J. Biochem. 2009;146(3)383–387
doi:10.1093/jb/mvp085

Siccanin Rediscovered as a Species-Selective Succinate Dehydrogenase Inhibitor

Tatsushi Mogi[1,*], Takuro Kawakami[2], Hiroyuki Arai[2], Yasuo Igarashi[2], Kazunobu Matsushita[3], Mihoko Mori[4], Kazuro Shiomi[4], Satoshi Ōmura[4], Shigeharu Harada[5] and Kiyoshi Kita[1]

[1]Department of Biomedical Chemistry, the University of Tokyo, Bunkyo-ku, Tokyo 113-0033; [2]Department of Biotechnology, the University of Tokyo, Bunkyo-ku, Tokyo 113-8657; [3]Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515; [4]Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, Minato-ku, Tokyo 108-8641; and [5]Department of Applied Biology, Kyoto Institute of Technology, Sakyo-ku, Kyoto 606-8585, Japan

Received and accepted May 20, 2009; published online June 8, 2009

To identify antibiotics targeting to respiratory enzymes, we carried out matrix screening of a structurally varied natural compound library with *Pseudomonas aeruginosa* membrane-bound respiratory enzymes. We identified a succinate dehydrogenase inhibitor, siccanin (IC_{50}, 0.9 μM), which is a potent antibiotic against some pathogenic fungi like *Trichophyton mentagrophytes* and inhibits their mitochondrial succinate dehydrogenase. We found that siccanin was effective against enzymes from *P. aeruginosa*, *P. putida*, rat and mouse mitochondria but ineffective or less effective against *Escherichia coli*, *Corynebacterium glutamicum*, and porcine mitochondria enzyme. Action mode was mixed-type for quinone-dependent activity and noncompetitive for succinate-dependent activity, indicating the proximity of the inhibitor-binding site to the quinone-binding site. Species-selective inhibition by siccanin is unique among succinate dehydrogenase inhibitors, and thus siccanin is a potential lead compound for new chemotherapeutics.

Key words: Antibiotics, complex II, matrix screening, respiratory chain, siccanin, succinate dehydrogenase.

Abbreviations: CIO, cyanide-insensitive oxidase; IC_{50}, the 50% inhibitory concentration; MQO, malate: quinone oxidoreductase; MQR, malate: quinone reductase; NDH1, H^+-translocating NADH dehydrogenase; NDH2, alternative NADH dehydrogenase; NQR, NADH: quinone reductase; Q_1, ubiquinone-1; SDH, succinate dehydrogenase; SQR, succinate: quinone reductase.

Pseudomonas aeruginosa is an opportunistic pathogen ubiquitous in nature, and able to survive in moist environments. *P. aeruginosa* is one of the leading causes of hospital-acquired infections, especially in intensive care units not only because of its high prevalence and severity but also because of its innate and acquired resistance to antimicrobial drugs (*1–3*). The increasing frequency of multidrug-resistant strains is a worldwide health problem, particularly in critically ill and immunocompromised patients (*1*, *3–5*). The empiric antimicrobial therapy of neutropenic patients (*6*) and the shortage of novel antibiotics are aggravating this problem (*7*). Carbapenems are one of the most effective antimicrobial agents and are most commonly used for infections due to *P. aeruginosa*. Carbapenem-resistant strains have already appeared but effective antimicrobial agents to them are currently unavailable. Thus, the prevalence of this pathogen poses a serious therapeutic problem (*8*, *9*) and chemotherapy directed against new classes of targets is an urgent need.

