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Cytotoxic and PTP1B inhibitory activities from *Erythrina abyssinica*

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ABSTRACT

Bioassay-guided fractionation of the EtOAc extract of the stem bark of *Erythrina abyssinica* (Leguminosae) resulted in the isolation of three new (**1**–**3**), along with 12 known (**4**–**15**) pterocarpan derivatives. Their chemical structures were determined by physicochemical and spectroscopic data analysis (IR, UV, $[\alpha]_D$, CD, 1D and 2D NMR, and MS data). All the isolates were evaluated for their inhibitory effects on protein tyrosine phosphatase-1B (PTP1B), as well as their growth inhibition on MCF7, tamoxifen-resistant MCF7 (MCF7/TAMR), adriamycin-resistant MCF7 (MCF7/ADR) and MDA-MB-231 breast cancer cell lines. Compounds which exhibited PTP1B inhibitory activity (IC_{50} values ranging from 4.2 ± 0.2 to 19.3 ± 0.3 μM) showed strong cytotoxic activity (IC_{50} values from 5.6 ± 0.7 to 28.0 ± 0.2 μM). Our data suggested that pterocarpans could be considered as new anticancer materials by PTP1B inhibition.

Breast cancer is the most common malignant tumor in women these days accounting for approximately 24% of all cancer. Despite the development of various treatments, such as chemotherapy, radiation therapy and surgery, breast cancer remains the second most lethal cancer for women. Tamoxifen is the most widely used selective estrogen receptor modulator (SERM) in hormone-dependent breast cancer therapy, and has made a substantial contribution to reducing the mortality rate in many developed countries. However, although tamoxifen is still considered for endocrine therapy in hormone-dependent breast cancer, its continuous use is associated with tumor resistance and increased risk of endometrial cancer.[1] Therefore, new types of compounds with suitable pharmacological properties for tamoxifen itself or its resistant cancer lines are needed to be discovered (Fig. 1).

Protein tyrosine phosphatase (PTP) superfamily coordinates with protein tyrosine kinases to regulate a vast array of cellular functions, including proliferation, differentiation, apoptosis and motility. Of the various PTPs, PTP1B plays a critical role in regulating body weight, glucose homeostasis by acting as a key negative regulator of insulin and leptin signaling pathway.[2] However, PTP1B also has recently drawn attention as an attractive target for anticancer, especially for the treatment of breast cancer.[3] PTP1B is overexpressed in human breast cancer, and inhibition of PTP1B delays erbB2-induced mammary tumorigenesis and protects from lung metastasis.[4] These findings raise the possibility that selective inhibition of PTP1B may be effective strategy for the treatment of human breast cancer.

As part of an ongoing investigation aimed at PTP1B inhibitors from plants, the genus *Erythrina* using in vitro assay on both the cytotoxic activity and PTP1B inhibitory activity were studied. The EtOAc extract of the stem bark of *Erythrina abyssinica* (Leguminosae) was found to exhibit significant activity on both (>50% inhibition at 30 μg/mL). The genus *Erythrina* (Leguminosae) is comprised of approximately 110 species of trees and shrubs that are widely distributed in tropical and subtropical regions with representative species being used in indigenous medicine.[5] Alkaloids, benzofurans, flavonoids, chalcone, and other pterocarpans have been reported as constituents of this genus, which have been found to possess a wide range of antioxidant, antimicrobial, cytotoxic, and anti-inflammatory activity.[6] Despite the number of studies on the genus *Erythrina*, there are no reports on the PTP1B inhibitory-related cytotoxic activity by the chemical constituents isolated from *E. abyssinica*. Bioassay-guided fractionation of an EtOAc-soluble extract of the stem bark of this plant led to the isolation of a series of pterocarpans, consisting of three new compounds, erythribyssins A–C (**1**–**3**), along with 12 known ones (**4**–**15**).[7] The structures of the known compounds were determined to be eryvarin K (**4**),[8] neorautenol (**5**),[9] erybreadin B (**6**),[10] 3,9-dihydroxy-4-prenyl-[6a*R*:11a*R*]pterocarpan (**7**),[11] folitenol (**8**),[12] erybreadin D (**9**),[13] erysubin E (**10**),[14] erybreadin C (**11**),[15] phaseollidin (**12**),[16] sophorapterocarpan A (**13**),[17] erythrabyssin II (**14**),[16,18,19] and erystagallin A (**15**),[20] from a comparison of the physical and spectroscopic data (IR, UV, $[\alpha]_D$, NMR, and MS) with those reported in the literature.

