

RESEARCH ARTICLE

Diaphorin, a polyketide produced by a bacterial symbiont of the Asian citrus psyllid, kills various human cancer cells

Atsushi Nakabachi^{1,2*}, Keiko Okamura¹

1 Electronics-Inspired Interdisciplinary Research Institute (EIIRIS), Toyohashi University of Technology, Toyohashi, Aichi, Japan, **2** Department of Environmental and Life Sciences, Toyohashi University of Technology, Toyohashi, Aichi, Japan

* nakabachi@eiiris.tut.ac.jp



OPEN ACCESS

Citation: Nakabachi A, Okamura K (2019) Diaphorin, a polyketide produced by a bacterial symbiont of the Asian citrus psyllid, kills various human cancer cells. PLoS ONE 14(6): e0218190. <https://doi.org/10.1371/journal.pone.0218190>

Editor: Richard Mankin, US Department of Agriculture, UNITED STATES

Received: April 27, 2019

Accepted: May 28, 2019

Published: June 10, 2019

Copyright: © 2019 Nakabachi, Okamura. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the manuscript and Supporting Information files.

Funding: This work was supported by Japan Society for the Promotion of Science (<https://www.jsps.go.jp>) KAKENHI grant number 26292174 to AN. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Diaphorin is a polyketide produced by *Candidatus Proffotella armatura* (*Betaproteobacteria*), an organelle-like defensive symbiont harbored by a plant sap-sucking insect, Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae). Diaphorin belongs to the pederin family, a group of compounds that share much of their core structure with that of pederin, which is characterized by two dihydropyran rings bridged by an *N*-acyl aminal. Most members of this family have potent antitumor activity, making them promising anticancer drug candidates. The present study assessed the therapeutic potential of diaphorin for its antitumor activity against 39 human cancer cell lines including those from breast, brain, colon, lung, skin, ovary, kidney, stomach, and prostate. The results showed that diaphorin had inhibitory activity against all 39 cancer cell lines tested. The GI₅₀, TGI, and LC₅₀ values ranged from 0.28 μM–2.4 μM, 1.6 μM–11 μM, and 7.5 μM→100 μM, respectively. These values are among the highest in the pederin family, indicating that the anticancer activity of diaphorin is milder than those of other pederin congeners. The inhibitory effects of diaphorin significantly differed among the distinct cancer types. The maximum difference was about 10-fold, which was similar to those of most other pederin congeners.

Introduction

A variety of invertebrate animals harbor endosymbiotic microbes to deter natural enemies, which makes them among the richest sources of biologically active secondary metabolites [1–6]. Pederin family compounds, mostly isolated from marine sponges, are polyketides that share much of their core structure with that of pederin, which is characterized by two dihydropyran rings bridged by an *N*-acyl aminal [7–12]. Most compounds in this family, including pederin, mycalamides, onnamides, theopederins, and psymberin, exhibit potent antitumor activity, making them promising as anticancer drug candidates [7–10,13–20]. Mycalamide A not only inhibited replication of various cancer cells *in vitro* but also improved the survival of mice bearing ascitic lymphomas and a variety of other ascitic and solid tumors [15]. Moreover, this compound exhibited cancer preventive properties, inhibiting epidermal growth factor

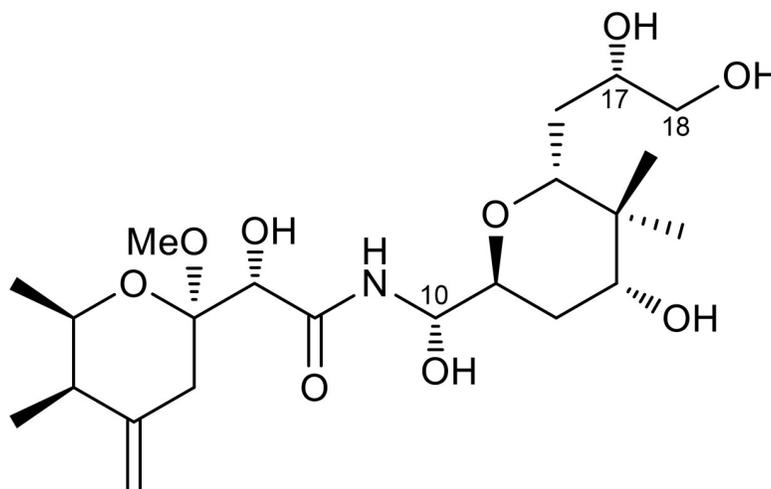


Fig 1. Chemical structure of diaphorin.

<https://doi.org/10.1371/journal.pone.0218190.g001>

(EGF)-induced neoplastic transformation in murine cells [20]. Analysis using 60 human cancer cell lines revealed that psymberin, also known as irciniastatin A [21], has an remarkably selective activity toward solid tumor cells, making it one of the most promising lead drug candidates among a variety of other possible leads from different structural classes [22].