*To whom correspondence should be addressed.
Tel: +81-3-5841-8202, Fax: +81-3-5841-3444,
E-mail: tmogi@m.u-tokyo.ac.jp

Pseudomonas aeruginosa PAO1 has a branched aerobic respiratory chain (*10*). Membrane-bound dehydrogenases [H^+-translocating NADH dehydrogenase (NDH1), alternative NADH dehydrogenase (NDH2), succinate dehydrogenase (SDH), malate: quinone oxidase (MQO)] are linked to cbb_3- and aa_3-type cytochrome *c* oxidases through cytochrome bc_1 or directly to cytochrome bo_3 quinol oxidase and cyanide-insensitive quinol oxidase (CIO), a variant of cytochrome *bd*. Because of the absence in mammalian mitochondria, NDH2, MQO and quinol oxidases are potential targets for new chemotherapeutics. Recently, we are carrying out screening of natural antibiotics in the Kitasato Institute for Life Sciences Chemical Library (*11*) with bacterial and parasitic protist respiratory enzymes and identified gramicidin S, LL-Z1272β and LL-Z1272ε as inhibitors of *Escherichia coli* cytochrome *bd* quinol oxidase, LL-Z1272γ, LL-Z1272δ and LL-Z1272ζ as inhibitors of *E. coli* cytochrome bo_3 quinol oxidase and trypanosome alternative oxidase (*12*, *13*), gramicidin S and scopafungin as inhibitors of *Gluconobacter oxydans* NDH2 (*14*), and LL-Z1272γ as an inhibitor of *G. oxydans* CIO (*15*). From these screening studies, we found that the inhibitory activity of antibiotics varied from species to species. Here we carried out matrix screening of the natural antibiotics library, which is rich in structural variations,

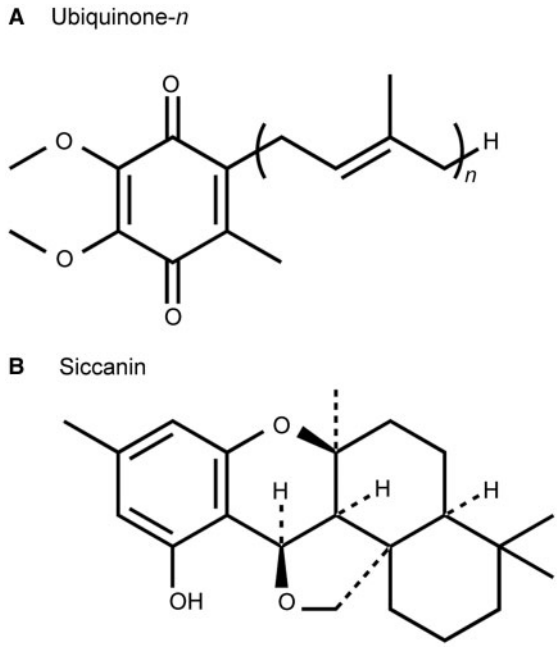

Fig. 1. **Structures of ubiquinone and siccanin.**

with *P. aeruginosa* respiratory dehydrogenases (NDH2, MQO and SDH) and CIO. We identified siccanin (Fig. 1) as a species-selective SDH inhibitor and showed that the site of action is likely the quinone-binding site.

EXPERIMENTAL PROCEDURES

Isolation of P. aeruginosa *Membranes*—*P. aeruginosa* PAO1 cells were grown until stationary phase in LB medium (1.0% tryptone, 0.5% yeast extract and 1.0% NaCl, pH 7.0) at 37°C. Cells were harvested, washed twice with 50 mM K-phosphate (pH 7.0), and suspended in the same buffer. The suspension was passed through a French pressure cell at 110 MPa. After removal of intact cells, membranes were precipitated by centrifugation at 69 000*g* for 60 min and suspended in10 mM K-phosphate (pH 7.0) containing 5 mM $MgCl_2$ and 10% sucrose.

Isolation of Other Bacterial Membranes and Mitochondria—Membranes from *Pseudomonas putida* HK5 (*15*), *Corynebacterium glutamicum* KY9714 (*16*) and *E. coli* GO103/pMFO2 (*12*) were prepared as described previously. Preparation of rat and mouse liver mitochondria (*17*) and porcine heart mitochondria (*18*) was described previously. Animal care and experimental procedures were performed according to the Guidelines for Animal Experimentation, the University of Tokyo.

