Investigation of 5-Nitrofuran Derivatives: Synthesis, Antibacterial Activity, and Quantitative Structure−Activity Relationships

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Three sets of antibacterial nitrofuran derivatives [set I, 5-R-substituted (*Z*)-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranones (R = OCH_3, H, CH_3, C_2H_5, nC_3H_7, Cl, Br, CN, and NO_2) and their 2-hydroxyphenyl and 2-acetoxyphenyl analogues; set II, 5-R-substituted (*E*)-1-(2-hydroxyphenyl)-3-(5-nitrofuryl)-2-propen-1-ones (R = H, CH_3, C_2H_5, Cl, and NO_2); and set III, 5-R-substituted (*E*)-1-(2-acetoxyphenyl)-3-(5-nitrofuryl)-2-propen-1-ones (R = H, CH_3; C_2H_5, Cl, and NO_2)] were prepared and tested against a Gram-positive (*Staphylococcus aureus,* strain ATCC-25923) and a Gram-negative bacterium (*Caulobacter crescentus,* strain NA 1000). QSAR equations derived for the IC_{50} values against both bacteria show negative contributions of two terms: an electronic one, expressed either by σ, the Hammett substituent constant, or by E, the cyclic voltametric reduction potential. Another term described by an indicator variable, I_{abs}, is assigned the value of 0 for set I compounds and the value of 1 for sets II and III. No important contribution of the hydrophobic factor was found. For the three sets, the QSAR regressions suggest that the same structural features describe the activities for both bacteria and that, although reduction is a necessary step, it should not be the determining one. These results agree with those found for the QSAR of 5-nitroimidazole analogues.

Introduction

The use of nitroheterocyclic drugs as antibacterial, antiprotozoal, and anticancer agents is well-established. The first nitroheterocyclic compounds to be introduced in chemotherapy were the nitrofurans, which have been extensively employed for a number of years. In common with other nitroheterocyclics, it is assumed that their cytotoxicity depends on the reduction of the nitro group which subsequently results in damage to DNA, although the exact mechanism of action is not yet completely understood.[1−3]

5-Nitrofurans and 5-nitroimidazoles are the classes of nitroheterocyclic drugs most used as antibacterials.[1] The distinguishing properties between these two subclasses are their reduction potentials. The 5-nitroimidazol derivatives show comparatively low reduction potentials: they can be reduced to the corresponding amino derivatives only under anaerobic conditions and their spectrum of activity is limited to anaerobic or facultative anaerobic bacteria. It has been proposed that the active damaging species, intermediate in the reduction process, is the protonated one-electron nitro radical anion, which explains the relative cytotoxicity of these drugs under hypoxia.[2] This intermediate species interacts with DNA, causing strand breaks[4] with simultaneous release of thymine and thymidine phosphates and helix destabilization. The efficiency of this interaction is proportional to the A + T content of the DNA.[5] In contrast, the mutagenicity of the 5-nitroimidazoles is related to the adduct formation of amino or hydroxyamino reduction products to DNA and is proportional to the C + G content.

On the other hand, 5-nitrofuran derivatives, which are easily reduced, show activity against both aerobic and anaerobic bacteria. Their proposed mechanism of action[1] seems to be even more complex than the one suggested for 5-nitroimidazoles. For *Escherichia coli*, two classes of nitroreductase enzymes were described.[6,7] One is oxygen sensitive, being capable of reducing nitrofurans only in absence of oxygen. The other is oxygen insensitive, reducing nitrofurans under aerobic conditions. Oxygen-sensitive nitroreductase promotes one-electron reduction of nitrofuran derivatives with the formation of the nitro radical anion. In the absence of oxygen, further reduction can occur, leading to the corresponding amino derivative,[6] while futile cycling[8] occurs in the presence of oxygen. The oxygen-insensitive nitroreductase imposes a different route for reduction of nitrofurans. First, a two or more electron reduction occurs and a nitro radical anion is not observed. Then, another reduction occurs, leading to a cyano derivative with opening of the nitrofuran ring.[6] *E. coli* resistant strains possess only the oxygen-sensitive nitroreductase. In other words, the absence of the oxygen-insensitive nitroreductase confers to *E. coli* resistance to nitrofurans under aerobic conditions. As to the mechanism of the antibacterial activity of the nitrofurans, although it was demonstrated that the nitrofurans can damage both DNA and proteins, the active intermediate species as well as the biological target are still unknown.

Thus, although there is a large amount of experimental work on nitrofurans, they remain an area of active research interest. Furthermore, most of these studies were not structured with the thought of doing QSAR.[9]

