Abenquines and their Synthetic Analogues Exert Algicidal Activity against Bloom-Forming Cyanobacteria

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ABSTRACT: Abenquines are natural quinones, produced by some *Streptomycetes*, showing the ability to inhibit cyanobacterial growth in the 1 to 100 µM range. To further elucidate their biological significance, the synthesis of several analogues (**4f**-**4h**, **5a**-**5h**) allowed us to identify some steric and electronic requirements for bioactivity. Replacing the acetyl by a benzoyl group in the quinone core and also changing the amino acid moiety with ethylpyrimidinyl or ethylpyrrolidinyl groups resulted in analogues 25-fold more potent than the natural abenquines. The two most effective analogues inhibited the proliferation of five cyanobacterial strains tested, with IC_{50} values ranging from 0.3 to 3 µM. These compounds may be useful leads for the development of an effective strategy for the control of cyanobacterial blooms.

Cyanobacteria, also referred to as blue-green algae, are the only prokaryotes able to perform oxygenic photosynthesis.[1] Widely distributed even in strikingly different and harsh habitats, they play a major role in both carbon and nitrogen global cycling, since several free-living strains can accomplish biological N_2 fixation.[2] In freshwater bodies and coastal waters under favorable conditions, *e.g.*, warm temperature, sunlight and increased N and P availability, some cyanobacteria are capable of blooming, rapidly multiplying and forming a dense surface scum.[3] This often leads to the establishment of anoxic conditions, altering the ecological structure and contributing to the ecosystem's decline.[4] Moreover, with the only exception of the genus *Spirulina/Arthrospira*, cyanobacteria produce a variety of potentially harmful toxins, targeting either the liver, the gastrointestinal apparatus or the nervous system.[5] The release of high concentrations of toxins in cyanobacterial blooms causes concern for public safety and human health, deteriorates freshwater quality affecting drinking water supplies, and threatens aquaculture and fisheries, potentially causing serious economic damage.[6] Since the frequency of cyanobacterial blooms is increasing as a consequence of both pollution and climate changes,[7] the development of efficient methods for bloom control is urgently required.

With this aim, physical[8] and chemical[9] strategies have been exploited. However, since the former are too costly to be applied on a large scale and the latter can cause further pollution, a biological approach to the problem would be a possibly better choice.[10] Under natural conditions, cyanobacterial proliferation seems to be limited by some algicidal bacteria that are believed to contribute to control and terminate blooms.[11] Many bacteria showing inhibitory properties *in vitro* against harmful cyanobacteria have been recently isolated whose activity was shown to rely on production and release of active substances.[12-16] Despite the large number of organisms with algicidal activity, the

development of effective biological strategies for bloom control requires isolation, characterization and inexpensive chemical synthesis of these active compounds.[17]

During the last decades the exploitation of microbial products and their synthetic analogues has proved a promising source of new bioactive compounds for agriculture, medicine, and the food industry.[18-21] Within the plethora of active principles identified so far, quinones are an important class of naturally occurring and synthetic compounds[22-25] with a variety of biological properties, such as antibacterial, antifungal, and antitumor activity.[26-28] Owing to their electron transfer properties,[29] they also have the ability of interfering with the photosynthetic electron transport chain, resulting in phytotoxic effects.[30–32]

Among natural quinones are the abenquines, a group of benzoquinones bearing both an *N*-acetyl and an amino acid moiety (Figure 1). These compounds were recently isolated from the fermentation broth of a *Streptomyces* sp. found in the Chilean highland of the Atacama desert,[33] and were synthesized for the first time by our group.[34] Abenquines were shown to possess only mild inhibitory properties against bacteria, dermatophytic fungi and phosphodiesterase type 4b.[33] However, abenquines A, B2 and D in the concentration range of 10^{-6} to 10^{-4} M were found to inhibit the growth of a model cyanobacterial strain, *Synechococcus elongatus* PCC 6301.[34]

In continuation of our interest in using natural products as a model for the development of new synthetic bioactive compounds,[35–39] we further investigated the potential of abenquines as algicides. With this aim, variations were introduced in their scaffold by replacing either the amino acid substituent with different amines, or adding a bulky group through the substitution of the acetyl for a benzoyl group (Figure 2). Here we report that, with the exception of abenquine C, both natural and synthetic abenquines were able to inhibit cyanobacterial growth, with some derivatives showing a 25-fold

increased effectiveness. The activity of the two most effective compounds was evaluated against various cyanobacterial strains, some heterotrophic microorganisms, and plants at either the seedling or the undifferentiated tissue level, pointing out a selectivity for photosynthetic bacteria.