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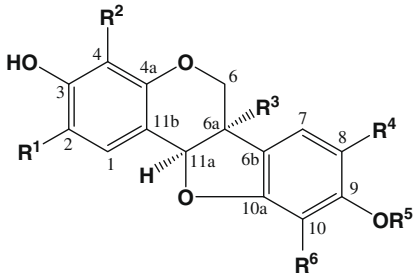
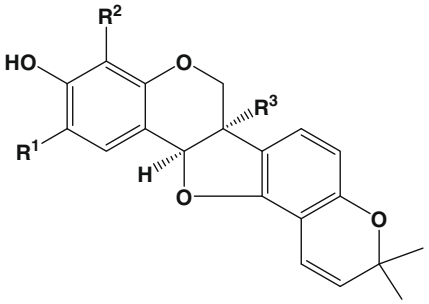




1 $R^1 = R^2 = R^4 = H$, $R^3 = OCH_3$, $R^5 = CH_3$, R^6 = prenyl group [$-CH_2-CH=C(CH_3)_2$]

2 $R^1 = R^2 = R^3 = R^4 = R^5 = H$, $R^6 = CHO$

4 R^1 = prenyl, $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = OCH_3$

6 $R^1 = R^3 = H$, R^2 = prenyl

7 $R^1 = R^3 = R^4 = R^5 = R^6 = H$, R^2 = prenyl

8 R^1 = prenyl, $R^2 = R^3 = H$

10 R^1 = prenyl, $R^2 = H$, $R^3 = OH$

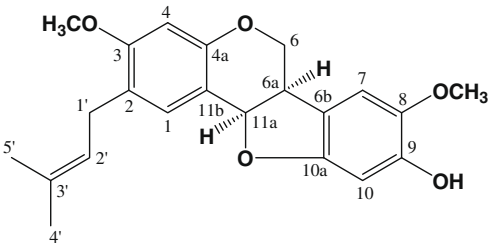
11 $R^1 = R^3 = R^5 = R^6 = H$, $R^2 = R^4$ = prenyl

12 $R^1 = R^2 = R^3 = R^4 = R^5$, R^6 = prenyl

13 $R^1 = R^2 = R^3 = R^5 = R^6 = H$, R^4 = prenyl

14 $R^1 = R^6$ = prenyl, $R^2 = R^3 = R^4 = R^5 = H$

15 $R^1 = R^6$ = prenyl, $R^2 = R^4 = H$, $R^3 = OH$, $R^5 = CH_3$



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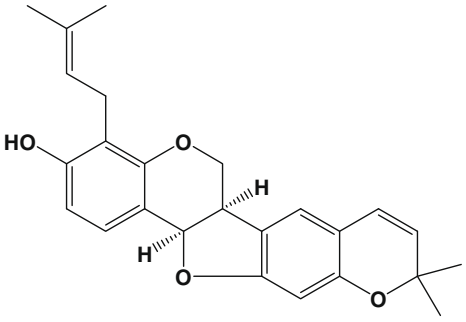

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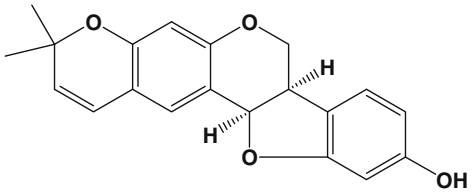

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Figure 1. Chemical structures of compounds **1**–**15** isolated from *Erythrina abyssinica*.