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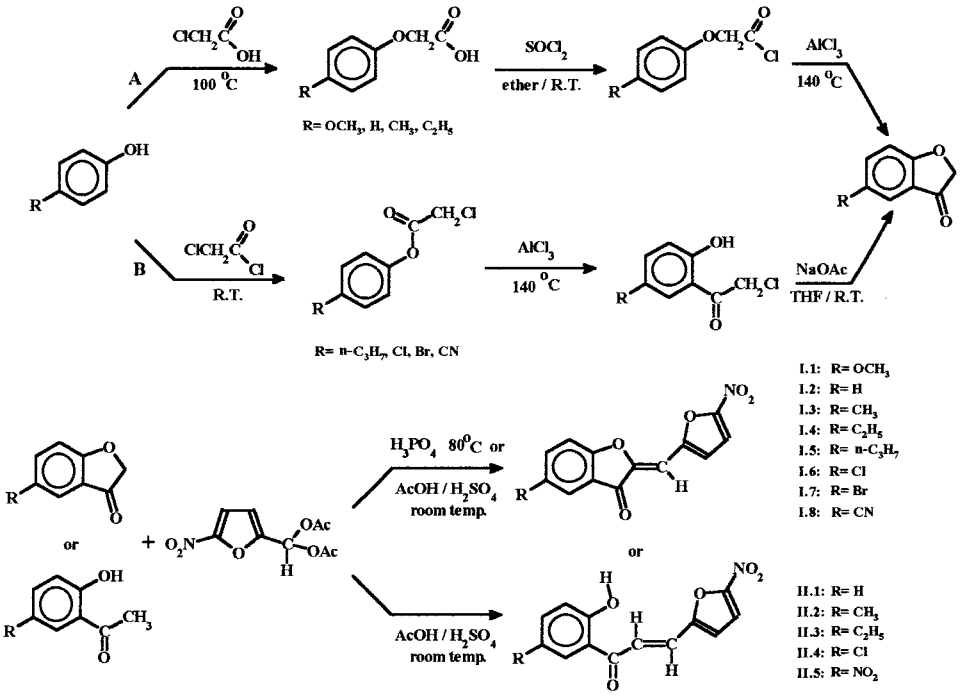
Scheme 1



In the present work, to gain an insight into the physicochemical and the structural features of this class of compounds that are important to the antibacterial activity, QSAR analyses of nine 5-R-substituted (*Z*)-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranones, set I, in which R = OCH_3, H, CH_3, C_2H_5, *n*-C_3H_7, Cl, Br, CN, and NO_2 were performed. Additionally, to verify the role of the benzofuran ring on activity, five 5′-R-substituted 1-(2-hydroxyphenyl)-3-(5-nitro-2-furyl)-2-propen-1-ones, set II, in which R = H, CH_3, C_2H_5, Cl, and NO_2, and their corresponding acetylated analogues, set III, in which R = H, CH_3, C_2H_5, Cl, and NO_2, were included in the analysis, using a mixed approach,[10,11] with the inclusion of an indicator variable, I_{abs}. The compounds have been tested against a Gram-positive (*Staphylococcus aureus*, strain ATCC-25923) and a Gram-negative bacterium (*Caulobacter crescentus*, strain NA 1000).

Results and Discussion

Chemistry. The compounds **I.1**−**I.7** of set I (where R = OCH_3, H, CH_3, C_2H_5, *n*-C_3H_7, Cl, and Br) were prepared by the condensation of the previously prepared appropriate 5-substituted-3(2*H*)-benzofuranone with commercially available 5-nitro-2-furfurylidene diacetate in orthophophoric acid following the procedure given by Albrecht et al.[12] (Scheme 1), while the cyano derivative, compound **I.8** was prepared by condensation in acetic/sulfuric acid following the procedure given by Nazarova and Ustimenko.[13] Although **I.2**, **I.3**, and **I.6** have been previously described,[14] their characterizations by elemental analyses and melting point determinations are presented in the Experimental Section for comparison. In this report new ^1H NMR data have been included for these known compounds.

As shown in Scheme 1, the 5-substituted-3(2*H*)-benzofuranones, in which R = OCH_3, H, CH_3, and Cl, have been previously described and were obtained from the commercially available appropriate para-substituted phenol following one of the two different routes, namely, route A, for R = OCH_3, H, CH_3, and C_2H_5 described by Kulkarni and Dwivedi,[15] or route B, for R= *n*-C_3H_7, Cl, Br, and CN described by Stefanye and Howard.[16] From our experience, these two routes summarize the most convenient synthetic paths leading to the 3(2*H*)-benzofuranones, considering each substituent.

The nitro derivative, compound **I.9**, was obtained by the treatment of the unsubstituted derivative, compound **I.2**, with concentrated nitric acid in acetic acid.

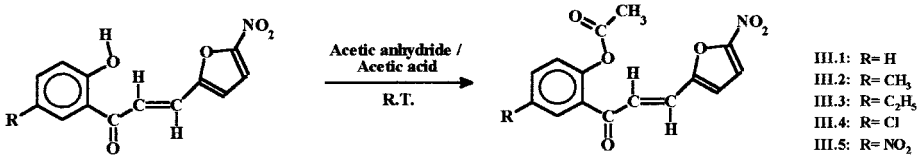
The compounds **II.1**−**II.5** of set II (where R = H, CH_3, C_2H_5, Cl, and NO_2) were obtained by the condensation of the corresponding 5-substituted-2-hydroxyacetophenone with 5-nitro-2-furfurylidene diacetate in a mixture of acetic/sulfuric acids following the procedure given by Nazarova and Ustimenko[13] (Scheme 1). Compound **II.1** has been previously described,[17] and its characterization by elemental analysis and melting point is provided in the Experimental Section for comparison. The 5-substituted-2-hydroxyacetophenones are all available commercially, except the 5-NO_2 substituted, which was obtained by treatment of the unsubstituted derivative, compound **II.1**, with concentrated nitric acid in acetic acid.[18]

The compounds **III.1**−**III.5** of set III (where R = H, CH_3, C_2H_5, Cl, and NO_2) were obtained by treatment of the appropriate set II analogues with acetic anhydride in acetic acid, (Scheme 2).