■ RESULTS AND DISCUSSION

Synthesis of Natural Abenquines and some Analogues. Abenquines (**4a-d**) and their analogues (**4e-h**; **5a-h**) were prepared as shown in Scheme 1.

In order to explore the ability of abenquines to inhibit cyanobacterial growth, a number of potentially active compounds was desired. Two parts of the molecule were modified, replacing either of the substituents in the core of the benzoquinone group (Figure 2). Initially the amino acid moiety was substituted with different amines, maintaining the acetamide group as part of the abenquine scaffold. The preparation of analogues (**4e-h**) involved the reaction of an equivalent amount of the different amines and quinone **3a**.[34,40]

Further modification on the abenquine structure involved replacement of the acetyl moiety by the bulkier group benzoyl (Figure 2). To obtain such analogues **5a-h**, the required intermediate quinone **3b** was prepared employing the same chemical procedure used for the synthesis of **3a**. Initially *N*-(2,5-dimethoxyphenyl)benzamide (**2b**) was prepared in quantitative yield by reaction of commercial 2,5-dimethoxyaniline (**1**) and benzoyl chloride. Further oxidation of **2b** with PIDA afforded the benzoquinone **3b** (Scheme 1). The benzoyl analogues of natural abenquines (**5a-d**) were then synthesized by reacting **3b** with different amino acids. The amino-analogues (**5e-h**) were synthesized in the same way by reaction of **3b** with different amines.[34]

The structures of compounds **4a-h** and **5a-h** were confirmed by extensive spectroscopic analysis. In the IR spectra, the typical signals corresponding to stretch vibration of NH_3^+ group in the region ν_{max} = 3554-2794 cm^{-1} were evident in the case of compounds bearing amino acid groups (**4a-d** and **5a-d**), whereas for the analogues carrying amine groups (**4e-h** and **5e-h**) the stretching vibration of N-H between $\bar{\upsilon}$ = 3379-3238 cm^{-1} was observed.[41] In ^1H NMR spectra, all acetamide compounds (**4a-h**) showed a signal as a singlet around δ = 2.06-2.22 corresponding to the methyl in the acetamide moiety. The signals of the aromatic hydrogen atoms corresponding to the benzoyl group in compounds **5a-h** were observed between δ = 7.50-7.90. In addition, all signals corresponding to residues of amines or amino acids in the high shielded region were confirmed in ^{13}C NMR spectra. Moreover, the carbonyls of amide and quinone were detected at δ = 165.83-171.62 and δ = 178.01-184.48, respectively. A detailed assignment of ^1H and ^{13}C NMR for all new compounds is presented in the experimental section. The assignments were possible by means of 2D NMR techniques when required, and the data were fully consistent with the proposed structures (See Supplementary Material for selected ^1H and ^{13}C NMR spectra).

Both Natural and Synthetic Abequines are Able of Inhibiting Cyanobacterial Growth (Table 1). When added at micromolar concentrations to the culture medium, abenquines A (**4a**), B2 (**4b**) and D (**4d**) inhibited the growth of the cyanobacterium *Synechococcus elongatus* PCC 6301 (IC_{50} ~ 10 µM). Abenquine C (**4c**) was less effective, causing 40%-inhibition at 100 µM (Figure S1).[34]

Analogues (**5a-d**) in which the acetyl moiety had been replaced with a benzoyl group, showed similar activity. However compound **5c**, analogue of the poorly active abenquine C (**4c**), was also inhibitory. Analogues containing a benzoyl group were

slightly less active than their acetyl counterparts when bearing aromatic amino acids (**5a**, **5d**), but the activity increased or did not change when an aliphatic amino acid was present (**5b**, **5c**). Compound **5e**, with an aromatic group in both sides, presented an IC_{50} > 100 μM whereas its acetyl-analogue **4e** was more active (IC_{50} = 2.0 μM).