Although most pederin congeners are derived from marine invertebrates, pederin itself is found exclusively from rove beetles of the genera *Paederus* and *Paederidus* (Coleoptera: Staphylinidae) [7–10,23,24]. Since the discovery of pederin in the mid 20th century [25], these beetles have long been a sole terrestrial source of pederin family members until diaphorin, a tri-*O*-desmethyl analog of pederin (Fig 1), was discovered from a plant sap-sucking insect, the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae)[26]. *D. citri* is an important pest of citrus worldwide and harbors the vertically transmitted intracellular symbiont *Candidatus Proffttella armatura* (*Betaproteobacteria*) [26–30]. *Proffttella* is an unprecedented organelle-like defensive symbiont with a drastically reduced genome of 460 bp, which produces diaphorin using the polyketide synthase (PKS) biosynthetic gene clusters [26]. A previous study showed that diaphorin has significant inhibitory activity against the rat neuroblastoma B104 and human HeLa cells [26]. In the present study, in an effort to further evaluate the therapeutic potential of diaphorin, we assessed the antitumor activities of diaphorin against various human cancers using a panel of 39 cancer cell lines prepared by the Japanese Foundation for Cancer Research.

Materials and methods

Preparation of diaphorin

Diaphorin was extracted from *D. citri*, purified, and quantified as previously described [30]. Briefly, adult *D. citri* were treated twice with methanol, and the extracts were combined and concentrated *in vacuo*. The residue was resuspended in methanol and purified in a Shimadzu (Kyoto, Japan) LC10 high-performance liquid chromatography (HPLC) system with an Inertsil ODS-3 C18 reversed-phase preparative column [5 μ m, 7.6 \times 150 mm, GL Science (Tokyo, Japan)] heated to 35°C. The mobile phase was isocratic 20% acetonitrile in water, with a flow rate of 1.5 mL/min. Diaphorin was detected at a wavelength of 200 nm. The purified samples were combined and dried *in vacuo*. Diaphorin was re-dissolved in methanol and quantified in an HPLC system as described above, except the mobile phase was 15% acetonitrile in water,

with a flow rate of 1.0 mL/min, and an Inertsil ODS-3 analytical column (5 μ m, 4.0 \times 250 mm, GL Science) was used.

Cell lines and cell culture

The human cancer cell line panel prepared by the Japanese Foundation for Cancer Research (Tokyo, Japan) consisted of 39 cell lines as follows [31]: breast cancer, BSY-1, HBC-4, HBC-5, MCF-7, and MDA-MB-231; central nervous system (CNS) cancer, SF-268, SF-295, SF-539, SNB-75, SNB-78, and U251; colon cancer, HCC-2998, HCT-116, HCT-15, HT-29, and KM-12; lung cancer, A549, DMS114, DMS273, NCI-H226, NCI-H23, NCI-H460, and NCI-H522; melanoma, LOX-IMVI; ovarian cancer, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; renal cancer, ACHN and RXF-631L; stomach cancer, MKN-A, MKN-B, MKN1, MKN45, MKN74, and St-4; prostate cancer, DU-145 and PC-3. Normal cell types were not included in the assay because they tend to respond to anticancer drugs with extreme phenotypes (e.g., fibroblasts being pan-resistant and renal epithelial cells being pan-sensitive), making them unsuitable as controls [32]. The cells were cultured in RPMI 1640 medium supplemented with 5% fetal bovine serum, 2 mM L-glutamine, penicillin (100 units/mL), and streptomycin (100 mg/mL) at 37°C in humidified air containing 5% CO₂.

Analysis of cell growth inhibition by diaphorin

Inhibition of the growth of human cancer cell lines was assessed using a standard sulforhodamine B (SRB) assay as described previously [33]. Briefly, cells in culture medium were inoculated into 96-well plates and incubated at 37°C for 24 h. Subsequently, 12 replicates of each cell line were fixed with trichloroacetic acid (TCA) to measure the cell population at the time of adding diaphorin (Tz: time zero). Serial dilutions (10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵, or 10⁻⁴ M final concentration) of diaphorin were then added to each cell line. After 48 h of additional incubation at 37°C, the plates were fixed with TCA, stained with SRB, and read with an automated microplate reader at a wavelength of 515 nm. Percentage growth inhibition was calculated as:

$$[(Ti - Tz)/(C - Tz)] \times 100, \text{ when } Ti \geq Tz$$

$$[(Ti - Tz)/Tz] \times 100, \text{ when } Ti < Tz$$

(Tz: the SRB absorbance at time zero, C: the SRB absorbance of the control cells receiving no diaphorin, Ti: the SRB absorbance of the test cells treated with diaphorin at the five concentration levels). The concentration of diaphorin resulting in a 50% reduction in the cell number increase as compared with the control (GI₅₀: 50% growth inhibition) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$. The concentration of diaphorin resulting in total growth inhibition (TGI) was calculated from $Ti = Tz$. The concentration of diaphorin resulting in a 50% reduction of the number of cells at the end of the treatment as compared with that at the beginning (LC₅₀: 50% lethal concentration) was calculated from $[(Ti - Tz)/Tz] \times 100 = -50$.