The overall lipophilicity of each nitrofuran derivative was assessed by the logarithm of its octanol/water partition coefficient, herein called log *P*. The values were calculated from reversed-phase HPLC capacity factors (log *k*′).[19−21] In addition, log *P* values were calculated by the CLOGP program.[22] The Hansch−Fujita hydrophobicity substituent constants, π, were taken from the literature.[23]

For all compounds, the values of the first peak reduction potential, *E*, were measured by cyclic voltammetry[24] in DMF; the LUMO energy values were

Scheme 2



calculated using the AM1 method and the MOPAC-6/QCPE[25] program. In addition, the Hammett σ as well as the Swain−Lupton $\mathscr{G}$ and $\mathscr{R}$ substituent constants for field and resonance effects were used as electronic parameters.[26]

The molecular volume, *V*, calculated by the Sybyl 6.3 program[27] was taken as the steric parameter, and the literature[23] molar refractivity values of the substituents at the 5-position of the benzofuran ring, MR, were taken as polarizability-related parameters, respectively. For correlation purposes the MR values were scaled by a factor of 0.1, in the QSAR analysis.[10]

Microbiology. The 50% growth inhibitory activity value, IC_{50}, of each compound was determined using two species of bacteria, *S. aureus (*ATCC-25923 strain) and *C. crescentus* (NA1000 strain), by the sequential broth macrodilution test.[28] Furthermore, for each compound of set I (**I.1**−**I.9**), the reduction of the nitrofuran derivatives mediated by *C. crescentus* cell extracts[28,29] using NADPH as cofactor was studied. For each compound, the profile of the initial velocity curve of nitrofuran consumption versus nitrofuran concentration was analyzed using the Michaelis−Menten kinetic model. For each compound, the maximum velocity of nitrofuran consumption values, V_{max}, in μM/min, and the values of $\log(1/IC_{50})$, expressed in μmol/mL, derived for correlation purposes, were taken as the biological parameters, indicating the inhibitory potency of the compounds.

QSAR Analysis. For the three sets of nitrofuran derivatives, a table containing the corresponding values of measured, calculated, or literature-reported physicochemical and biological parameters are available as Supporting Information.

Although the compounds of set II and their corresponding esters (set III) have similar activities, it was demonstrated by HPLC analysis that no hydrolysis takes place in phosphate buffer (pH 6.9) as well as in culture medium for the bacteria *C. crescentus* (PYE), except for compound **III.5** (R=NO_2), which is readily hydrolyzed in phosphate buffer.

Despite having different cell wall structures, both *C. crescentus*, a Gram-negative bacterium, and *S. aureus*, a Gram-positive bacterium, exhibited similar susceptibilities to the action of the studied nitrofuran derivatives. The correlation observed between the $\log(1/IC_{50})$ values determined against *S. aureus* and *C. crescentus* respectively (Eq 1, Figure 1) suggests that similar structural features contribute to the two antibacterial activities, for all tested compounds, excluding **II.5** and **III.5**.

All compounds except **II.5** and **III.5**:

$$\log(1/IC_{50})_{S.\ aureus} = 1.01(\pm0.26)\ \log(1/IC_{50})_{C.\ crescentus} + 0.72(\pm0.61)$$

$$n = 12,\ r = 0.937,\ s = 0.252,\ F = 72.104,\ Q^2 = 0.837,\ \text{s-PRESS} = 0.291 \quad (1)$$

The structural features that lead to more active compounds against *S. aureus* also make them more active against *C. crescentus.* Equation 1 shows a coefficient value statistically the same as 1.0. This indicates that the nitrofuran structural features that determine the variation of the inhibitory potency values for the series studied here were probably not related to properties that are associated with different permeabilities of the bacteria cell walls.

For compounds **I.1**−**I.9** of set I tested against *S. aureus*, the single most important physicochemical property that could explain the variance in the growth inhibitory activity was expressed by any one of several electronic parameters. Thus, either the electronic substituent constant (σ_p, $\mathscr{G}$, and $\mathscr{R}$) or the first reduction potential (*E*) could explain alone about 80% (*r* around 0.9) of the variance of growth inhibitory activity, as observed respectively in eq 2, 3, or 4.

Set I:

$$\log(1/IC_{50})_{S.\ aureus} = -1.30(\pm0.42)\sigma_p + 3.43(\pm0.17)$$

$$n = 9,\ r = 0.941,\ s = 0.189,\ F = 54.062,\ Q^2 = 0.829,\ \text{s-PRESS} = 0.231 \quad (2)$$

$$\log(1/IC_{50})_{S.\ aureus} = -1.19(\pm0.69)\mathscr{G} - 1.47(0.84)\mathscr{R} + 3.38(\pm0.28)$$

$$n = 9,\ r = 0.944,\ s = 0.199,\ F = 24.548,\ Q^2 = 0.805,\ \text{s-PRESS} = 0.267 \quad (3)$$

$$\log(1/IC_{50})_{S.\ aureus} = -6.27(\pm3.66)E + 1.87(\pm0.85)$$

$$n = 9,\ r = 0.837,\ s = 0.306,\ F = 16.394,\ Q^2 = 0.489,\ \text{s-PRESS} = 0.399 \quad (4)$$

Equations 2−4 suggest that compounds that bear substituents that are electron acceptors were less active than those that are either unsubstituted or bear electron donors. The same holds true for compounds that were more readily reduced. In contrast, more active compounds were found in nitrofuran analogues that bear electron-donor substituents, which leads to less easily reducible compounds. The addition of MR, a polarizability-related parameter, improves the correlation when compared with those obtained with only an electronic term (eqs 2−4). As seen in the eq 5, 95% of the variance of the biological activity has been explained.