Compound **1** was obtained as yellow amorphous powder with the molecular formula $C_{22}H_{24}O_5$, as determined by the HR-EI mass spectrum ($[M]^+$, *m*/*z* 368.1628).[21a] The IR spectrum of compound **1** suggested the presence of OH functional groups at 3406 cm^{-1}, 2927 (C–C), 1468 and 1193–1119, and C–O stretching vibrations at 1061–1041 cm^{-1}. The UV spectral data, ^1H and ^{13}C NMR spectra of compound **1** showed signals assignable to a methylene, a methine and one aliphatic quaternary carbon with an oxygen function [δ_H 4.07 (1H, d, *J* = 11.5 Hz), 4.32 (1H, d, *J* = 11.5 Hz), and 5.54 (1H, s)]; [δ_C 69.2 (C-6), 80.9 (C-6a), 82.6 (C-11a)]. This suggests that compound **1** is a pterocarpan derivative with an oxygenated functional group attached to C-6a.[16,20] Two methoxy groups, one prenyl group, two *ortho*-coupled aromatic protons [δ 7.20 (1H, d, *J* = 8.0 Hz) and 6.59 (1H, d, *J* = 8.0 Hz)], and one ABX-type aromatic spin system at [δ 7.33 (1H, d, *J* = 8.5 Hz), 6.26 (1H, d, *J* = 2.5 Hz), and 6.59 (1H, dd, *J* = 2.5, 8.5 Hz)] were observed in the ^1H and ^{13}C NMR spectra (acetone-d_6). These assignments resembled those of erythrabyssin I,[16] except for the signal of the methoxy group at C-6a. Placement of methoxy group to C-6a was further confirmed by an HMBC experiment, revealing correlation between the methoxy signal from δ_H 3.12 (3H, s) to the carbon at δ_C 80.9 (C-6a). The positions of the prenyl moiety and the methoxy group attached on aromatic ring of compound **1** were also determined by HMBC experiments with δ_H 3.81 (3H, s) and C-9 (160.8); H_2-1′ and C-9, C-10; H_2-6 and C-4a, C-6a, C-11a; H-11a and C-1, C-4a, C-11b, C-6b; H-1 and C-11a, C-3, C4a; H-4 and C-3, C-4a, C-11b; H-7 and C-6a, C-9, C-10a (Fig. 2). Compound **1** contains two chiral centers at C-6a and C-11a, which were considered to possess either *R*,*R* or *S*,*S* configurations from the stereochemical environment around the C-6a and C-11a.[22] It has been suggested that the absolute configuration of a pterocarpan compound might be presumed from the sign of its optical rotation.[22] Levorotatory pterocarpans have 6a*R* and 11a*R* configurations, while the dextrorotatory ones have 6a*S* and 11a*S* configurations.[23] In the case of compound **1**, because the specific optical rotation value was −205° (*c* = 0.03, MeOH), the absolute configuration at C-6a and C-11a was assigned to *S* from its negative optical rotation value.[16,20,23,24] The circular dichroic (CD) spectrum of compound **1** revealed a positive Cotton