Statistical analysis

Data were analyzed using R statistical computing software (version 3.4.2) [34].

Results and discussion

Growth inhibitory activity of diaphorin against 39 human cancer cell lines

A total of 39 human tumor cell lines were treated with diaphorin at final concentrations of 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵, and 10⁻⁴ M (S1 Table). The dose-response curves (Fig 2) showed that diaphorin had significant dose-dependent patterns of inhibitory activity against all 39 cancer cell lines tested (Tukey-Kramer test, $p < 0.05$). The concentrations of diaphorin required to

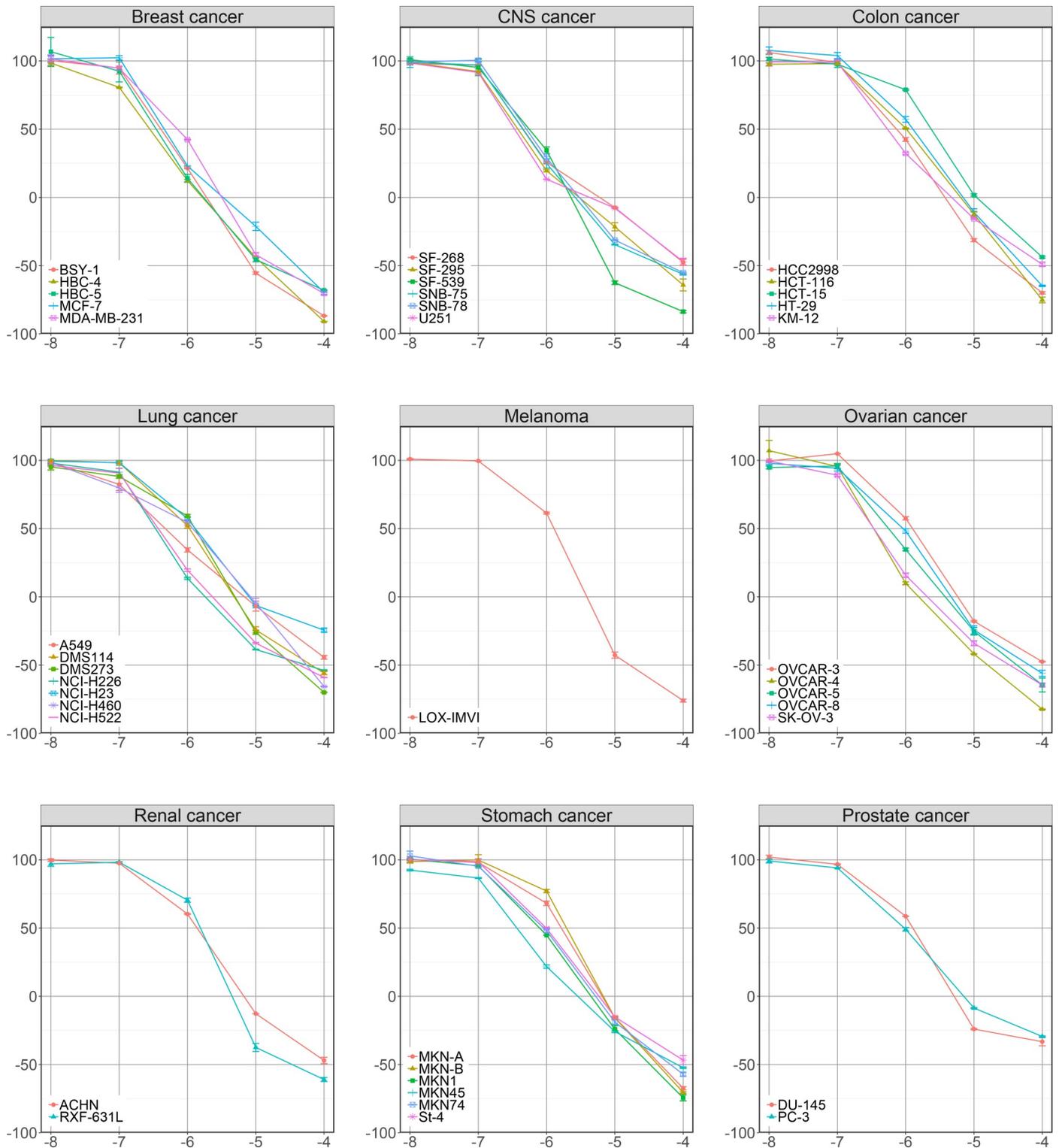


Fig 2. Dose response curves of the 39 human cancer cell lines treated with diaphorin.