Set I:

$$\log(1/IC_{50})_{S.\ aureus} = -1.37(\pm0.34)\sigma_p - 0.34(\pm0.34)\text{MR} + 3.69(\pm0.29)$$

$$n = 9,\ r = 0.971,\ s = 0.143,\ F = 50.215,\ Q^2 = 0.874,\ \text{s-PRESS} = 0.214 \quad (5)$$

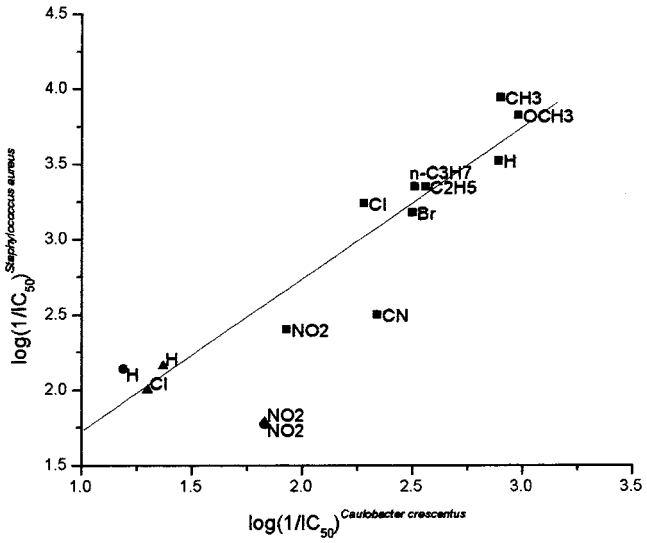


Figure 1. Plot of values of log(1/IC_{50}) obtained for a Gram-positive (*S. aureus*, strain ATCC-25923) and a Gram-negative bacteria (*C. crescentus*, strain NA 1000) for compounds of sets I (■), II (●), and III (▲).

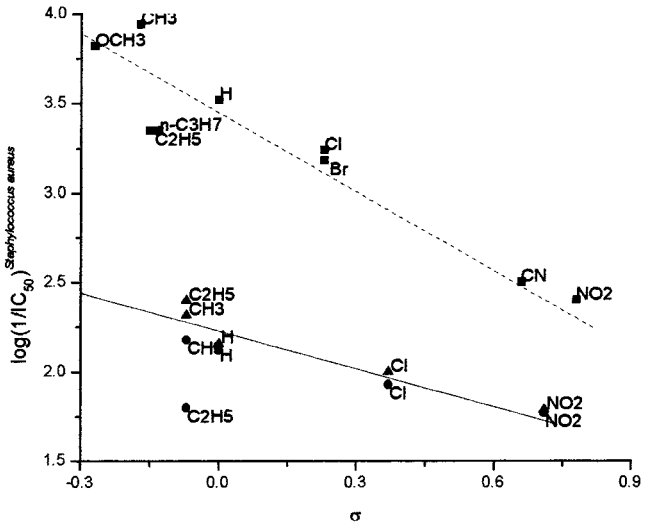


Figure 2. Plot of values of log(1/IC_{50}) for *S. aureus* (strain ATCC-25923) determined experimentally for compounds of sets I (■), II (●), and III (▲) versus the appropriate values of the σ Hammett electronic substituent constant, respectively, σ_p for set I (eq 2) and σ_m for sets II and III compounds (eq 6).

Despite the observed improvement in eq 5 when compared with eq 2−4, the contribution associated with the MR term is much less significant than the one observed for the electronic term. In eq 5, the coefficient value is approximately −0.3 and is poorly determined, i.e., the contribution of the MR term is not significant at a 95% confidence interval. Furthermore, it has to be emphasized that MR is often correlated with volume and/or log *P*. This correlation has been observed in this set of compounds (for set I, $r \sim 0.5$). Thus, it is not possible to ensure that MR will account only for a single polarizability, steric, or lipophilic term. The results obtained suggest that either a more bulky or a more polarizable or a more lipophilic substituent seems to lead to less active derivatives. Figure 2 shows the correlation between the measured growth inhibitory activity values observed for *S. aureus* and the corresponding electronic substituent constant values. For all compounds of sets II and III, excluding compound **II.3**, better models have been found using the substituent constant σ_m or $\mathscr{G}$ as the electronic descriptor, as observed in eq 6 and 7.

Sets II and III, except compound **II.3**:

$$\log(1/IC_{50})_{S.\ aureus} = -0.63(\pm 0.20)\sigma_m + 2.21(\pm 0.07)$$

$$n = 9,\ r = 0.945,\ s = 0.077,\ F = 57.892,\ Q^2 = 0.832,\ \text{s-PRESS} = 0.096 \quad (6)$$

$$\log(1/IC_{50})_{S.\ aureus} = -0.70(\pm 0.26)\mathscr{G} + 2.24(\pm 0.09)$$

$$n = 9,\ r = 0.926,\ s = 0.089,\ F = 41.971,\ Q^2 = 0.780,\ \text{s-PRESS} = 0.111 \quad (7)$$

The analysis of the coefficients of eqs 3 and 7 suggests that for compounds of sets II and III, the relative contributions of the inductive/field terms to the biological activity are smaller than those observed for compounds of set I, although they cannot be considered statistically different at a 95% confidence level. For the compounds of sets II and III, there was no contribution from a resonance term. The growth inhibitory activity values observed for compounds of sets II and III are about one logarithmic unit lower than those observed for the corresponding compounds of set I. As presented in the table available as Supporting Information, the lower activity values could not be explained exclusively by substituent polar/electronic descriptors expressing the effects of the introduction of a substituent in the rigid benzofuran ring analogue.