Screening of a Natural Antibiotics Library—Effects of natural antibiotics (*11*) on NADH: quinone reductase (NQR), malate: quinone reductase (MQR) and succinate: quinone reductase (SQR) activities of the membranes were examined at 10 μg/ml in 50 mM Tris–HCl (pH 7.4) containing 1 mM $MgCl_2$ and 5 mM KCN (buffer A) with SpactraMax Plus^{384} high-throughput spectrophotometer (Molecular Devices) by monitoring the decrease of NADH ($\varepsilon_{340} = 6220\,M^{-1}cm^{-1}$) at 340 nm for NQR or the decrease of the oxidized form of ubiquinone-1 (Q_1; $\varepsilon_{275} = 12\,300\,M^{-1}cm^{-1}$) at 275 nm for MQR and SQR. Reactions were started by addition of 50 μl of the ice-cold membrane suspension (~120 μg protein/ml in buffer A) to 100 μl of the ice-cold reaction mixture, buffer A containing 15 μg/ml of the natural compounds, 0.15 mM Q_1 and an electron donor (0.3 mM NADH, 15 mM L-malate or Na-succinate), in UV-transparent 96-well microplates. The reaction mixture was mixed for 5 sec and the absorbance at 340 or 275 nm was determined before and after 10 min incubation at 25°C. Screening with rat liver mitochondria dehydrogenases was done in 50 mM Tris–HCl (pH 7.4) containing 1 mM $MgCl_2$ and 2 mM KCN. Screening with CIO was done in 50 mM Tris–HCl (pH 7.4) containing 1 mM $MgCl_2$ by using 0.1 mM ubiquinol-1 as an electron donor.

Measurement and Analysis of SQR Activity—After 5-min incubation with inhibitors, the SQR activity of the membranes was determined in buffer A with V-650 spectrophotometer (JASCO, Tokyo) at 25°C. Kinetic analysis and determination of the 50% inhibitory concentration (IC_{50}) were described in (*12*).

RESULTS

Identification of Inhibitors for P. aeruginosa *SDH*—For identification of novel and potent inhibitors of *P. aeruginosa* MQO, NDH1/NDH2 and SDH, we examined effects of 304 microbial compounds in the Kitasato Institute for Life Sciences Chemical Library (*11*) on the ubiquinone-1 reductase activity. Screening with membrane-bound dehydrogenases by 10-min end point assay resulted in the identification of siccanin as a potent SDH inhibitor (residual activity, 21 ± 1%) (Table 1). At a final concentration of 10 μg/ml, siccanin was only one compound which reduced the residual activity to <50% of the control. We found no other compound with the potent inhibitory activity on *P. aeruginosa* NQR and MQR. Furthermore, the independent screening with rat liver mitochondria NDH1 and SDH revealed that there was no potent NDH1 and SDH inhibitor in the library except siccanin (residual activity, 28 ± 5%) (Table 1). Screening with CIO resulted in the identification of rather weak inhibitors, ascofuranone (58 ± 2%) and antimycin A (56 ± 9%), which are known inhibitors of quinol oxidase (*13*, *15*).

Determination of IC50 Value of SDH for Siccanin—At 10 μg/ml of siccanin, we examined effects on the initial velocity of the SQR activity of bacterial membranes and mitochondria in the presence of 0.1 mM Q_1 and 10 mM succinate. We found that SDHs of *P. aeruginosa* (residual activity 13%) and *P. putida* (>1%) membranes, and of mouse (>7%) and rat (19%) liver mitochondria were sensitive to siccanin. In contrast, SDHs of *E. coli* (77%) and *C. glutamicum* (97%) membranes and of porcine heart mitochondria (106%) showed the insensitivity. Then, we determined the IC_{50} value of SDH from *P. aeruginosa* (0.9 μM), *E. coli* (210 μM), rat liver mitochondria (9 μM) and porcine heart mitochondria (860 μM) (Fig. 2). These results indicate that siccanin is the

Table 1. **Effects of siccanin on *P. aeruginosa* and rat mitochondria membrane-bound enzymes determined by 10-min end point assay.**

Enzyme	Residual activity (%)	
	P. aeruginosa membranes	Rat mitochondria
SDH	21 ± 1	28 ± 5
NDH	87 ± 2	108 ± 11
MQO	111 ± 18	NA
CIO	73 ± 3	NA

NA: not applicable, because of the absence of MQO and CIO.