<https://doi.org/10.1371/journal.pone.0218190.g002>

Table 1. Indices showing the inhibitory activity of diaphorin against the 39 human cancer cell lines.

Cell line	GI ₅₀ (μM)	TGI (μM)	LC ₅₀ (μM)
Breast cancer			
BSY-1	0.40	1.9	8.4
HBC-4	0.28	1.6	13
HBC-5	0.35	1.7	16
MCF-7	0.46	3.3	40
MDA-MB-231	0.71	3.2	19
CNS cancer			
SF-268	0.46	6.0	>100
SF-295	0.38	2.9	46
SF-539	0.56	2.3	7.5
SNB-75	0.45	2.7	52
SNB-78	0.51	3.0	61
U251	0.34	4.2	>100
Colon cancer			
HCC2998	0.74	3.8	31
HCT-116	1.0	6.3	40
HCT-15	2.4	11	>100
HT-29	1.3	7.1	54
KM-12	0.54	4.7	>100
Lung cancer			
A549	0.47	7.0	>100
DMS114	1.1	4.8	65
DMS273	1.3	5.0	35
NCI-H226	0.34	1.8	55
NCI-H23	1.3	8.1	>100
NCI-H460	1.2	8.3	55
NCI-H522	0.37	2.3	43
Melanoma			
LOX-IMVI	1.3	3.9	17
Ovarian cancer			
OVCAR-3	1.3	5.8	>100
OVCAR-4	0.35	1.6	16
OVCAR-5	0.56	3.8	43
OVCAR-8	0.93	4.7	67
SK-OV-3	0.34	2.1	34
Renal cancer			
ACHN	1.4	6.6	>100
RXF-631L	1.5	4.5	34
Stomach cancer			
MKN-A	1.6	6.4	45
MKN-B	2.0	6.8	43
MKN1	0.78	4.4	32
MKN45	0.37	2.9	84
MKN74	0.93	5.3	67
St-4	1.0	5.8	>100
Prostate cancer			
DU-145	1.3	5.1	>100
PC-3	0.96	7.1	>100

<https://doi.org/10.1371/journal.pone.0218190.t001>

exert the growth inhibitory activity were at the micromolar level, which is much higher than those reported for other members of the pederin family including pederin, mycalamides, onnamides, theopederins, psymberin, and their synthetic analogs that exhibit antitumor activity at nanomolar or even subnanomolar concentrations [9,10,13–20]. The anticancer activities of pederin congeners are primarily attributed to their ability to bind to ribosomes and inhibit protein synthesis [9,18,19,35,36]. Moreover, the C10 methoxy group of pederin and its analogs is postulated to be important for ribosome binding through its hydrogen bonding and its effects on conformation [9]. Diaphorin is a tri-*O*-desmethyl analog of pederin (Fig 1), where the methoxy groups at the C10, C17, and C18 of pederin are replaced by hydroxyl groups in diaphorin [26,37]. This alteration increases the hydrophilicity of the compound while enabling the occurrence of putative conformational changes and hydrogen bonding required for ribosome binding. Analyses of structure–activity relationships in this family support the idea that greater affinity toward ribosomes is associated with lower hydrophilicity [10], which most likely accounts for the notably milder activity of diaphorin compared with other pederin congeners.

The x-axis represents log₁₀ molar concentrations of diaphorin added to the culture medium. The y-axis represents the percentage growth of the cells during the diaphorin treatment. The growth percentage value of 100 represents the growth of the control cells receiving no diaphorin, the value of 0 represents no net growth throughout the treatment period, and the value of -100 indicates that all cells are killed at the end of the experiment. Error bars represent standard errors.