In addition, with respect to the first reduction potential and log *P* values, the same range of variations has been observed for the compounds in each set, namely in sets I−III. Furthermore, for the studied compounds and the models derived until now, lipophilicity does not seem to be a very important property for growth inhibitory activity.

To have a model that describes the biological activity of the compounds of sets I−III simultaneously, it is necessary to consider the mixed approach,[10] in which an indicator variable, I_{abs} is considered. The introduction of an indicator of variable, I_{abs}, in the analysis allows[11] one to describe a model for sets I−III in one unique equation, eq 8. I_{abs} indicates the absence of the benzofuran ring substructure in the compounds of sets II and III. By definition, this variable assumes respectively the values of 1 (unity) for the compounds of sets II and III and 0 (zero) for compounds of set I. In eq 8, $\mathscr{R}$ was set to 0 for compounds of sets II and III. This has been done considering that the resonance effect, described by $\mathscr{R}$, is only observed for compounds of set I (eq 3) and not for compounds of sets II and III (eq 7). Thus, in eq 8 the contribution of the resonance term is only applied to compounds of set I.

Sets I−III:

$$\log(1/IC_{50})_{S.\ aureus} = -0.82(\pm 0.38)\mathscr{G} - 1.59(\pm 0.70)\mathscr{G} - 1.04(\pm 0.21)I_{abs} + 3.26(\pm 0.20)$$

$$n = 19,\ r = 0.970,\ s = 0.195,\ F = 78.429,\ Q^2 = 0.905,\ \text{s-PRESS} = 0.246 \quad (8)$$

The actual values of the growth inhibitory activity evaluated against *S. aureus* and the corresponding

values estimated from eq 8 for compounds of sets I−III are presented in Table 1.

The model described by eq 8 tell us that for compounds in which there is no benzofuran ring moiety one should subtract about one logarithm unit of activity. However, this approach does not explain which physicochemical property of this moiety is the relevant one to explain the difference in activities of set I as compared to sets II and III.

Sets I−III:

$$\log(1/IC_{50})_{S.\ aureus} = -0.67(\pm0.20)\Sigma\sigma - 1.31(\pm0.19)I_{abs} + 3.49(\pm0.15)$$

$$n = 19,\ r = 0.968,\ s = 0.195,\ F = 117.428,\ Q^2 = 0.909,\ \text{s-PRESS} = 0.233 \quad (9)$$

In eq 9, $\Sigma\sigma$ describes the overall contribution of the electronic effect transmitted by the substituent. For the compounds of sets II and III, $\Sigma\sigma$ values have been considered equal to those described by the σ_m constant, while for the compounds of set I the $\sigma_m + \sigma_p$ expression is used.

For *C. crescentus*, similar models have been derived using compounds of set I, as presented in eq 10. Thus, the coefficients in eq 10 suggest that there is a similar important negative contribution of the electronic term describing the variance in the biological activity. A second variable could also be considered in the analysis, such as MR, volume, or log *P*. Equations 10 and 11 describe the models for which the contribution of a MR or a lipophilic term is analyzed.

Set I:

$$\log(1/IC_{50})_{C.\ crescentus} = -0.83(\pm0.35)\sigma_p - 0.36(\pm0.35)\text{MR} + 2.93(\pm0.31)$$

$$n = 9,\ r = 0.924,\ s = 0.150,\ F = 17.569,\ Q^2 = 0.730,\ \text{s-PRESS} = 0.205 \quad (10)$$

$$\log(1/IC_{50})_{C.\ crescentus} = -1.14(\pm0.37)\sigma_p - 0.29(\pm0.19)\log P^a + 3.68(\pm0.68)$$

$$n = 9,\ r = 0.955,\ s = 0.117,\ F = 30.744,\ Q^2 = 0.806,\ \text{s-PRESS} = 0.173 \quad (11)$$

To take into account the three sets of compounds simultaneously, similar models have been obtained with the introduction of the indicator variable I_{abs} (eq 12).

All compounds, except **II.5** and **III.5**:

$$\log(1/IC_{50})^{C.\ crescentus} = -0.73(\pm0.39)\sigma_p - 1.30(\pm0.28)I_{abs} + 2.64(\pm0.15)$$

$$n = 12,\ r = 0.964,\ s = 0.188,\ F = 59.128,\ Q^2 = 0.875,\ \text{s-PRESS} = 0.250 \quad (12)$$

Table 1 shows the measured activity evaluated for *C. crescentus* and the values predicted by the model described in eq 12, for all the compounds of sets I−III, excluding compounds **II.5** and **III.5**. The two nitro-substituted derivatives, respectively **II.5** and **III.5**, are outliers in the proposed model, i.e., their inhibitory activity values are higher than those predicted by eq 12. This observation could be explained considering that compound **II.5** is the only one in the set that seems to be ionized at pH conditions used for biological determinations (pH 7.0), showing a low log *P* value as can be seen in the table available as Supporting Information. On the other hand, it was observed that the second outlier, **III.5**, undergoes hydrolysis very easily.