Interestingly, when the amino acid moieties were replaced with some amino groups, the biological activity of abenquines was significantly enhanced. In particular, the presence of groups such as ethylpyrimidinyl and ethylpyrrolidinyl (as in compounds **4f-g**) caused a ten-fold increase of the inhibitory potential (Figure S1), with IC_{50} values lower than 1 μM (Table 1). The more aliphatic benzamide analogues **5f-g** showed a higher effectiveness, causing almost complete suppression of cyanobacterial growth at 1 μM (Figure S1).

The Most Effective Abenquine Analogues Show Selective Toxicity for Photosynthetic Bacteria. Compounds **5f** and **5g** were evaluated against a wide range of cyanobacteria. Data, summarized in Table 2, showed that all strains were highly susceptible to these quinones, with IC_{50} values ranging from 0.3 to 3.0 μM. Interestingly, the three unicellular, non-nitrogen-fixing strains belonging to the order *Chroococcales* were significantly more sensitive than the two filamentous, nitrogen-fixing strains of the order *Nostocales*. The growth of *Microcystis aeruginosa*, which is believed the most dangerous toxin-producing species,[42] was inhibited at concentrations about one order of magnitude lower than those found effective in the case of some natural inhibitors recently described, comprising two algicidal diketopiperazines produced by a *Chryseobacterium* sp.,[15] the active 3-methylindole isolated from an algicidal strain of *Aeromonas* sp.,[16] and the pyrazine-1,4-diones purified from an algicidal *Bacillus* sp.[14]

Due to their electron transfer properties,[29] the toxicity of these abenquines could depend on their ability to interfere with the photosynthetic electron transport chain, as previously shown for other quinones.[30-32] If so, plants and eukaryotic algae would be most likely susceptible to their activity, representing a drawback for the use in the environment to control cyanobacterial blooms. Thus the effect of **5f** and **5g** on the growth of rice (a monocotyledon) and rapeseed (a dicotyledonous species) were evaluated under either photoautotrophic (seedlings) or heterotrophic (suspension cultured cells) conditions. Results showed a negligible effect at 3 and 10 µM for seedling and cultured cells, respectively. Concentrations in the range from 10^{-7} to $3x10^{-6}$ M, levels at which the growth of cyanobacteria is progressively reduced, did not exert any phytotoxic effect.

Compound **5f** and **5g** were evaluated against *Escherichia coli*, *Lactobacillus casei* and *Saccharomyces cerevisiae*. Data showed that the growth of the eukaryotic microorganism in a mineral medium was substantially unaffected up to 100 µM, whereas that of *E. coli* was strongly inhibited in the 1 to 100 µM range, with IC_{50} values of 13 and 23 µM for **5f** and **5g**, respectively. The growth of *L. casei* was also affected, although to a lesser extent (Table 2). As often observed in the case of antimetabolic compounds, when the experiment was repeated growing the bacteria in organic media the sensitivity was much lower, with only a slight growth inhibition at 100 µM.

Moreover, preliminary data showed a significant cytotoxicity of some abenquines against non-malignant NIH 3T3 mouse fibroblasts (Table 2). However, in the case of the most active compounds **5f** and **5g** a selectivity index was evident (SI = 5.5 and 7.0 respectively, Table S1) between the effect on *Synechococcus elongatus* PCC 6301 and that on mouse cells.

The Physicochemical Properties of the Most Effective Abenquines Comply with the Tice's Rule-of-five. To be developed as a new pesticide, a bioactive compound should have some specific properties.[43-45] Pesticides must be absorbed by pests, be transported to the target site and finally interact with the molecular target. All these steps may be greatly influenced by solubility, lipophilicity, stability and acid-base character of a compound.[45] To help characterizing the suitability of a substance as a potential pesticide, a set of rules based on key molecular structure descriptors and lipophilicity have been defined.[43] These parameters were thus calculated for abenquines **4a-d** and their analogues **4e-h** and **5a-h** (Table 3).

In general, the molecular mass should range between 150 and 500.[43] Increasing molecular mass has frequently been observed to correlate with lower solubility and poorer penetration through membranes. In Tice's rule, the logarithm of the octanol/water partition coefficient (Log *P*) should be between 1.2 and 2.4; log *P* values for compounds **4e-h** are lower than 1.0, but those of compounds **5f** and **5g** fall in the suitable range. All the compounds present suitable numbers of hydrogen-bond acceptors and rotatable bonds (Table 3), and most abenquines and their analogues have a suitable number of hydrogen-bond donors. The most effective compounds **5f** and **5g** present molecular structure descriptors and lipophilicity values that seem on the whole compatible with a potential use as pesticides.