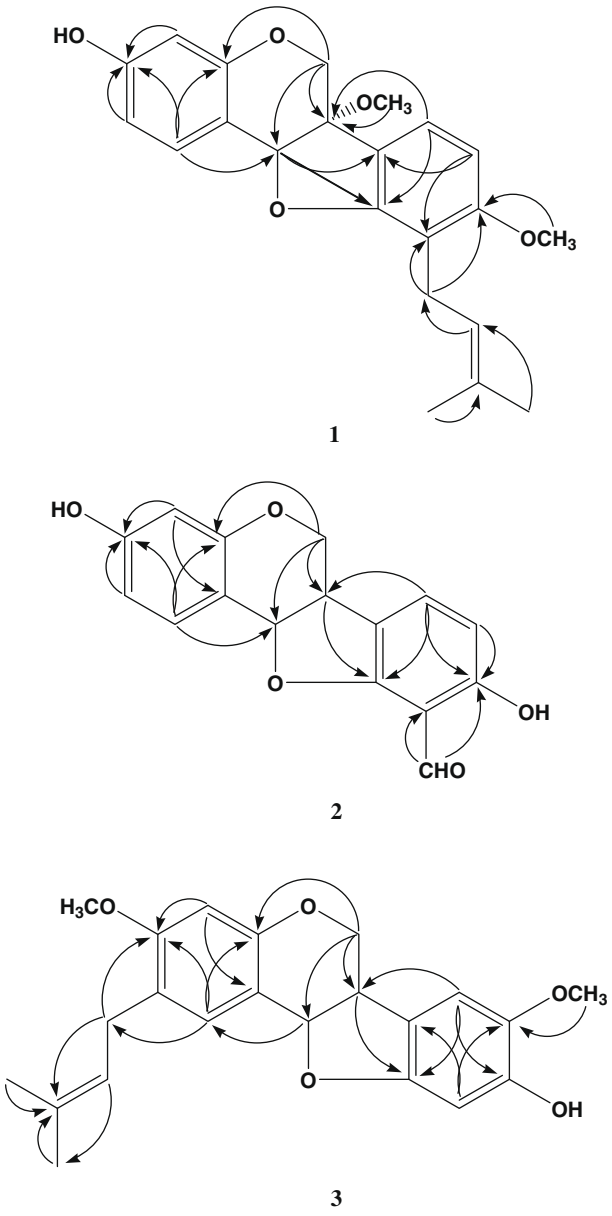

Figure 2. Key HMBC correlations of new compounds **1**–**3**.

effect [MeOH, λ_{max} = 295 nm ($\Delta\varepsilon$ = +0.98)], indicating an absolute configuration of 6a*S* and 11a*S*.[24] Based on the above data, compound **1** was identified as (6a*S*,11a*S*)-3-hydroxy-6a,9-dimethoxy-10-(3′,3′-dimethylallyl)pterocarpan, a new natural product named erythribyssin A.

Compound **2** was isolated as a yellowish amorphous powder and showed absorption maxima at 208 nm (log ε = 4.80), 228 nm (log ε = 4.46), 277 nm (log ε = 4.32) and 363 nm (log ε = 3.71) in the UV spectrum. The IR spectrum of compound **2** showed absorption bands at 3418 (OH), 2924 (C–C), 1650 (CHO), and 1418, 1160, 1041 cm^{-1} ascribable to aromatic ring. The molecular formula for compound **2**, $C_{16}H_{12}O_5$, was determined from the molecular ion peaks (*m*/*z* 284.0688) observed by high-resolution EI-MS measurement.[21b] The ^{1}H NMR spectrum of compound **2** showed four signals at δ 4.32 (1H, dd), 3.72 (1H, m), and 3.72 (1H, m), and 5.80 (1H, d), which were reminiscent of a pterocarpan skeleton.[19,23] The presence of 1,2,4-trisubstituted benzene [δ 7.38 (1H, d), 6.59 (1H, dd), and 6.38 (1H, d)] and *ortho*-coupled aromatic protons [δ 7.55 (1H, d, *J* = 8.0 Hz) and 6.41 (1H, d, *J* = 8.0 Hz)] on rings A and D in the ^{1}H and ^{13}C NMR spectra was assigned. The ^{13}C NMR spectrum showed 16 carbons, of which 15 were assigned to the pterocarpan skeleton. The remaining one carbon signal at δ_C 193.5 and the corresponding proton signal δ_H 10.14 (1H, s) was characteristic of an aldehyde group. The assignment of the aldehyde group at C-10 was supported by the HMBC experiment, which indicated a correlation from the aldehyde proton at δ_H 10.14 to the aromatic quaternary carbon at C-10 (δ_C 108.3) (see Table 1 and Fig. 2). The absolute stereochemistry at C-6a and C-11a was found to be *R* from the negative optical rotation value [−110° (*c* = 0.02, MeOH)].[15,16,23] From the above data, compound **2** was determined to be **[**3,9-dihydroxy-(6a*R*,11a*R*)-10-formyl]pterocarpan, named as erythribyssin B.