Table 1. Experimental and Calculated Values of $\log(1/IC_{50})$ for Compounds of Sets I−III Tested Against *S. aureus* and *C. crescentus* Bacteria, Respectively

		$\log(1/IC_{50})$					
		S. aureus			C. crescentus		
compd	R	exp	calcd[a]	Δ*c*	exp	calcd[b]	Δ*c*
I.1	OCH_3	3.82	3.92	−0.10	2.98	2.84	0.14
I.2	H	3.52	3.26	0.26	2.89	2.64	0.25
I.3	CH_3	3.94	3.54	0.40	2.90	2.76	0.14
I.4	C_2H_5	3.35	3.50	−0.15	2.56	2.75	−0.19
I.5	*n*-C_3H_7	3.35	3.48	−0.13	2.51	2.73	−0.22
I.6	Cl	3.24	3.22	0.02	2.28	2.47	−0.19
I.7	Br	3.18	3.24	−0.04	2.50	2.47	0.03
I.8	CN	2.50	2.61	−0.11	2.34	2.16	0.18
I.9	NO_2	2.40	2.53	−0.13	1.93	2.07	−0.14
II.1	H	2.14	2.23	−0.09	1.19	1.34	−0.15
II.2	CH_3	2.18	2.22	−0.04	nd[d]	1.47	
II.3	C_2H_5	1.80	2.23	−0.43	nd[d]	1.45	
II.4	Cl	1.93	1.88	0.05	nd[d]	1.17	
II.5	NO_2	1.77	1.69	0.08	1.83	0.77	1.06[e]
III.1	H	2.16	2.23	−0.07	1.37	1.34	0.03
III.2	CH_3	2.32	2.22	0.10	nd[d]	1.47	
III.3	C_2H_5	2.40	2.23	0.17	nd[d]	1.45	
III.4	Cl	2.00	1.88	0.12	1.30	1.17	0.13
III.5	NO_2	1.79	1.69	0.10	1.83	0.77	1.06[e]

[a] Values calculated by eq 8. [b] Values calculated by eq 12. [c] Values of the difference between the logarithm of measured and calculated values. [d] Values not determined. [e] Outliers in the proposed models.

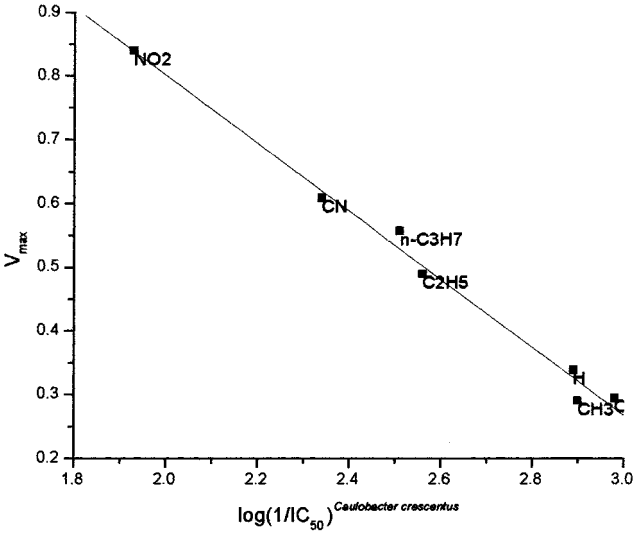


Figure 3. Plot of observed values of velocity of reduction of nitrofuran derivatives of (■) set I by NADPH mediated by *C. crescentus* extracts against the corresponding observed/experimental values of $\log(1/IC_{50})$ for *C. crescentus*.

For all compounds of set I, excluding **I.6** and **I.7**, a correlation was observed between the velocity of reduction of the nitrofurans by NADPH mediated by nitroreductases present in an unpurified *C. crescentus* extract and the inhibitory activity, as shown in eq 13 and Figure 3. Equation 13 indicates that the more potent compounds are those that have been slowly reduced by *C. crescentus* cells. Although the reduction is considered an essential step for the activity, eq 13 suggests that

the activity should be mainly determined by the reactivity or stability of an intermediate generated by the reduction.

Set I, except compounds **I.6** and **I.7**:

$$\log(1/IC_{50})_{C.\ crescentus} = -1.86(\pm 0.21)V_{max} + 3.49(\pm 0.11)$$
$$n = 7,\ r = 0.995,\ s = 0.041,\ F = 496.47,$$
$$Q^2 = 0.982,\ \text{s-PRESS} = 0.055 \quad (13)$$

Conclusions

QSAR analyses performed for compounds of set I have demonstrated the importance of the electronic properties of the substituents of the nitrofuran derivatives for inhibitory growth activity evaluated against *S. aureus* and *C. crescentus*. The inhibitory activity seems to be inversely related to the velocity with which the compounds are reduced in the interior of the cells. These derived QSAR models are similar to those obtained for the antibacterial activity of the 5-nitroimidazole derivatives,[30,31] where the protonated nitro anion radical has been proposed as the damaging species with DNA as the target. Furthermore, the derived QSAR models were related to the stability/reactivity of the reduced intermediate formed rather than the reduction of the initial nitro compound, suggesting the possibility of parallels in their mechanisms of action. Nevertheless, DNA has not been clearly demonstrated to be the unique target, because nitrofurans can also damage proteins. Although it has been demonstrated that aerobic reduction of nitrofurans does not form nitro anion radicals, the derived QSAR shows that the stability/reactivity of reduced intermediates may be the most prominent factor regulating the activity of the studied 5-nitrofurans. The differences in biological activity observed for compounds of set I versus sets II and III may reflect differences at the level of compound−receptor interaction. Nevertheless, the QSAR analyses performed in this report suggest that the benzofuranic moiety is an important structural feature contributing to the activity. For the set of compounds studied, less active analogues have been detected when this structural feature is absent.

