our lead compound **4**. Both **12** and **4** caused 100% reversion of the resistant cell line to doxorubicin. From the results in Table 3, it is clear

Table 3. MDR reversal

Compd at 1.0 μM	Vinblastine IC_{50}, (μM)[a]	% reversion[b] (gain in sensitivity)[c]	Doxorubicin IC_{50}, (μM)[a]	% reversion[b] (gain in sensitivity)[c]
8	0.05	8 (2)	0.4	23 (2)
9	0.03	13 (3)	0.2	45 (4)
10	0.04	10 (3)	0.3	30 (2)
11	0.009	44 (11)	0.2	45 (4)
12	0.006	67 (17)	0.09	100 (8)
4	0.006	67 (17)	0.09	100 (8)
Verapamil	0.04	10 (3)	0.3	30 (2)

[a]IC_{50} (μM) of vinblastine or doxorubicin against the MDR resistant cell line HCT116/VM46 in the presence of 1 μM of the indicated compound. IC_{50} values in the absence of added compound are 0.1 μM (vinblastine) and 0.7 μM (doxorubicin). For the wild-type HCT116 cell line not subject to MDR, IC_{50} values are 0.004 μM (vinblastine) and 0.09 μM (doxorubicin).
[b]Percent reversion is measured as wild type $IC_{50}(-)$/MDR $IC_{50}(+) \times 100$.
[c]Gain in sensitivity is measured as MDR $IC_{50}(-)$/MDR $IC_{50}(+)$ [(−) = without added drug, (+) = with added drug].

Table 4. MDR reversal

Compd at 1.0 μM	Vinblastine IC_{50}, (μM)[a]	% reversion[b] (gain in sensitivity)[c]	Doxorubicin IC_{50}, (μM)[a]	% reversion[b] (gain in sensitivity)[c]
14	0.13	1 (1)	5.6	2 (1)
14 (7.5 μM)	0.02	5 (4)	0.6	17 (11)
16	0.03	3 (3)	0.8	13 (8)
16 (7.5 μM)	0.02	5 (4)	0.7	14 (10)
18	0.001	100 (80)	0.1	100 (67)
18 (7.5 μM)	0.0001	1000 (800)	0.009	1100 (744)
Verapamil	0.004	25 (20)	0.4	25 (17)

[a]IC_{50} (μM) of vinblastine or doxorubicin against the MDR resistant cell line HCT116/VM46 in the presence of 1 μM of the indicated compound. IC_{50} values in the absence of added compound are 0.08 μM (vinblastine) and 6.7 μM (doxorubicin). For the wild-type HCT116 cell line not subject to MDR, IC_{50} values are 0.001 μM (vinblastine) and 0.1 μM (doxorubicin).
[b]Percent reversion is measured as wild type $IC_{50}(-)$/MDR $IC_{50}(+) \times 100$.
[c]Gain in sensitivity is measured as MDR $IC_{50}(-)$/MDR $IC_{50}(+)$ [(−) = without added drug, (+) = with added drug].

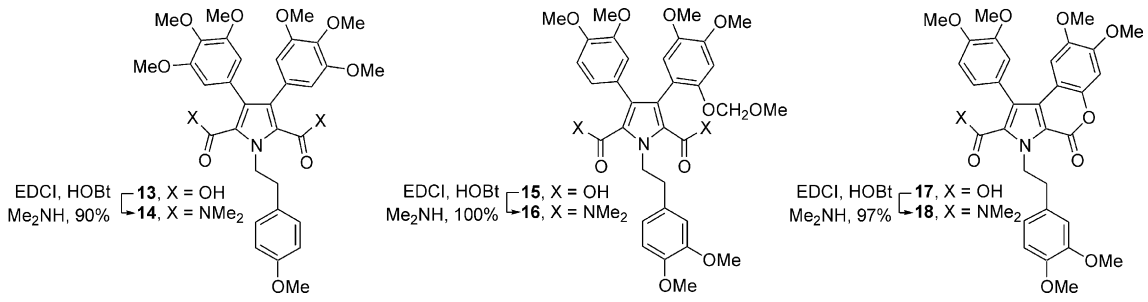


Figure 2.