Compound **3** was obtained as brown powder with the molecular formula $C_{22}H_{24}O_5$, as determined by the HR-EI mass spectrum ([M]$^+$, *m*/*z* 368.1624). The IR spectrum of compound **3** suggested the presence of OH functional groups at 3408 cm^{-1}, 2928 (C–C), 1657 and 1625, 1161–1033 cm^{-1}. Its UV spectrum showed absorption maxima at 208 nm (log ε = 4.80), 228 nm (log ε = 4.46), and 277 nm (log ε = 4.32).[21c] The ^{1}H NMR spectrum of compound **3** showed characteristic signals of a pterocarpan skeleton[19,23] with four typical signals at δ_H 4.27 (1H, dd), 3.57 (1H, t), and 3.51 (1H, m), and 5.41 (1H, d). A methoxy group [δ_H 3.79 (3H, s), δ_C 57.5], one prenyl group and four aromatic protons at δ_H 7.16, 7.01, 6.43, 6.34 (each 1H, s) were observed in the ^{1}H and ^{13}C NMR spectra of **3**. All of these observations were resembled with those of compound **4**,[8] a pterocarpan isolated from the same source.[7] The major difference was that an additional methoxy group [δ_H 3.81 (3H, s), δ_C 55.9] was found in compound **3**. HMBC correlations were observed between one methoxy proton (δ_H 3.79) to C-8 (δ_C 142.9), the other methoxy proton (δ_H 3.81) to C-3 (δ_C 159.4) confirmed the arrangement of these two methoxy groups. A negative optical rotation value was evidenced for the absolute stereochemistry of compound **3** to be (6a*R*:11a*R*).[22] Thus, compound **3** was established as 3,9-dimethoxy-2-prenyl-(6a*R*,11a*R*)pterocarpan, a new natural product named erythribyssin C.

For investigation of the biological properties, the isolated compounds (**1**–**15**) were evaluated for their inhibitory effects on protein tyrosine phosphatase-1B (PTP1B), as well as their growth inhibition on MCF7, tamoxifen-resistant MCF7 (MCF7/TAMR), adriamycin-resistant MCF7 (MCF7/ADR) and MDA-MB-231 breast cancer cell lines (Tables 2 and 3).[25,26] Among the isolates, compound **6**, **8**, **9**, **11**, and the new compound erythribyssin A (**1**) which significantly inhibited PTP1B (IC_{50} values ranging from 4.2 ± 0.2 to 19.3 ± 0.3 μM) showed strong cytotoxic activity against four cancer cell lines, with IC_{50} values ranging from 5.6 ± 0.7 to 22.7 ± 0.5 μM. Especially, compound **6** was twice as potent against MCF7/TAMR and MCF7/ADR breast cancer cell lines when it compared with tamoxifen used as the positive control, also showed strongest activity against PTP1B enzyme. Erybreadin B (**6**), folitenol (**8**) and erybreadin D (**9**) which is the presence of a 2,2-dimethylpyran ring fused to the C-9 or C-10 position could be considered to be more stronger activities. Most of the isolates bearing a prenyl group gave the inhibitory effects on both breast cancer cells and protein tyrosine phosphatase-1B, while the absence of this moiety displayed non-activity in both assay systems. Interestingly, the cytotoxic activity of folitenol (**8**) was showed by disappearance of a hydroxy group (6a-OH) of erysubin E (**10**). Overall, the new compounds, erythribyssins B (**2**) and C (**3**), and the other known compounds were not active in cytotoxic activity assay, showed weak or no inhibitory activity against PTP1B (Tables 2 and 3). From the data obtained, pterocarpans with prenyl group are promised to be a new class of PTP1B inhibitors and anticancer agents. Therefore, it is suggested that compounds reducing PTP1B activity or the genetic expression levels can be used for treating breast cancer.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of new compounds **1**–**3** in acetone-d_6