Experimental Section

Chemistry. Melting points were determined in a Thomas-Scientific Koffler hot stage apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer 2400 CHN analyzer and are within ±0.4% of the theoretical values of the indicated elements. ^{1}H NMR spectra were recorded on a AC-200 or on a DPX-300 Bruker spectrometer, and all spectra were obtained in $CDCl_3$ as solvent; Chemical shifts are reported in δ (ppm) units relative to tetramethylsilane (TMS). IR spectra, in KBr, were recorded on a Perkin-Elmer-283 spectrophotometer. Mass spectra were obtained on a Finnigan instrument, model INCOS50. All spectra and elemental analyses were obtained at the Analytical Laboratory of the Chemistry Institute of University of São Paulo, Brazil, and are in accordance with the structure given.

HPLC analyses, for the log *P* value determinations, were performed in a Shimadzu chromatograph equipped with a Rheodyne injection valve, a 9A pump, a SPD-6AV variable wavelength UV−vis detector (set either at 223 or 254 nm for column calibration purposes or at 422, 376, and 365 nm for the log *P* measurements respectively for compounds of sets I, II, and III), a SCL-6B system controller, and a C-R4A registrator and analyzer. Calculations of log *P* values were obtained by ClogP program for Windows (version 1.0.0, 1995; Biobyte). Calculations of molecular volume values, *V*, were performed by the Sybyl 6.3 program.[27]

Cyclic voltamograms, for the study of the reduction process and measurements of the first reduction peak values, *E* (in Volts), were obtained by employing a Princeton applied research 273A potentiostat/galvanostat and a Houston Instrument 2000 XY registrator. A 0.1 M tetraethylammonium perchlorate solution in DMF has been used as electrolyte solution.[32] The concentration of each nitrofuran derivative was typically 0.5 mg/mL and the applied potential varied from + 0.5 to −1.5 V with a scan rate of 200 mV/s. A mercury microelectrode was employed as the working electrode. A platinium sheet has been used as auxiliary electrode and a Ag/AgI/ 0.04 M $[N(n\text{-}C_4H_9)_4]^+I^-$ reference electrode was used, with both in 0.1 M tetraethylammonium perchlorate, in DMF.

All chemicals were obtained from commercial sources and were used without previous purification, except for the 5-substituted-3(2*H*)-benzofuranones, which were prepared from commercially available appropriate para-substituted phenols following one of the two different routes given by Kulkarni and Dwivedi[15] (Scheme 1, route A, for R= OCH_3, H, CH_3, and C_2H_5) or Stefanye and Howard[16] (Scheme 1, route B, for R= n-C_3H_7, Cl, Br, and CN). 5-Nitro-2-hydroxyacetophenone was prepared from the 2-hydroxyacetophenone by the procedure described by Joshi and Singh.[18] Solvents were used without purification except where indicated.

General Procedure for the Preparation of 5-Substituted (*Z*)-2-(5-Nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranones[12] (I.1−I.7) (Scheme 1). Equimolar amounts of the appropriate 5-substituted 3(2*H*)-benzofuranone (3−48 mmol) and 5-nitro-2-furfurylidene diacetate in orthophosphoric acid (85% w/w, 7−90 mL) were stirred for 5 h at 80 °C. After standing for 16 h at room temperature the mixture was poured into crushed ice. The insoluble product was filtered off and recrystallized from ethyl acetate, unless otherwise indicated. Yields were 20−65%.

***(Z)*-5-Methoxy-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (I.1):** mp 198−200 °C; ^{1}H NMR (200 MHz) δ 7.21−7.27 (m, 4H), 7.45, (d, J = 3.9 Hz, 1H), 6.78 (s, 1H) 3.58, (s, 3H); MS m/z 287 (M^+), 241 ($M^+ - NO_2$). Anal. ($C_{14}H_9NO_6$) C, H, N.

***(Z)*-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (I.2).** mp 170−178 °C (recrystallized from acetone) (lit.[14] mp 170 °C); ^{1}H NMR (200 MHz) δ 6.79 (s, 1H), 7.26 (d, J = 4.0 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.32, (t, J = 7.8 Hz, 1H), 7.45 (d, J = 4.0 Hz, 1H), 7.72 (td, J = 7.8 Hz, 1.4 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H); MS m/z 257 (M^+), 211 ($M^+ - NO_2$). Anal. ($C_{13}H_7NO_5$) C, H, N.