Table 5. Cytotoxic activity

Compd	IC_{50} (μM)	
	HCT116 (wild type)	HCT116/VM46 (MDR)
1	40	60
2	90	>100
3	6	40
4	30	40
5	>100	>100
6	>100	>100
8	25	25
9	100	75
10	70	45
11	75	85
12	100	40
14	>100	>100
16	45	60
18	25	20

that shortening of the alkyl tether between the pyrrole core and the aromatic ring is detrimental to the MDR modifier activity and, in the **8**–**12** series, a progressive increase in activity is observed as the chain is extended.

In our original work,[7] we established that the hydrolysis product of methyl ester **3** showed no MDR modifier activity in vitro. Since methyl esters are readily hydrolyzed in vivo, we were interested in examining the more stable amide derivatives of our leads for biological activity. Dimethylamides **14**, **16**, and **18** (Fig. 2) were prepared by condensation of the requisite carboxylic acids[13] with dimethylamine (EDCI, HOBt, 90–100%). Like **1**–**6**, amides **14**, **16**, and **18** did not exhibit significant cytotoxic activity (Table 5). Each dimethylamide analogue was tested for MDR modifier activity, and showed remarkable differences in activity. Neither bis-dimethylamide **14** or **16** showed any activity as a resistance modifier, whereas dimethylamide **18** (1 μM) caused 100% reversion of the MDR cell line toward both vinblastine and doxorubicin. In addition, when treated with higher concentrations of **18** (7.5 μM), the MDR cell line became approximately 10 times more sensitive to vinblastine and doxorubicin than the wild type cell line. The origin of this hypersensitization with **18** (1000% at 7.5 μM) has not yet been established, but naively might be suggested to result from a synergy resulting from its modest cytotoxic activity (IC_{50} = 20 μM) and P-gp inhibition. However, this hypersensitization, albeit not as pronounced, is also observed with both **5** and **6** (400% reversion at 7.5 μM),[8] which lack intrinsic cytotoxic activity as well as **3** and **4** (300% reversion at 7.5 μM),[7] suggesting additional mechanisms are likely responsible for the effects. Importantly, this remarkable hypersensitivity toward conventional therapeutics induced by **18** not only provides the opportunity for their use in the treatment of MDR tumors, but preliminary in vivo studies also suggest lower therapeutic doses of the conventional therapeutics might be capable of use against sensitive tumors, thereby avoiding their dose-related toxicities.[14] Consequently, **18** is presently undergoing detailed in vivo evaluations in animal models with both MDR and wild type HCT116 and results of these studies will be disclosed in due time.[14,15]

Acknowledgements

We gratefully acknowledge the financial support of the National Institutes of Health (CA42056), The Skaggs Institute of Chemical Biology, and Bristol-Myers Squibb and ARCS for predoctoral fellowships (D.R.S.). D.R.S. is a Skaggs Fellow.

References and Notes

1. Wiese, M.; Pajeva, I. K. *Curr. Med. Chem.* **2001**, *8*, 685.
2. Ambudkar, S. V.; Dey, S.; Hrycyna, C. A.; Ramachandra, M.; Pastan, I.; Gottesman, M. M. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 361.
3. Gottesman, M. M.; Pastan, I. *Annu. Rev. Biochem.* **1993**, *62*, 385.
4. Pearce, H. L.; Safa, A. R.; Bach, N. J.; Winter, M. A.; Cirtain, M. C.; Beck, W. T. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5128. Pearce, H. L.; Winter, M. A.; Beck, W. T. *Adv. Enzyme Regul.* **1990**, *30*, 357.
5. For recent disclosures, see: Ekins, S.; Kim, R. B.; Leake, B. F.; Dantzig, A. H.; Schuetz, E. G.; Lan, L.-B.; Yasuda, K.; Shepard, R. L.; Winter, M. A.; Schuetz, J. D.; Wikel, J. H.; Wrighton, S. A. *Mol. Pharmacol.* **2002**, *61*, 964 and 974. Pajeva, I. K.; Wiese, M. *J. Med. Chem.* **2002**, *45*, 5671, and references cited therein.
6. Kang, H.; Fenical, W. *J. Org. Chem.* **1997**, *62*, 3254.
7. Boger, D. L.; Soenen, D. R.; Boyce, C. W.; Hedrick, M. P.; Jin, Q. *J. Org. Chem.* **2000**, *65*, 2479.
8. Boger, D. L.; Boyce, C. W.; Labroli, M. A.; Sehon, C. A.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 54.
9. Fürstner, A.; Krause, H.; Thiel, O. R. *Tetrahedron* **2002**, *58*, 6373.
10. Palermo, J. A.; Brasco, M. F. R.; Seldes, A. M. *Tetrahedron* **1996**, *52*, 2727.
11. Lam, P. Y. S.; Vincent, G.; Clark, C. G.; Deudon, S.; Jadhav, P. K. *Tetrahedron Lett.* **2001**, *42*, 3415.