Position	1		2		3	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	133.1	7.33, d, 8.5	133.3	7.38, d, 8.0	133.1	7.16, s
2	110.9	6.59, dd, 8.5, 2.5	112.1	6.59, dd, 2.0, 8.0	113.1	
3	160.0		160.2		159.4	
4	103.7	6.26, d, 2.5	104.2	6.38, d, 2.0	98.6	6.43, s
4a	157.2		158.1		156.0	
6	69.2	4.32, d, 11.5	67.1	4.32, m	67.3	4.27, dd, 4.5, 16.5
		4.07, d, 11.5		3.72, m		3.57, t-like, 16.5
6a	80.9		42.8	3.72, m	41.3	3.51, m
6b	117.6		106.3		117.9	
7	123.4	7.20, d, 8.0	120.1	7.55, d, 8.0	110.5	7.01, s
8	104.6	6.59, d, 8.0	109.0	6.41, d, 8.0	142.9	
9	160.8		162.7		148.6	
10	113.5		108.3		100.0	6.34, s
10a	160.9		164.1		155.1	
11a	82.6	5.54, s	82.2	5.80, d, 7.5	78.9	5.41, d, 6.5
11b	114.1		110.9		124.2	
1	23.1	3.19, d, 8.0			28.5	3.25, d, 7.0
2	123.1	5.13, t-like, 7.5			123.8	5.29, m
3	131.7				132.3	
4	25.9	1.56, s			17.9	1.72, s
5	17.9	1.68, s			26.0	1.72, s
3-OCH_3					55.9	3.81, s
6a-OCH_3	50.9	3.12, s				
9-OCH_3	56.4	3.81, s			57.5	3.79, s
10-CHO			193.5	10.14, s		

Table 2
Inhibitory activities of compounds **1**–**15** against PTP1B

Compounds	Inhibitory activity[a]
Erythribyssin A (**1**)	19.3 ± 0.3
Erythribyssin B (**2**)	>30
Erythribyssin C (**3**)	>30
Eryvarin K (**4**)	>30
Neorautenol (**5**)	7.6 ± 0.9
Erybreadin B (**6**)	4.2 ± 0.2
3,9-Dihydroxy-4-prenyl-[6a*R*;11a*R*] pterocarpan (**7**)	19.5 ± 1.5
Folitenol (**8**)	7.8 ± 0.5
Erybreadin D (**9**)	6.4 ± 0.6
Erysubin E (**10**)	8.8 ± 0.5
Erybreadin C (**11**)	7.3 ± 0.1
Phaseollidin (**12**)	>30
Sophorapterocarpan A (**13**)	>30
Erythrabyssin II (**14**)	>30
Erystagallin A (**15**)	20.8 ± 1.5
RK-682[b]	4.5 ± 0.5
Ursolic acid[b]	3.6 ± 0.2

[a] Results are expressed as IC_{50} values (μM), determined by regression analyses and expressed as the mean ± SD of three replicates.
[b] Positive control.

Table 3
Cytotoxic activities of compounds **1**–**15** against breast cancer cells

Compounds	Cell lines/IC_{50} (μM)[a]			
	MCF7	MCF/TAMR	MCF/ADR	MDA-MB-231
Erythribyssin A (**1**)	19.4 ± 1.2	12.0 ± 1.9	16.1 ± 0.6	28.0 ± 0.2
Erybreadin B (**6**)	11.8 ± 0.5	6.2 ± 0.2	5.6 ± 0.7	7.7 ± 0.05
Folitenol (**8**)	—	18.8 ± 2.2	10.8 ± 1.5	16.7 ± 1.3
Erybreadin D (**9**)	—	7.8 ± 1.0	12.7 ± 0.05	—
Erybreadin C (**11**)	14.8 ± 1.5	9.1 ± 0.3	20.1 ± 1.4	22.7 ± 0.05
Tamoxifen[b]	11.4 ± 0.9	11.1 ± 0.8	10.9 ± 1.1	12.4 ± 0.8

[a] New compounds, erythribyssins B (**2**) and C (**3**), and the other known compounds were weak or not active in this assay system (IC50 >30 μM).
[b] Positive control.