The GI₅₀, TGI, and LC₅₀ values are summarized in Table 1. The GI₅₀ values varied 10-fold, ranging from 0.28 μM for the HBC-4 breast cancer cells to 2.4 μM for the HCT-15 colon cancer cells. The TGI values ranged from 1.6 μM for the HBC-4 breast cancer cells and the OVCAR-4 ovarian cancer cells to 11 μM for the HCT-15 colon cancer cells. The LC₅₀ values varied more than 10-fold; the most sensitive cell line was the SF-539 CNS cancer cells with an LC₅₀ value of 7.5 μM followed by the BSY-1 breast cancer cells with an LC₅₀ value of 8.4 μM. The least potent inhibitory activities with LC₅₀ values greater than 100 μM were observed for the SF-268 and U251 CNS cancer cells, the HCT-15 and KM-12 colon cancer cells, the A549 and NCI-H23 lung cancer cells, the OVCAR-3 ovarian cancer cells, the ACHN renal cancer cells, the St-4 stomach cancer cells, and the DU-145 and PC-3 prostate cancer cells. In these cell lines, treatment with 100 μM diaphorin did not result in a 50% reduction of the cells at the end of the treatment as compared with that at the beginning. In terms of the three parameters, GI₅₀, TGI, and LC₅₀, the HCT-15 colon cancer cells were the least sensitive of the 39 cell lines. This is reasonable because HCT-15 is a multidrug-resistant cell line, which expresses the multidrug-resistance related genes MDR1 and MRP that are involved in the efflux of cytotoxic substances including therapeutic drugs [38]. The mean graphs of log GI₅₀, TGI, and LC₅₀ visualize the relative sensitivity of each cell line against diaphorin (Fig 3).

The sensitivity toward diaphorin significantly differed among the distinct cancer types (Friedman test: Friedman chi-squared = 9.4821, df = 1, *p* = 0.002075). The maximum difference was about 10-fold, which is similar to those observed for most other pederin congeners. Psymberin is the only exception to this within the pederin family thus far, showing more than a 10,000-fold difference in its inhibitory activity toward distinct cancer types. Indeed, several melanoma, breast, and colon cancer cell lines were highly sensitive (LC₅₀ < 2.5 nM) to psymberin, whereas six leukemia cell lines were invariably insensitive (LC₅₀ > 25 μM), indicating a >10⁴-fold difference in inhibitory activities [22]. Psymberin is the only pederin congener that lacks the A-ring oxane and the 1, 3-dioxane ring, which may account for the remarkably selective inhibitory profile of this compound [22].