Conclusions. The replacement in the quinone core of the acetyl with a benzoyl moiety and of the amino acid with an ethylpyrimidinyl or an ethylpyrrolidinyl group increased the inhibitory potential of natural abenquines up to 25-fold, and the most active analogues exerted their effects in the 0.1 to 1 µM range. Therefore, these substances appear as promising tools toward the control of cyanobacterial blooms.

■ EXPERIMENTAL SECTION

General Experimental Procedures. All reagents were purchased from Sigma-Aldrich (Milwaukee, Wisconsin, USA) and were used without any purification; solvents were acquired from Fluka (Rio de Janeiro-Brazil). Analytical thin layer chromatography (TLC) was performed on silica gel 60 F_{254} 0.2 mm thick plates (Merck). Flash column chromatography was performed using silica gel 230-400 mesh. All compounds were fully characterized by EI-MS, ^{1}H NMR and ^{13}C NMR spectroscopy. Mass spectra were recorded on a Finnigan MAT LCQ 7000 or Sciex API2000 under electro spray ionization (ESI). Elemental analyses were measured on a Foss-Heraeus Vario EL unit. The ^{1}H and ^{13}C-NMR spectra were recorded on a Varian Mercury 300 spectrometer at 300 and 75 MHz, or on a Bruker Avance at 400 MHz and 100 MHz. Chemicals shifts (δ) are reported from tetramethylsilane with the solvent resonance as the internal standard.

Synthesis. Compounds **2a, 3a,** and **4a-e** were synthesized using previously published methods.[34] For synthesis of analogues **4f-h**, a round bottom flask was charged with 2-acetamido-1,4-benzoquinone (**3a**) (192 mg, 1.1 mmol) and dichloromethane (5 mL) at room temperature. To this solution the appropriate amine (1.1 mmol) was added and the resultant reaction mixture was stirred for 2 h, followed by removal of the solvent under reduced pressure. The obtained residue was purified by silica gel column chromatography (silica gel, Hex/EtOAc 1:1 v/v and EtOAc/MeOH 1:0.5 v/v). The physical and spectroscopic data for all compounds are in the Supplementary Material.

*N-(2,5-dimethoxyphenyl)benzamide (**2b**).* To a round-bottomed flask at 0 °C, under magnetic stirring, were added 2,5-dimethoxyaniline **1** (2.0 g, 13.1 mmol), trimethylamine (2.1 mL, 15.2 mmol) and dichloromethane (50 mL). To this solution

was added benzoyl chloride (1.8 mL, 15.2 mmol), and the temperature was increased to 25 ºC. After 2 h the mixture was quenched by addition of water. The organic phase was separated and further washed with saturated aqueous $NaHCO_3$ solution and brine. After that it was dried over $MgSO_4$. filtered and concentrated under reduced pressure to give the compound as a brown solid. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate:hexane 1:3 v/v, to give the title compound. The physical and spectroscopic data and ^{1}H and ^{13}C spectra for compound **2b** are in the Supplementary Material.

*2-benzamido-1,4-benzoquinone (**3b**).* To a round-bottomed flask were added 2,5-dimethoxyphenyl)benzamide **2b** (1.0 g, 3.9 mmol), water (25 mL) and MeOH (625 μL). To the suspension was added phenyliodine diacetate (1.9 g, 5.9 mmol) and the resultant mixture was stirred stirred for 1.5 h, before it was diluted with water (50 mL) and extracted with dichloromethane. (3 x 50 mL). The combined extracts were washed with water, saturated aqueous sodium hydrogen carbonate solution, and dried over Na_2SO_4. Then, the solution was filtered and the solvent was concentrated under reduced pressure. The crude product was used without further purification.