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7. The dried stem bark of *Erythrina abyssinica* (5 kg) collected in Uganda was extracted with MeOH at room temperature. The EtOAc-soluble fraction (88 g) was found to be most active (50% inhibition at 30 μg/mL) among the solvent fractions. This fraction was fractionated by silica gel column chromatography (20 × 80 cm; 63–200 μm particle size) using a gradient of hexane:acetone (from 20:1 to 0:1), to yield five combined fractions according to their TLC profiles. Fraction 2 (4 g) was subjected to reversed phase C18 (RP-18) column chromatography (9.0 × 60 cm; 40–63 μm particle size) and eluted with MeOH:H_2O (from 5:5 to 5:0, 2 L for each step) to afford five sub-fractions (Fr.2-1 to Fr.2-5). Further purification of Fr.2-1 by semi-preparative Gilson HPLC systems [using RS Tech Optima Pak C18 column (10 × 250 mm, 10 μm particle size); mobile phase MeCN:H_2O (51:49); flow rate 2 mL/min; UV-detection at 205 and 254 nm] resulted in the isolation of compounds **4** (12.7 mg; t_R = 34.5 min) and **5** (7.8 mg; t_R = 43.5 min), respectively. Fr.2-3 was purified using a Gilson HPLC system [RS Tech Optima Pak C18 column (10 × 250 mm, 10 μm particle size); eluted with 63% Acetonitrile (MeCN) in H_2O + 0.1% formic acid; flow rate 2 mL/min; UV-detection at 205 and 254 nm] resulting in the isolation of compounds **6** (39.2 mg; t_R = 26.5 min) and **1** (9.8 mg; t_R = 32.5 min), respectively. Fraction 3 (8 g) was also subjected to reversed phase C18 (RP-18) column chromatography (9.0 × 60 cm; 40–63 μm particle size) eluted with MeOH:H_2O (from 40:60 to 100:0, 2 L for each step) to afford five sub-fractions (Fr.3-1 to Fr.3-5) according to their similar of TLC patterns. Further purification of fraction Fr.3-1 by HPLC using an isocratic

solvent system of 53% MeCN in H_2O led to the isolation of compounds **2** (3.8 mg, t_R = 17.5 min), **7** (66 mg, t_R = 23.2 min) and **8** (3.5 mg, t_R = 25.5 min), respectively. Compounds **9** (2.5 mg, t_R = 30.1 min), **10** (17.2 mg, t_R = 34.9 min) and **11** (3.4 mg, t_R = 36.5 min) were isolated from Fr.3-4 by using semi-preparative Gilson HPLC, with 60% MeCN in H_2O + 0.1% formic acid as the mobile phase. Fraction Fr.4 [eluted with hexane:acetone (from 2:1 to 1:1), 7.2 g] was subjected to reversed phase C18 (RP-18) column chromatography (9.0 × 60 cm; 40–63 μm particle size) using a stepwise gradient of MeOH in H_2O (from 35:65 to 100:0; 2 L for each step) to yield five sub-fractions (Fr.4-1 to Fr.4-5). Further purification of Fr.4-3 (185.0 mg) by semi-preparative HPLC [RS Tech Optima Pak C18 column (10 × 250 mm, 10 μm particle size); mobile phase MeCN:H_2O (34:66); flow rate 2 mL/min; UV-detection at 205 and 254 nm] resulted in the isolation of compounds **12** (1.6 mg; t_R = 41.5 min), **13** (11.2 mg; t_R = 49.9 min) and **3** (9.8 mg; t_R = 58.5 min), respectively. Compounds **14** (5.2 mg, t_R = 37.9 min) and **15** (12.5 mg, t_R = 37.9 min) were isolated from fraction Fr.4-5 [eluted with MeOH:H_2O (80:20), 171.1 mg] using preparative HPLC with 70% MeCN in H_2O + 0.1% formic acid as the mobile phase and detection at 205 and 254 nm.