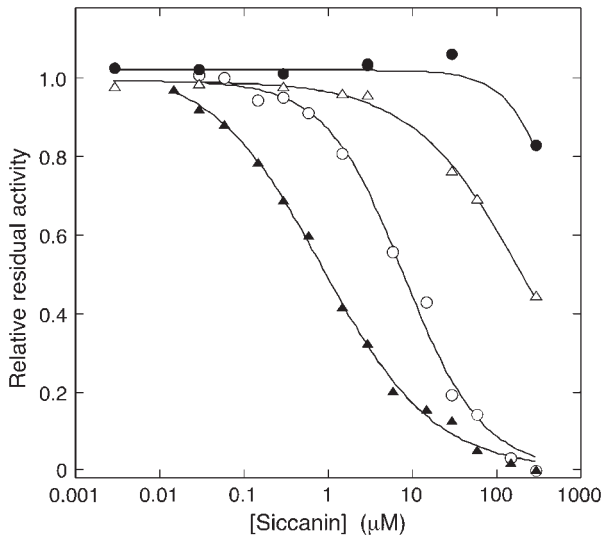

Fig. 2. **Dependence of SQR activity of *P. aeruginosa* and rat mitochondria SDH on the siccanin concentration.** SQR activity was determined in the presence of 0.1 mM Q_1, 10 mM Na-succinate and siccanin and IC_{50} values were estimated to be 0.87 ± 0.09 (*P. aeruginosa* membranes; filled triangle), 9.3 ± 1.0 (rat mitochondria; open circle), 208 ± 14 (*E. coli* membranes; open triangle) and 861 ± 822 μM (porcine mitochondria; filled circle) by using the equation: relative residual activity = $A/[1 + ([\text{Inhibitor}]/IC_{50})^n]$, where A is the total amplitude (~1) and n is the Hill coefficient.

species-selective SDH inhibitor, rather than the universal SDH inhibitor.

Kinetic Analysis of Inhibition of SDH by Siccanin—Structure of siccanin is partially similar to ubiquinone molecule (Fig. 1) and the site of action is likely the quinone-binding site in the SDH complex. Q_1-dependent SQR activity of the control followed substrate inhibition kinetics with the apparent K_m value of 20 μM (Q_1) and the inhibition constant K_{si} of 210 μM (Fig. 3A). The K_m value for Q_1 was comparable to 10 μM of *E. coli* SDH, which followed the Michaelis-Menten kinetics (data not shown). Siccanin inhibited the Q_1-dependent SQR activity in mixed type mode with $K_{i1} = 2$ μM and $K_{i2} = 6$ μM. In contrast, succinate-dependent SQR activity of the control followed the simple Michaelis-Menten kinetics with the apparent K_m value of 0.15 mM for Na-succinate (at 0.1 mM Q_1) (Fig. 3B). Siccanin inhibited the succinate-dependent SQR activity noncompetitively with the K_i value of 6 μM. It should be noted that K_{i1}