For the preparation of analogues **5a-d**, to a round-bottomed flask was added the required amino acid (0.31 mmol) and sodium bicarbonate (52 mg, 0.62 mmol), dissolved in 5 mL of EtOH. Then, 2-benzamido-1,4-benzoquinone (**3b**) (70 mg, 0.31 mmol) was added and the mixture was stirred at reflux for 2 h. Then the reaction mixture was filtered to remove the sodium bicarbonate salt, and washed with ethanol. Therefore, the resulting residual mixture was acidified to pH 4-5 with hydrochloric acid 1 M. The mixture was extracted with EtOAc. The organic extracts were combined, and the resulting organic phase was dried over Na_2SO_4, filtered and the solvent concentrated

under reduced pressure. The obtained residue was purified by silica gel column chromatography eluting at first with Hex/EtOAc 1:1 v/v and then with acetone/methanol 1:1 v/v.

Analogues **5e-5h** were synthetized by combination of *2-benzamido-1,4-benzoquinone* (**3b**) (150 mg, 0.6 mmol) in dichloromethane (5 mL) and the appropriate amine (0.6 mmol) in a round bottom flask, at room temperature and stirring for 2 h. The reaction mixture was concentrated under reduced pressure and the obtained residue was purified by column chromatography (silica gel, Hex/EtOAc 3:1 v/v). The physical and spectroscopic data and ^{1}H and ^{13}C spectra for all compounds are in the Supplementary Material.

Cyanobacterial Cultures. Cyanobacterial strains, as listed in Table 2, were grown in Bg11 mineral medium as previously described.[47] Growth was followed by measuring chlorophyll concentration. Culture aliquots (0.5−1.0 mL) were withdrawn, and cells were sedimented by centrifugation for 3 min at 14000 x *g*. Pellets were resuspended with 1.0 mL of methanol, and solubilization was allowed to proceed for 30 min in the dark, with occasional mixing. Samples were then centrifuged again, and chlorophyll content in the supernatant was estimated spectrophotometrically at 663 nm. Late log-grown cells were sedimented by centrifugation 5 min at 4,000 x *g*, and used to inoculate 96-well plates, 0.2 mL per well, to an initial density of about 1.0 mg L^{-1} chlorophyll. Aliquots (2 μL) of suitable dilutions of a given compound in DMSO were added so as to obtain final concentrations ranging from 0.1 to 100 μM. A complete randomized design with four replications was adopted. Cell growth in each well was followed for one week by daily determination of absorbance using a Ledetect 96 plate reader (Labexim, Lengau, Austria) equipped with a LED plugin at 660 nm. Following

logarithmic transformation of data, growth constants were calculated, and expressed as percent of the mean value for controls (no less than 8 replications) treated with the same volume of DMSO. Mean values ± SE over replicates are reported. The concentrations causing 50% inhibition (IC_{50}) and their confidence limits were estimated by nonlinear regression analysis using Prism 6 for Windows, version 6.03 (GraphPad Software).

Bacteria and Yeast Cultures. *Escherichia coli* strain BL21(DE3)pLysS was grown at 37 °C in either standard Luria Broth or Davis and Mingioli medium. The effect of abenquines on its growth was evaluated as described for cyanobacteria, except that the inoculum was made to an initial density of about 0.1 Abs (600 nm), and growth was followed for 6 h by determining at 30 min intervals the increase in absorbance using the plate reader equipped with a LED plugin at 600 nm. *Lactobacillus casei* strain DSM 20011 was grown at 35 °C in either *optimum point medium*[48] or in basal minimal medium.[49] Cultures were inoculated to an initial density of about 0.2 Abs (600 nm), and growth was followed for 10 h at 1-h intervals, as indicated. Baker yeast Ura3$^-$ strain S23344C was grown at 30 °C in either standard Yeast Carbon Base (without amino acids, and with 1.0 g L^{-1} ammonium sulfate as the nitrogen source and 50 mg L^{-1} uracil) or Yeast Extract Peptone Dextrose medium. Growth was followed for 10 h by determining every 60 min the absorbance at 600 nm.