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21. Physical and spectroscopic data of new compounds: (a) *Erythribyssin A* (**1**): yellow amorphous powder; $[\alpha]_D^{25}$: −205 (*c* 0.03, MeOH); IR (KBr) υ_{max} cm^{-1}: 3406, 2927, 1468, 1193, 1119, 1061, 1041; UV (MeOH) λ_{max} nm (log ε): 209 (4.79), 233 (4.23), 282 (3.81); CD (*c* 0.1, MeOH) $[\theta]_{230}$ − 18.7, $[\theta]_{295}$ + 0.98; ^1H (500 MHz) and ^{13}C (125 MHz, acetone-d_6) NMR data, see Table 1; HREIMS *m*/*z* 368.1628 $[M]^+$ (calcd for $C_{22}H_{24}O_5$, 368.1624). (b) *Erythribyssin B* (**2**): yellowish amorphous powder; $[\alpha]_D^{25}$: −110 (*c* 0.02, MeOH); IR (KBr) υ_{max} cm^{-1}: 3418, 2924, 1650, 1622, 1418, 1160, 1041; UV (MeOH) λ_{max} nm (log ε): 208 (4.80), 228 (4.46), 277 (4.32), 363 (3.71); ^1H (500 MHz) and ^{13}C (125 MHz, acetone-d_6) NMR data, see Table 1; HREIMS *m*/*z* 284.0688 $[M]^+$ (calcd for $C_{16}H_{12}O_5$, 284.0685). (c) *Erythribyssin C* (**3**): $[\alpha]_D^{25}$: −16.7 (*c* 0.01, MeOH); IR (KBr): υ_{max} 3408, 2928, 1657, 1625, 1161–1033 cm^{-1}; UV (*c* 0.02, MeOH) λ_{max} nm (log ε): 208 (4.80), 228 (4.46), 277 (4.32) nm; ^1H (500 MHz) and ^{13}C (125 MHz, acetone-d_6) NMR data, see Table 1; HREIMS *m*/*z* 368.1624 $[M]^+$, (calcd $C_{22}H_{24}O_5$ 368.1620).
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25. (a) *Cell culture*: The screening cell lines (MCF7 and MDA-MB-231 human breast carcinoma cells, and the multidrug-resistant cell lines MCF7/TAMR and MCF7/ADR) were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. All the media used were DMEM supplemented with 10% heat-inactivated fetal bovine serum, 4.5 g/L D-glucose, 100 mg/L sodium pyruvate and L-glutamine. The cells were subcultured every 3 days using the standard trypsinization procedure. (b) Cytotoxicity assay: The cell viability was assessed using a 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate (WST-1) based cytotoxicity assay kit to determine the IC_{50} of the isolated compounds (Daeil lab service Co., Ltd, Korea). In these assays, 1×10^4 (MCF7, MCF/TAMR, MDA-MB-231) or 1.5×10^4 (MCF/ADR) cells in 100 μL of the culture medium per well were seeded in 96-well plates and allowed to adhere for 24 h prior to treatment. The final concentration of DMSO in the culture medium was maintained at 0.05% (v/v) to avoid solvent toxicity. Subsequently, 10 μL of the kit solution was added to each well of the plate and the absorbance was measured at 450 nm using an ELISA reader. The survival percentages are defined as the absorbance in the experiment well compared to that in the control wells. The cytotoxicity results are expressed as the mean ± standard deviation and represent the concentration inhibiting 50% cell growth (IC_{50}). Each experiment was carried out three times in triplicate. (c) PTP1B assay: PTP1B (human, recombinant) was purchased from BIOMOL International LP (USA) and the enzyme activity was measured using *p*-nitrophenyl phosphate (*p*-NPP) as a substrate. To each 96-well (final volume: 200 μL) were added 2 mM *p*-NPP and PTP1B (0.05–0.1 μg) in a buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) with or without test compounds. Following incubation at 37 °C for 30 min, the reaction was terminated with 10 M NaOH. The amount of produced *p*-nitrophenol was estimated by measuring the absorbance at 405 nm. The nonenzymatic hydrolysis of 2 mM *p*-NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme.
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