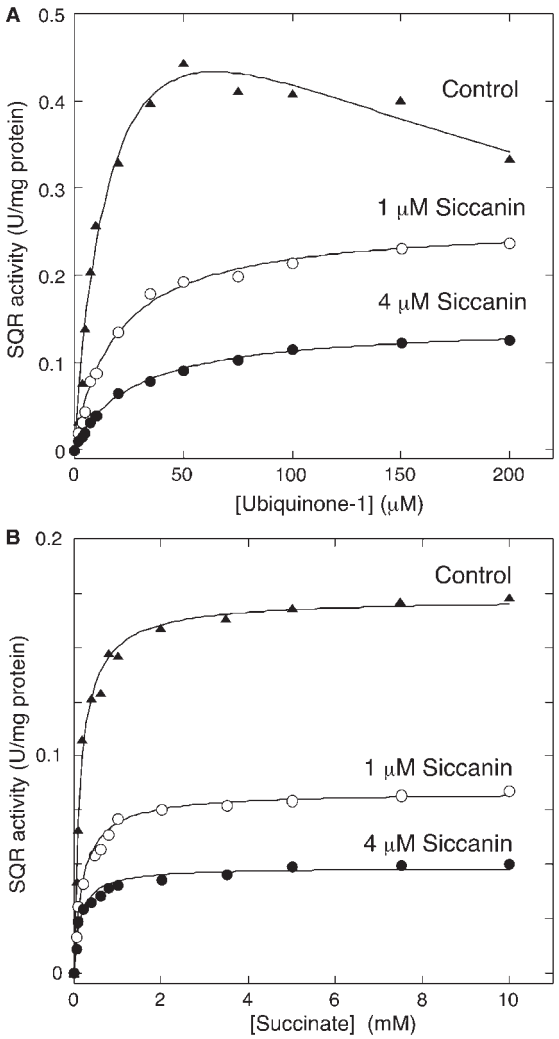

Fig. 3. **Kinetic analysis of inhibition of *P. aeruginosa* SDH by siccanin.** (A) SQR activity was determined as a function of the Q_1 concentration in the absence (filled triangle) or presence of 1 (open circle) and 4 μM (filled circle) siccanin. After 5 min incubation with 10 mM succinate and siccanin, reactions were started by addition of Q_1. Data points were average values from duplicate assay. Data were fitted to the substrate inhibition kinetics (control) or the Michaelis–Menten kinetics (in the presence of siccanin). Apparent K_m, V_{max} and K_{si} values for the control were estimated to be 19.5 ± 3.6 μM (Q_1), 0.853 ± 0.085 U/mg protein, and 214 ± 57 μM, respectively. In the presence of siccanin, Apparent K_m and V_{max} values were estimated to be 20.4 ± 2.6 μM and 0.330 ± 0.019 U/mg protein (1 μM siccanin) and 27.4 ± 1.3 μM and 0.176 ± 0.003 U/mg protein (4 μM siccanin). By assuming mixed type inhibition, K_m, V_{max}, K_{i1} and K_{i2} were estimated to be 19.0 ± 1.6 μM (Q_1), 0.29 + 0.1 U/mg protein, 1.8 ± 0.1 and 5.8 ± 1.7 μM, respectively. (B) SQR activity was determined as a function of the succinate concentration in the absence (filled triangle) or presence of 1 (open circle) and 4 μM (filled circle) siccanin. After 5 min incubation with 0.1 mM Q_1 and siccanin, reactions were started by addition of succinate. Data points were average values from duplicate assay. Data were fitted to the Michaelis-Menten kinetics and the apparent K_m and V_{max} values were estimated to be 0.15 ± 0.01 mM and 0.172 ± 0.002 U/mg protein (control), 0.21 ± 0.01 mM and 0.083 ± 0.001 U/mg protein (1 μM siccanin), and 0.15 ± 0.02 mM and 0.048 ± 0.001 U/mg protein (4 μM siccanin), respectively. By assuming noncompetitive inhibition and the K_m value (succinate) of 0.15 mM, the K_i value was estimated to be 6.4 ± 1.9 μM.

and K_{i2} of the Q_1-dependent reaction are comparable or identical to IC_{50} and K_i of the succinate-dependent reaction, respectively. These results indicate that siccanin binds to a hydrophobic binding pocket, which is within or close to the quinone-binding site.