12. Monnet, M.-O.; Prévost, P.; Dupas, G.; Bourguignon, J.; Quéguiner, G. *Tetrahedron* **1993**, *49*, 5831. Kreipl, A. T.; Reid, C.; Steglich, W. *Org. Lett.* **2002**, *4*, 3287.
13. Carboxylic acids **13** and **17** were prepared as described in refs 8 and 7 respectively. Carboxylic acid **15** was prepared by KOH (10 equiv) hydrolysis of **2** in dioxane/MeOH/H_2O (4/2/1) at 70 °C (2 days, 56%).
14. Chou, T.-C.; Guan, Y.; Soenen, D. R.; Danishefsky, S. J.; Boger, D. L. *Proc. Natl. Acad. Sci. U.S.A.* Submitted for publication.
15. Characterization of all new compounds: for **8**, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.97–6.90 (m, 3H), 6.39 (s, 4H), 3.95 (s, 3H), 3.90 (s, 3H), 3.83 (s, 6H), 3.66 (s, 12H), 3.51 (s, 6H); IR (film) ν_{max} 2833, 1716, 1234, 1125 cm^{-1}; MALDIFTMS (DHB) *m/z* 674.2207 ($M+Na^+$, $C_{34}H_{37}NO_{12}$ requires 674.2208). For **9**, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.80 (m, 2H), 6.69 (dd, $J=1.8$, 8.1 Hz, 1H), 6.26 (s, 4H), 5.91 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.81 (s, 6H), 3.64 (s, 12H), 3.62 (s, 6H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 163.0, 153.6, 150.3, 149.9, 138.3, 132.1, 131.5, 130.6, 125.2, 120.2, 112.8, 112.2, 109.3, 60.6, 56.5, 56.1, 52.1, 49.5; IR (film) ν_{max} 2823, 1716, 1239, 1125 cm^{-1}; MALDIFTMS (DHB) *m/z* 665.2470 ($C_{35}H_{39}NO_{12}^+$ requires 665.2467). For **10**, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.83 (br s, 2H), 6.75 (s, 1H), 6.23 (s, 4H), 4.87 (t, $J=6.8$ Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.82 (s, 6H), 3.64 (s, 18H), 3.10 (t, $J=7.6$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.2, 152.5, 149.1, 148.0, 137.0, 131.1, 130.5, 130.0, 124.1, 121.3, 112.5, 111.4, 108.0, 61.1, 56.2, 56.1, 56.0, 51.8, 48.9, 38.1; IR (film) ν_{max} 2820, 1713, 1231, 1123 cm^{-1}; MALDIFTMS (DHB) *m/z* 702.2540 ($C_{36}H_{41}NO_{12}+Na^+$ requires 702.2521). For **11**, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.80 (m, 3H), 6.25 (s, 4H), 4.66 (t, $J=7.6$ Hz, 2H), 3.91 (s, 3H), 3.87 (s, 3H), 3.82 (s, 6H), 3.64 (s, 18H), 2.71 (t, $J=7.6$ Hz, 2H), 2.21 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.3, 152.5, 149.0, 147.5, 137.0, 134.0, 130.4, 130.0, 124.0, 120.4, 111.8, 111.3, 108.0, 61.1, 56.2, 56.13, 56.06, 51.9, 47.3, 33.5, 32.8; IR (film) ν_{max} 2833, 1714, 1463 cm^{-1}; MALDIFTMS (DHB) *m/z* 716.2683 ($C_{37}H_{43}NO_{12}+Na^+$ requires 716.2677). For **12**, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.79 (d, $J=8.2$ Hz, 1H), 6.74 (m, 2H), 6.24 (s, 4H), 4.66 (t, $J=7.6$ Hz, 2H), 3.89 (s, 3H), 3.85 (3H), 3.81 (s, 6H), 3.643 (s, 6H), 3.635 (s, 12H), 2.64 (t, $J=7.6$ Hz, 2H), 1.90 (m, 2H), 1.72 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.3, 152.5, 149.0, 147.4, 137.1, 135.0, 130.3, 130.0, 124.0, 120.4, 111.9, 111.4, 108.1, 61.1, 56.3, 56.1, 56.0, 51.8, 47.3, 35.2, 31.9, 28.7; IR (film) ν_{max} 2827, 1717, 1461, 1445, 1435, 1409 cm^{-1}; MALDIFTMS (DHB) *m/z* 707.2939 ($C_{38}H_{45}NO_{12}^+$ requires 707.2936). For **14**, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.21 (d, $J=8.5$ Hz, 2H), 6.84 (d, $J=8.5$ Hz, 2H), 6.30 (s, 4H), 4.57 (m, 1H), 3.93 (m, 1H), 3.82 (s, 6H), 3.78 (s, 3H), 3.62 (s, 12H), 3.25 (m, 1H), 2.97 (s, 6H), 2.83 (m, 1H), 2.49 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.5, 158.5, 153.0, 136.8, 130.4, 130.3, 129.6, 126.0, 121.9, 114.1, 106.6, 61.2, 56.1, 55.5, 48.1, 38.3, 37.7, 35.0; IR (film) ν_{max} 2927, 1622, 1455, 1240 cm^{-1}; MALDIFTMS (DHB) *m/z* 698.3054 ($C_{37}H_{45}N_3O_9+Na^+$ requires 698.3048). For **16**, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86–6.68 (m, 6H), 6.59 (s, 1H), 6.52 (dd, $J=1.4$, 8.2 Hz, 1H), 4.65 (br m, 1H), 4.35 (br, 1H), 4.00 (br m, 1H), 3.90 (s, 4H), 3.85 (s, 6H), 3.83 (s, 3H), 3.72 (br, 2H), 3.63 (s, 3H), 3.21 (br m, 4H), 2.95 (s, 3H), 2.89 (br s, 4H), 2.48 (s, 3H), 2.43 (s, 3H); IR (film) ν_{max} 2935, 1622, 1463, 1259 cm^{-1}; MALDIFTMS (DHB) *m/z* 728.3128 ($C_{38}H_{47}N_3O_{10}+Na^+$ requires 728.3153). For **18**, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.07 (br m, 2H), 6.92 (m, 4H), 6.80 (dd, $J=1.5$, 8.2 Hz, 1H), 6.76 (d, $J=7.9$ Hz, 1H), 4.80 (m, 1H), 4.62 (br m, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.56 (br s, 3H), 3.15 (m, 2H), 2.88 (s, 3H), 2.35 (s, 3H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 163.1, 155.4, 150.7, 150.5, 150.3, 149.3, 147.2, 146.9, 137.2, 131.8, 126.3, 123.6, 122.0, 118.5, 116.4, 114.6, 113.8, 113.0, 110.7, 106.1, 101.7, 56.4, 56.23, 56.20, 56.11, 56.08, 49.2, 38.2, 34.5; IR (film) ν_{max} 2935, 2831, 1713, 1631, 1456, 1154 cm^{-1}; MALDIFTMS (DHB) *m/z* 617.2500 ($C_{34}H_{36}N_2O_9+H^+$ requires 617.2494).
16. Keller, R. P.; Altermatt, H. J.; Nooter, K.; Poschmann, G.; Laissue, J. A.; Bollinger, P.; Hiestand, P. C. *Int. J. Cancer* **1992**, *50*, 593.