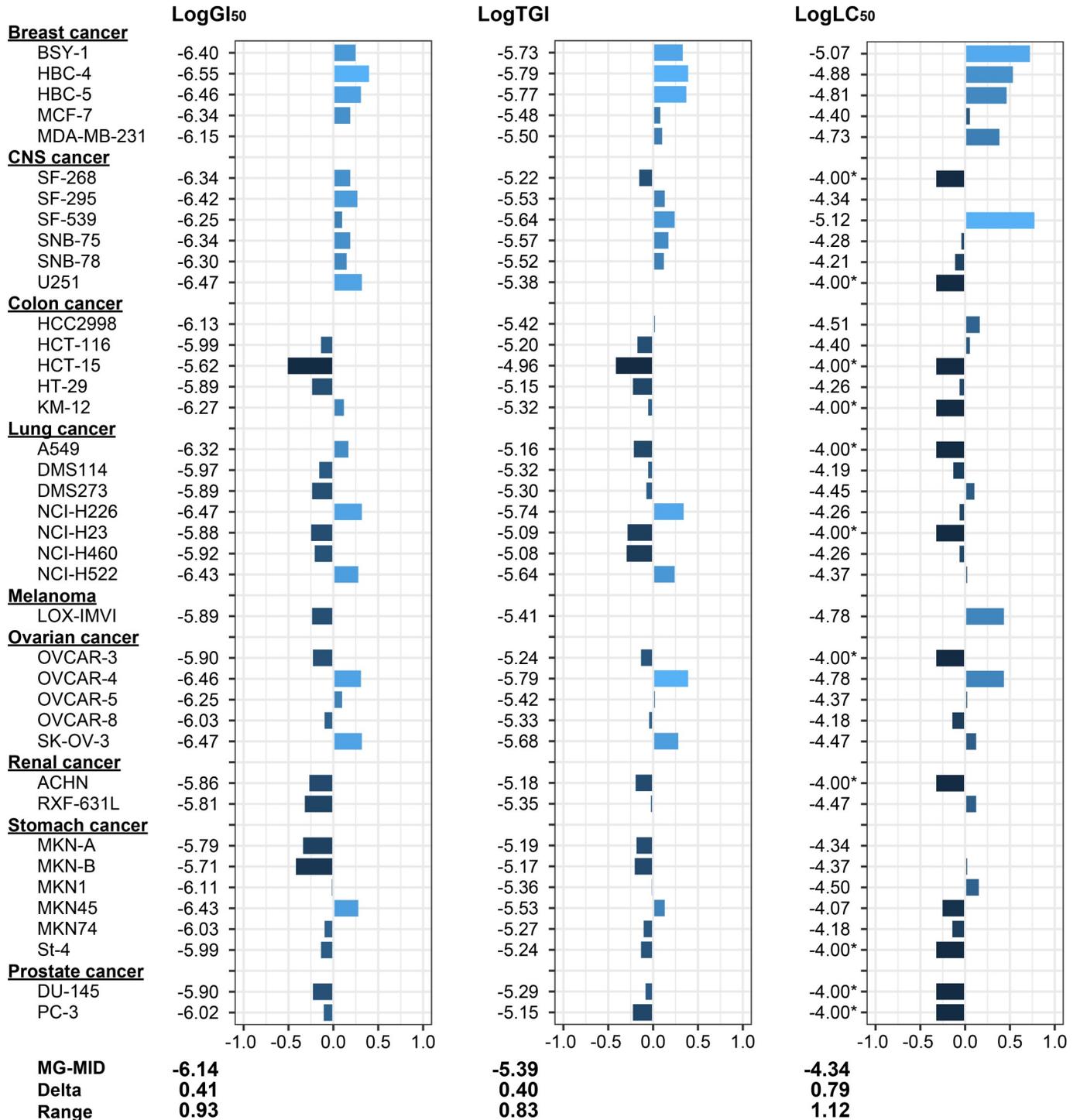


Fig 3. Mean graphs showing the relative sensitivity of each cell line against diaphorin. The logarithms of GI₅₀, TGI, and LC₅₀ are indicated for each cell line. The x-axis represents the difference between the mean of the parameters for all 39 cell lines and the parameters for each cell line. Namely, columns extending to the right of center (mean) indicate that the corresponding cell lines are more sensitive to diaphorin than the average of the 39 cell lines, and columns extending to the left indicate that the cell lines are more resistant to diaphorin than the average. MG-MID: the mean of log GI₅₀, TGI, or LC₅₀ for the 39 cell lines. Delta: the difference between the MG-MID and the log GI₅₀, TGI, or LC₅₀ of the most sensitive cell line. Range: the difference between the log GI₅₀, TGI, or LC₅₀ of the most resistant cell line and those of the most sensitive one. Asterisks (*) indicate that the log LC₅₀ values were greater than -4.00 (10⁻⁴ M), the maximum concentration of diaphorin tested, at which the treatment did not result in 50% reduction of the cells at the end of the treatment as compared with those at the beginning.

<https://doi.org/10.1371/journal.pone.0218190.g003>

The present study revealed that diaphorin inhibits a wide variety of human cancer cells. The selectivity of the inhibitory effect of diaphorin against distinct cancer types was only moderate, suggesting that this compound is less promising as a drug lead compared with psymberin. However, the notably milder inhibitory activity of diaphorin compared with other pederin analogs may make it worthwhile to explore its use for other potential pharmaceutical applications.

Supporting information

S1 Table. Absorbance of sulforhodamine B at a wavelength of 515 nm.
(XLSX)

Acknowledgments

This research was done with technical support from the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Koto, Tokyo, Japan.

Author Contributions

Conceptualization: Atsushi Nakabachi.

Formal analysis: Atsushi Nakabachi.

Funding acquisition: Atsushi Nakabachi.

Investigation: Atsushi Nakabachi, Keiko Okamura.

Project administration: Atsushi Nakabachi.

Supervision: Atsushi Nakabachi.

Validation: Atsushi Nakabachi.

Writing – original draft: Atsushi Nakabachi.

Writing – review & editing: Atsushi Nakabachi.

References

1. Schwartzmann G, Brondani da Rocha A, Berlinck RG, Jimeno J. Marine organisms as a source of new anticancer agents. *Lancet Oncol.* 2001; 2: 221–225. PMID: [11905767](#)
2. Moran NA, McCutcheon JP, Nakabachi A. Genomics and evolution of heritable bacterial symbionts. *Annu Rev Genet.* 2008; 42: 165–190. <https://doi.org/10.1146/annurev.genet.41.110306.130119> PMID: [18983256](#)
3. Hentschel U, Piel J, Degnan SM, Taylor MW. Genomic insights into the marine sponge microbiome. *Nat Rev Microbiol.* 2012; 10: 641–654. <https://doi.org/10.1038/nrmicro2839> PMID: [22842661](#)
4. Freeman MF, Gurgui C, Helf MJ, Morinaka BI, Uria AR, Oldham NJ, et al. Metagenome mining reveals polytheonamides as posttranslationally modified ribosomal peptides. *Science (80-).* 2012; 338: 387–390. <https://doi.org/10.1126/science.1226121> PMID: [22983711](#)
5. Wilson MC, Mori T, Rückert C, Uria AR, Helf MJ, Takada K, et al. An environmental bacterial taxon with a large and distinct metabolic repertoire. *Nature.* 2014; 506: 58–62. <https://doi.org/10.1038/nature12959> PMID: [24476823](#)
6. Engl T, Kroiss J, Kai M, Nechitaylo TY, Svatos A, Kaltenpoth M. Evolutionary stability of antibiotic protection in a defensive symbiosis. *Proc Natl Acad Sci U S A.* 2018; 115: E2020–E2029. <https://doi.org/10.1073/pnas.1719797115> PMID: [29444867](#)
7. Piel J, Butzke D, Fusetani N, Hui D, Platzer M, Wen G, et al. Exploring the chemistry of uncultivated bacterial symbionts: antitumor polyketides of the pederin family. *J Nat Prod.* 2005/03/25. 2005; 68: 472–479. <https://doi.org/10.1021/np049612d> PMID: [15787465](#)