DISCUSSION

Siccanin has been isolated in 1962 from the culture broth of the fungus *Helminthosposium siccans* Drechsler, a parasitic organism of rye grass (*Lolium multiflorum* Lam) (*19*). It possesses an unusual *cis*,*syn*,*cis*-fused alicyclic ring system (Fig. 1) (*20*, *21*) and exhibits the potent antifungal activity, in particular against several pathogenic fungi [*Trichophyton* (the cause of a skin infection, trichophytosis), *Epidermophyton* and *Microsporum*] (*19*, *22*). Clinical tests by topical application proved the effectiveness of siccanin against superficial fungal infections (*23*). Siccanin at 3 μg/ml completely inhibited the growth of *Trichophyton mentagrophytes* (IC_{50} = 0.3 μg/ml) and the primary site of action of siccanin was identified as SDH (IC_{50} = 0.03 μg/ml (~90 nM)) (*24*). In contrast, concentrations up to 45 μM were hardly inhibitory to succinate oxidation by whole cells of the basidiomycete *Ustilago maydis* (*25*).

Here we rediscovered siccanin as the species-selective SDH inhibitor. Siccanin inhibited bacterial SDH from *P. aeruginosa* and *P. putida* but not SDH from *E. coli*, and *C. glutamicum*. In mitochondria, siccanin inhibited rodent SDH but not porcine SDH. Thus, structure and action mode of siccanin are unique among known SDH inhibitors. Mixed type inhibition of Q_1-dependent SQR activity suggests the proximity of the siccanin-binding site to the ubiquinone-binding site. SDH consists of four subunits (SDH1~SDH4) (*26*) and amino-acid residues responsible for quinone binding (*27*, *28*) are conserved in SDH2, SDH3 and SDH4. Sequence analysis of SDH examined in this study did not result in the identification of amino acid residues that determine the sensitivity against siccanin (data not shown). Future structural studies on a siccanin bound form of SDH would provide a clue for understanding the inhibition mechanism by siccanin and facilitate the structure-based drug design.

IC_{50} of the *P. aeruginosa* growth has been reported to be 50 μg/ml (~150 μM) (*19*), and we obtained a similar value (>100 μg/ml) for the static culture in LB medium. Notably, a difference in the siccanin sensitivity between *P. aeruginosa* and porcine SDH is high as 1000 (Fig. 2). Complex II (SDH and fumarate reductase) is often used for anaerobic respiration in the adaptation of pathogenic bacteria and parasites to host environments (*29*, *30*), thus siccanin is a promising lead compound targeting to SDH. An enantioselective and biomimetic total synthesis of (–)-siccanin has been already established in 2003 (*31*). As discussed recently (*32*–*35*), revival of old antibiotics (e.g. polymyxins) is an alternative choice to combat against infection with multidrug-resistant bacteria. Siccanin is one of such neglected old antibiotics, and we hope that synthetic analogs would be used as potent chemotherapeutics with SDH as a novel drug target.