Plant Cell Cultures and Seedlings. Rice (*Oryza sativa* L. cv Gigante Vercelli) and rapeseed (*Brassica napus* L., cv. Zeruca) seedlings and suspension-cultured cells were grown as described.[50-51] To measure the effect of abenquines on culture growth, cell samples were withdrawn from the stock cultures in the early stationary growth phase and used to inoculate 100 mL culture flasks containing 25 mL

of fresh medium with or without the test compound, to a density of about 1.0 mg mL^{-1} (dry weight). After 8 days of incubation, when untreated controls reached the late exponential phase of growth, cells were harvested by vacuum filtration, and the dry weight increase was determined on each sample after drying in an oven at 90°C for 48 h. At least four replicates were carried out for each treatment. Data were expressed as percent values of untreated controls. In the case of seedling growth, seeds were sown in Magenta GA7 vessels containing 50 mL of agarized (6‰) half-strength MS medium containing or not the test compound, 16 seeds for vessel. A randomized complete block design with three replications was used, each block consisting of 5 vessels of 4 abenquine rates and untreated controls. Destructive harvest was carried out 14 to 16 days after sowing, when controls had reached the three-leaf stage, and each seedling was weighed individually ($40 \leq n \leq 48$, depending on germination rate).

■ ASSOCIATED CONTENT

Supporting Information

Spectroscopic data and ^{1}H and ^{13}C NMR spectra for synthetic abenquines, and their effects on model cyanobacterial strain and plant species. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Table 1. Concentrations of natural and synthetic abenquines able to inhibit by 50% (IC_{50}) the growth of the model cyanobacterial strain *Synechococcus elongatus* PCC 6301.

Compound	**IC_{50} (µM)****	**Compound**	**IC_{50} (µM)**
4a*	9.6 ± 2.2	**5a**	27.7 ± 3.0
4b*	13.8 ± 1.9	**5b**	13.5 ± 1.0
4c*	> 100	**5c**	25.9 ± 1.8
4d*	11.7 ± 2.6	**5d**	26.2 ± 1.6
4e*	2.0 ± 0.2	**5e**	> 100
4f	0.8 ± 0.1	**5f**	< 0.50
4g	0.6 ± 0.1	**5g**	< 0.50
4h	3.8 ± 0.6	**5h**	3.1 ± 0.2

*Data quoted from reference 34.

** The IC_{50} values in µg/mL is presented in Table S2.

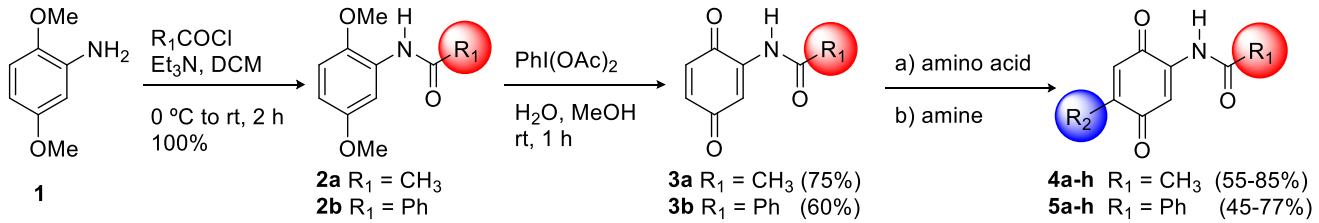
Table 2. Concentrations of **5f** and **5g** able to inhibit by 50% the growth of various organisms.

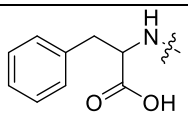
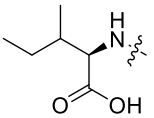
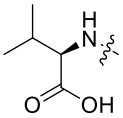
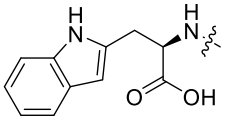
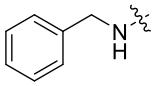
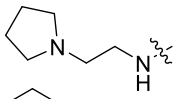
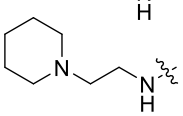
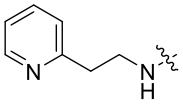
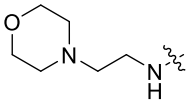
Species	**Compound 5f IC_{50} (µM) ***	**Compound 5g IC_{50} (µM)**
Nostoc sp. PCC 7524-1	2.4 ± 0.1	2.6 ± 0.2
Anabaena sp. PCC 7120	3.1 ± 0.3	1.7 ± 0.2
Microcystis aeruginosa PCC 7941	0.8 ± 0.1	0.4 ± 0.0
Synechocystis sp. PCC 6803	0.4 ± 0.0	0.4 ± 0.0
Synechococcus elongatus PCC 6301	0.4 ± 0.0	0.3 ± 0.0
Oryza sativa L., seedlings	> 100	> 100
Oryza sativa L., cultured cells	> 100	> 100
Brassica napus L., seedlings	> 100	> 100
Brassica napus L., cultured cells	> 100	> 100
Escherichia coli, mineral medium	13.4 ± 0.7	22.7 ± 1.2
Escherichia coli, organic medium	> 100	> 100
Lactobacillus casei, mineral medium	62.7 ± 8.9	83.1 ± 12.2
Lactobacillus casei, organic medium	> 100	> 100
Saccharomyces cerevisiae, mineral medium	> 100	> 100
Saccharomyces cerevisiae, organic medium	> 100	> 100
non-malignant mouse fibroblasts (NIH 3T3)	2.0	2.1