8. Piel J. Bacterial symbionts: prospects for the sustainable production of invertebrate-derived pharmaceuticals. *Curr Med Chem*. 2006; 13: 39–50. <https://doi.org/10.2174/092986706775197944> PMID: 16457638
9. Wan S, Wu F, Rech JC, Green ME, Balachandran R, Horne WS, et al. Total synthesis and biological evaluation of pederin, psymberin, and highly potent analogs. *J Am Chem Soc*. 2011; 133: 16668–16679. <https://doi.org/10.1021/ja207331m> PMID: 21902245
10. Mosey RA, Floreancig PE. Isolation, biological activity, synthesis, and medicinal chemistry of the pederin/mycalamide family of natural products. *Nat Prod Rep*. 2012; 29: 980–995. <https://doi.org/10.1039/c2np20052j> PMID: 22772477
11. Kampa A, Gagunashvili AN, Gulder TAM, Morinaka BI, Daolio C, Godejohann M, et al. Metagenomic natural product discovery in lichen provides evidence for a family of biosynthetic pathways in diverse symbioses. *Proc Natl Acad Sci U S A*. 2013; 110: E3129–E3137. <https://doi.org/10.1073/pnas.1305867110> PMID: 23898213
12. Kust A, Mareš J, Jokela J, Urajová P, Hájek J, Saurav K, et al. Discovery of a pederin family compound in a non-symbiotic bloom-forming cyanobacterium. *ACS Chem Biol*. 2018; 13: 1123–1129. <https://doi.org/10.1021/acscchembio.7b01048> PMID: 29570981
13. Soldati M, Fioretti A, Ghione M. Cytotoxicity of pederin and some of its derivatives on cultured mammalian cells. *Experientia*. 1966; 22: 176–178. PMID: 5959934
14. Brega A, Falaschi A, De Carli L, Pavan M. Studies on the mechanism of action of pederine. *J Cell Biol*. 1968/03/01. 1968; 36: 485–496. Available: <http://www.ncbi.nlm.nih.gov/pubmed/5650903> <https://doi.org/10.1083/jcb.36.3.485> PMID: 5650903
15. Burres NS, Clement JJ. Antitumor activity and mechanism of action of the novel marine natural products mycalamide-A and -B and onnamide. *Cancer Res*. 1989; 49: 2935–2940. PMID: 2720652
16. Matsunaga S, Fusetani N, Nakao Y. Eight new cytotoxic metabolites closely related to onnamide A from two marine sponges of the genus *Theonella*. *Tetrahedron*. 1992; 48: 8369–8376.
17. Richter A, Kocienski P, Raubo P, Davies DE. The in vitro biological activities of synthetic 18-O-methyl mycalamide B, 10-epi-18-O-methyl mycalamide B and pederin. *Anticancer Drug Des*. 1997; 12: 217–227. PMID: 9154112
18. Lee K, Nishimura S, Matsunaga S, Fusetani N, Horinouchi S, Yoshida M. Inhibition of protein synthesis and activation of stress-activated protein kinases by onnamide A and theopederin B, antitumor marine natural products. *Cancer Sci*. 2005; 96: 357–364. <https://doi.org/10.1111/j.1349-7006.2005.00055.x> PMID: 15958059
19. Dang Y, Schneider-poetsch T, Eyley DE, Jewett JC, Bhat S, Rawal VH, et al. Inhibition of eukaryotic translation elongation by the antitumor natural product Mycalamide B. *RNA*. 2011; 17: 1578–1588. <https://doi.org/10.1261/rna.2624511> PMID: 21693620
20. Dyshlovoy SA, Fedorov SN, Kalinovsky AI, Shubina LK, Bokemeyer C, Stonik VA, et al. Mycalamide A shows cytotoxic properties and prevents EGF-induced neoplastic transformation through inhibition of nuclear factors. *Mar Drugs*. 2012; 10: 1212–1224. <https://doi.org/10.3390/md10061212> PMID: 22822368
21. Pettit GR, Xu J, Chapuis J, Pettit RK, Tackett LP, Doubek DL, et al. Antineoplastic agents. 520. Isolation and structure of irciniastatins A and B from the Indo-Pacific marine sponge *Ircinia ramosa*. *J Med Chem*. 2004; 47: 1149–1152. <https://doi.org/10.1021/jm030207d> PMID: 14971894
22. Cichewicz RH, Valeriote FA, Crews P. Psymberin, a potent sponge-derived cytotoxin from *Psammocinia* distantly related to the pederin family. *Org Lett*. 2004; 6: 1951–1954. <https://doi.org/10.1021/ol049503q> PMID: 15176791
23. Kellner RLL, Dettner K. Differential efficacy of toxic pederin in deterring potential arthropod predators of *Paederus* (Coleoptera: Staphylinidae) offspring. *Oecologia*. 1996; 107: 293–300. <https://doi.org/10.1007/BF00328445> PMID: 28307257
24. Piel J. A polyketide synthase-peptide synthetase gene cluster from an uncultured bacterial symbiont of *Paederus* beetles. *Proc Natl Acad Sci U S A*. 2002; 99: 14002–14007. <https://doi.org/10.1073/pnas.222481399> PMID: 12381784
25. Pavan M, Bo G. Pederin, toxic principle obtained in the crystalline state from the beetle *Paederus fuscipes* Curt. *Physiol Comp Oecol*. 1953; 3: 307–312.
26. Nakabachi A, Ueoka R, Oshima K, Teta R, Mangoni A, Gurgui M, et al. Defensive bacteriome symbiont with a drastically reduced genome. *Curr Biol*. 2013; 23: 1478–1484. <https://doi.org/10.1016/j.cub.2013.06.027> PMID: 23850282
27. Nakabachi A, Nikoh N, Oshima K, Inoue H, Ohkuma M, Hongoh Y, et al. Horizontal gene acquisition of *Liberibacter* plant pathogens from a bacteriome-confined endosymbiont of their psyllid vector. *PLoS One*. 2013; 8: e82612. <https://doi.org/10.1371/journal.pone.0082612> PMID: 24349319