FUNDING

Grants-in-aid for Scientific Research (C) (20570124 to T.M.) and Creative Scientific Research (18GS0314 to K.K.) from the Japan Society for the Promotion of Science and by a grant-in-aid for Scientific Research on Priority Areas (18073004 to K.K.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Bukholm, G., Tannaes, T., Kjelsberg, A.B., and Smith-Erichsen, N. (2002) An outbreak of multidrug-resistant *Pseudomonas aeruginosa* associated with increased risk of patient death in an intensive care unit. *Infect. Control Hosp. Epidemiol.* **23**, 441–446
2. Obritsh, M.D., Fish, D.N., MacLaren, R., and Jung, R. (2004) National surveillance of antimicrobial resistance in *Pseudomonas aeruginosa* isolates obtained from intensive care unit patients from 1993 to 2002. *Antimicrob. Agents Chemother.* **48**, 4606–4610
3. Paterson, D.L. (2006) The epidemiological profile of infections with multidrug resistant *Pseudomonas aeruginosa* and *Acinetobacter species*. *Clin. Infect. Dis.* **43** (Suppl. 2), S43–S48
4. Aloush, V., Navon-Venezia, S., Seigman-Igra, Y., Cabili, S., and Carmeli, Y. (2006) Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. *Antimicrob. Agent Chemother.* **50**, 43–48
5. Sanders, C.C. and Sanders, W.E. Jr. (1992) Beta-lactam resistance in gram-negative bacteria: global trends and clinical impact. *Clin. Infect. Dis.* **15**, 824–839
6. Bodey, G.P., Bolivar, R., Fainstein, V., and Jadeja, L. (1983) Infections caused by *Pseudomonas aeruginosa*. *Rev. Infect. Dis.* **5**, 279–313
7. Talbot, G.H., Bradley, J., Edwards, J.E. Jr., Gilbert, D., Scheld, M., and Barlett, J.G. (2006) Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin. Infect. Dis.* **42**, 657–668
8. Bonfiglio, G., Carciotto, V., Russo, G., Stefani, S., Schito, G.C., Debbia, E., and Nicoletti, G. (1998) Antibiotic resistance in *Pseudomonas aeruginosa*: an Italian survey. *J. Antimicrob. Chemother.* **41**, 307–310
9. Livermore, D.M. (2004) The need for new antibiotics. *Clin. Microbiol. Infect.* **10** (Suppl. 4), 1–9
10. Matsushita, K., Yamada, M., Shinagawa, E., Adachi, O., and Ameyama, M. (1980) Membrane-bound respiratory chain of *Pseudomonas aeruginosa* grown aerobically. *J. Bacteriol.* **141**, 389–392
11. Ui, H., Ishiyama, A., Sekiguchi, H., Namatame, M., Nishihara, A., Takahashi, A., Shiomi, K., Otoguro, K., and Ōmura, S. (2007) Selective and potent *in vitro* antimalarial activities found in four microbial metabolites. *J. Antibiot.* **60**, 220–222
12. Mogi, T., Ui, H., Shiomi, K., Ōmura, S., and Kita, K. (2008) Gramicidin S identified as a potent inhibitor for cytochrome *bd*-type quinol oxidase. *FEBS Lett.* **582**, 2299–2302
13. Mogi, T., Ui, H., Shiomi, K., Ōmura, S., Miyoshi, H., and Kita, K. (2009) Antibiotics LL-Z1272 identified as novel inhibitors discriminating bacterial and mitochondrial quinol oxidases. *Biochim. Biophys. Acta* **1787**, 129–133
14. Mogi, T., Matsushita, K., Miyoshi, H., Ui, H., Shiomi, K., Ōmura, S., and Kita, K. (2009) Identification of new