* The IC_{50} values in µg/mL is presented in Table S3.

Table 3. Physicochemical properties of natural abenquines and their analogues.

Compound	Mol Weight	Log *P*	H-acceptors	H-donors	nRotb
4a	328.32	-0.26	7	3	6
4b	294.31	-0.44	7	3	6
4c	280.28	-0.95	7	3	5
4d	367.36	0.20	8	4	6
4e	270.29	0.89	5	2	4
4f	277.32	-0.08	6	2	5
4g	291.35	0.43	6	2	5
4h	285.30	0.12	6	2	5
5a	390.39	1.41	7	3	7
5b	356.38	1.23	7	3	7
5c	342.35	0.73	7	3	6
5d	429.43	1.87	8	4	7
5e	332.36	2.56	5	2	5
5f	339.39	1.59	6	2	6
5g	353.42	2.10	6	2	6
5h	355.39	1.04	7	2	6
Tice's rule	≥ 150 and ≤ 500	≤ 3.5	≥ 2 and ≤ 12	≤ 3	≤ 12



R_2	Compound (Yield)	Compound (Yield)
	**4a** (68%)[a,c]	**5a** (58%)[a]
	**4b** (81%)[a,c]	**5b** (65%)[a]
	**4c** (70%)[a,c]	**5c** (77%)[a]
	**4d** (55%)[a,c]	**5d** (45%)[a]
	**4e** (72%)[b,c]	**5e** (60%)[b]
	**4f** (71%)[b]	**5f** (75%)[b]
	**4g** (85%)[b]	**5g** (70%)[b]
	**4h** (70%)[b]	---
	---	**5h** (62%)[b]

a) Amino acid, $NaHCO_3$, EtOH, reflux, 2h.
b) Amine, DCM, rt, 2h.
c) Data corresponding to the previous work.[34]

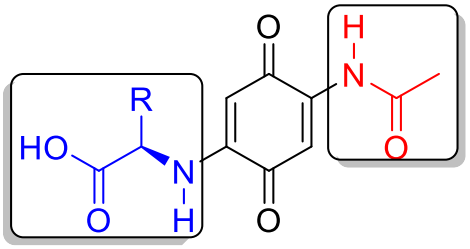
Scheme 1. Synthesis of natural abenquines (**4a-d**) and their analogues (**4e-h**, **5a-h**).

Captions to figures

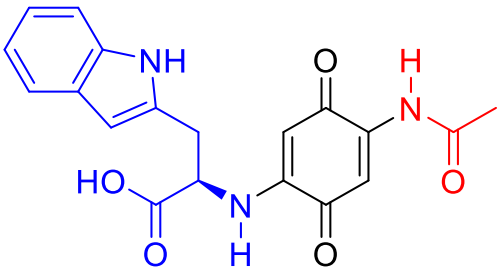
Figure 1. Structure of naturally occurring abenquines.

Figure 2. Variations introduced in the abenquine scaffold. Analogues were obtained by either replacing the amino acid substituent with different amines in the core of benzoquinone group, or adding a bulky group through the substitution of the acetamide moiety with a benzamide group.

Figure 1



Abenquine **A** (R= benzyl)
Abenquine **B1** (R= isobutyl)
Abenquine **B2** (R= *sec*-butyl)
Abenquine **C** (R= isopropyl)



Abenquine **D**

Figure 2