28. Nakabachi A. Horizontal gene transfers in insects. *Curr Opin Insect Sci.* 2015; 7: 24–29. <https://doi.org/10.1016/j.cois.2015.03.006>
29. Dan H, Ikeda N, Fujikami M, Nakabachi A. Behavior of bacteriome symbionts during transovarial transmission and development of the Asian citrus psyllid. *PLoS One.* 2017; 12: e0189779. <https://doi.org/10.1371/journal.pone.0189779> PMID: 29240843
30. Yamada T, Hamada M, Floreancig P, Nakabachi A. Diaphorin, a polyketide synthesized by an intracellular symbiont of the Asian citrus psyllid, is potentially harmful for biological control agents. *PLoS One.* 2019; 14: e0216319. <https://doi.org/10.1371/journal.pone.0216319> PMID: 31048920
31. Yamori T. Panel of human cancer cell lines provides valuable database for drug discovery and bioinformatics. *Cancer Chemother Pharmacol.* 2003; 52: S74–S79. <https://doi.org/10.1007/s00280-003-0649-1> PMID: 12819939
32. Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. *Nat Rev Cancer.* 2006; 6: 813–823. <https://doi.org/10.1038/nrc1951> PMID: 16990858
33. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Natl Cancer Inst.* 1991; 83: 757–766. <https://doi.org/10.1093/jnci/83.11.757> PMID: 2041050
34. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>. [Internet]. 2017.
35. Nishimura S, Matsunaga S, Yoshida M, Hirota H, Yokoyama S, Fusetani N. 13-Deoxytedanolide, a marine sponge-derived antitumor macrolide, binds to the 60S large ribosomal subunit. *Bioorganic Med Chem.* 2005; 13: 449–454. <https://doi.org/10.1016/j.bmc.2004.10.012> PMID: 15598566
36. Gürel G, Blaha G, Steitz TA, Moore PB. Structures of triacetyloleandomycin and mycalamide A bind to the large ribosomal subunit of *Haloarcula marismortui*. *Antimicrob Agents Chemother.* 2009; 53: 5010–5014. <https://doi.org/10.1128/AAC.00817-09> PMID: 19738021
37. Szebenyi DM, Kriksunov I, Howe KJ, Ramsey JS, Hall DG, Heck ML, et al. Crystal structure of diaphorin methanol monosolvate isolated from *Diaphorina citri* Kuwayama, the insect vector of citrus greening disease. *Acta Cryst.* 2018; E74: 445–449. <https://doi.org/10.1107/S2056989018002992> PMID: 29765742
38. Iwahashi T, Okochi E, Ono K, Sugawara I, Tsuruo T, Mori S. Establishment of multidrug resistant human colorectal carcinoma HCT-15 cell lines and their properties. *Anticancer Res.* 1991; 11: 1309–1312. PMID: 1679619