inhibitors for alternative NADH dehydrogenase (NDH-II). *FEMS Microbiol. Lett.* **291**, 157–161
15. Mogi, T., Ano, Y., Nakatsuka, T., Muroi, A., Miyoshi, H., Migita, C.T., Ui, H., Shiomi, K., Ōmura, S., Kita, K., and Matsushita, K. (2009) Biochemical and spectroscopic properties of cyanide-insensitive quinol oxidase from *Gluconobacter oxydans*. *J. Biochem.* **146**, in press (doi: 10.1093/jb/mvp067)
16. Nantapong, N., Kugimiya, K., Toyama, H., Adachi, O., and Matsushita, K. (2004) Effect of NADH dehydrogenase-disruption and over-expression on respiration-related metabolism in *Corynebacterium glutamicum* KY9714. *Appl. Microbiol. Biotechnol.* **66**, 187–193
17. Miyadera, H., Shiomi, K., Ui, H., Yamaguchi, Y., Masuma, R., Tomoda, H., Miyoshi, H., Osanai, A., Kita, K., and Ōmura, S. (2003) Atpenins, potent and specific inhibitors of mitochondrial complex II (succinate-ubiquinone oxidoreductase). *Proc. Natl Acad. Sci. USA* **100**, 473–477
18. Huo, X., Su, D., Wang, A., Zhai, Y., Xu, J., Li, X., Bartlam, M., Sun, F., and Rao, Z. (2007) Preliminary molecular characterization and crystallization of mitochondrial respiratory complex II from porcine heart. *FEBS J.* **274**, 1524–1529
19. Ishibashi, K. (1962) Studies on antibiotics from *Helminthosposium* sp. fungi. VII. Siccanin, a new antifungal antibiotic produced by *Helminthosposium siccans*. *J. Antibiot.* **A15**, 161–167
20. Hirai, K., Okuda, S., Nozoe, S., and Iitaka, Y. (1969) The crystal and molecular structure of siccanin *p*-bromobenzenesulphonate. *Acta Crystallogr.* **B25**, 2630–2638
21. Iitaka, Y., Ishibashi, K., and Shirasaka, M. (1967) The structure of siccanin. *Tetrahedron Lett.* **23**, 2177–2179
22. Arai, M., Nose, K., Nakahara, M., Kitahara, N., and Naito, A. (1968) Antimicrobial properties of siccanin. *Ann. Sankyo Res. Lab.* **20**, 80–88
23. Kitano, N., Kondo, F., Kusano, K., and Ishibashi, K. (1976) Fungicidal compositions for dermatomycosis of animals. US Patent 3974291
24. Nose, K. and Endo, A. (1971) Mode of action of the antibiotic siccanin on intact cells and mitochondria of *Trichophyton mentagrophytes*. *J. Bacteriol.* **105**, 176–184
25. Georgopoulos, S.G., Alexandri, E., and Chrysayi, M. (1972) Genetic evidence for the action of oxathiin and thiazole derivatives on the succinic dehydrogenase system of *Ustilago maydis* mitochondria. *J. Bacteriol.* **110**, 809–817
26. Cecchini, G. (2003) Function and structure of Complex II of the respiratory chain. *Annu. Rev. Biochem.* **72**, 77–109
27. Yankovskaya, V., Horsefield, R., Tornroth, S., Luna-Chavez, C., Miyoshi, H., Leger, C., Byrne, B., Cecchini, G., and Iwata, S. (2003) Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science* **299**, 700–704
28. Sun, F., Huo, X., Zhai, Y., Wang, A., Xu, J., Su, D., Bartlam, M., and Rao, Z. (2005) Crystal structure of mitochondrial respiratory membrane protein complex II. *Cell* **121**, 1043–1057
29. Boshoff, H.I.M. and Barry, C.E. 3rd. (2005) Tuberculosis-Metabolism and respiration in the absence of growth. *Nature Rev. Microbiol.* **3**, 70–80
30. Saruta, F., Kuramochi, T., Nakamura, K., Takamiya, S., Yu, Y., Aoki, T., Sekimizu, K., Kojima, S., and Kita, K. (1995) Stage-specific isoforms of complex II (succinate-ubiquinone oxidoreductase) in mitochondria from the parasitic nematode, *Ascaris suum*. *J. Biol. Chem.* **270**, 928–932
31. Trost, B.M., Shen, H.C., and Surivet, J. (2003) An enantioselective biomimetic total synthesis of (–)-siccanin. *Angew. Chem.* **115**, 4073–4077
32. Falagas, M.E. and Kasiakou, S.K. (2005) Colistin: the revival of polymyxins for the management of multidrug–resistant Gram-negative bacterial infections. *Clin. Infect. Dis.* **40**, 1333–1341
33. Evans, M.E., Feola, D.J., and Rapp, R P. (1999) Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram negative bacteria. *Ann. Pharmacother.* **33**, 960–967
34. Levin, A.S., Barone, A.A., Penço, J., Santos, M.V., Marinho, I.S., Arruda, E.A., Manrique, E.I., and Costa, S.F. (1999) Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clin. Infect. Dis.* **28**, 1008–1011
35. Li, J., Nation, R.L., Turnidge, J.D., Milne, R.W., Coulthard, K., Rayner, C.R., and Paterson, D.L. (2006) Colistin: the re-emerging antibiotic for multidrug-resistant gram-negative bacterial infections. *Lancet Infect. Dis.* **6**, 589–601