***(Z)*-5-methyl-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (I.3).** mp 184−186 °C (lit.[14] mp 178 °C); ^{1}H NMR (200 MHz) δ 2.41 (s, 3H), 6.73 (s, 1H), 7.20 (d, J = 9.3 Hz, 1H), 7.22 (d, J = 3.7 Hz, 1H), 7.43 (d, J = 3.7 Hz, 1H), 7.50 (dd, J = 9.3 Hz, 1.7 Hz, 1H), 7.55 (s broad, 1H); MS m/z 271 (M^+), 225 ($M^+ - NO_2$). Anal. ($C_{14}H_9NO_5$) C, H, N.

***(Z)*-5-Ethyl-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (I.4).** mp 144−146 °C; ^{1}H NMR (200 MHz) δ 1.27 (t, J = 7.6 Hz, 3H), 2.42 (q, J = 7.6 Hz, 2H), 6.76 (s, 1H), 7.25 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 4.0 Hz, 1H), 7.44 (d, J = 4.0 Hz, 1H), 7.54 (dd, J = 8.4 Hz, 1.9 Hz, 1H), 7.60 (s broad, 1H); MS m/z 285 (M^+), 239 ($M^+ - NO_2$). Anal. ($C_{15}H_{11}NO_5$) C, H, N.

***(Z)*-5-Propyl-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (I.5).** mp 138−142 °C; ^{1}H NMR (200 MHz) δ 0.95 (t, J = 7.5 Hz, 3H), 1.66 (m, J = 7.5 Hz, 2H), 2,65 (t, J = 7.5 Hz, 2H), 6.76 (s, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 4.3 Hz, 1H), 7.45 (d, J = 4.3 Hz, 1H), 7.52 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 7.59 (d, J = 2.0 Hz, 1H); MS m/z 299 (M^+), 253 ($M^+ - NO_2$). Anal. ($C_{16}H_{13}NO_5$) C, H, N.

***(Z)*-5-Chloro-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (I.6).** mp 242−244 °C (lit.[17] mp 228 °C); ^{1}H NMR (200 MHz) δ 6.84 (s, 1H), 7.32 (d, J = 8.8 Hz, 1H), 7.25 (d, J = 3.9 Hz, 1H), 7.46 (d, J = 3.9 Hz, 1H), 7.67 (dd, J = 8.8 Hz, 2.2 Hz, 1H), 7.78 (d, J = 2.2 Hz, 1H); MS m/z 293 (M + 2), 291 (M^+), 245 ($M^+ - NO_2$). Anal. ($C_{13}H_6NO_5Cl$) C, H, N, Cl.

***(Z)*-5-Bromo-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (I.7).** mp 215−220 °C; ^{1}H NMR (200 MHz) δ 6.83 (s, 1H), 7.27 (d, J = 8.8 Hz, 1H), 7.25 (d, J = 4.0 Hz, 1H), 7.45 (d, J = 4.0 Hz, 1H), 7.80 (dd, J = 8.8 Hz, 2.2 Hz, 1H), 7.93 (d, J = 2.2 Hz, 1H); MS m/z 337 (M + 2), 335 (M^+), 289 ($M^+ - NO_2$). Anal. ($C_{13}H_6NO_5Br$) C, H, N.

***(Z)*-5-Cyano-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (I.8)** was prepared by following the general procedure described for the preparation of 5-substituted (*E*)-1-(2-hydroxyphenyl)-3-(5-nitrofuryl)-2-propen-1-ones and using equimolar amounts of the appropriate 5-substituted-3(2*H*)-benzofuranone (Scheme 1): mp 271−274 °C; ^{1}H NMR (200 MHz) δ 6.93 (s, 1H), 7.27 (d, J = 4.0 Hz, 1H), 7.46 (d, J = 4.0 Hz, 1H), 7.51 (d, J = 8.6 Hz, 1H), 7.98 (dd, J = 8.6 Hz, 1.5 Hz, 1H), 8.14 (d, J = 1.5 Hz, 1H); MS m/z 282 (M^+), 236 ($M^+ - NO_2$). Anal. ($C_{14}H_6N_2O_5$) C, H, N.

Preparation of *(Z)*-5-Nitro-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (I.9). A mixture of (*Z*)-5-nitro-2-(5-nitrofuran-2-ylmethylene)-3(2*H*)-benzofuranone (**I.2**) (0.78 mmol) and concentrated sulfuric acid (2 mL) was stirred and chilled in an ice water bath. After the mixture was cooled, nitric acid (0.1 mL, 65% w/w) was add dropwise. The mixture was kept at room temperature for 1 h and then poured into crushed ice. The insoluble product was filtered off and purified by column chromatography (silica gel 60, 70−230 mesh; eluent benzene): yield 22%; mp 244−245 °C; ^{1}H NMR (200 MHz) δ 6.89 (s, 1H), 7.22 (d, J = 3.9 Hz, 1H), 7.41 (d, J = 3.9 Hz, 1H), 7.47 (d, J = 9.0 Hz, 1H), 8.57 (dd, J = 9.0 Hz, 2.5 Hz, 1H), 8.66 (d, J = 2.5 Hz, 1H); MS m/z 302 (M^+), 256 ($M^+ - NO_2$). Anal. ($C_{13}H_6N_2O_7$) C, H, N.

General Procedure for the Preparation of 5-Substituted *(E)*-1-(2-Hydroxyphenyl)-3-(5-nitrofuryl)-2-propen-1-ones[13] (II.1−II.5) (Scheme 1).

A solution of 5-nitro-2-furfurylidene diacetate (10−60 mmol) in a mixture of acetic acid (6−30 mL) and concentrated sulfuric acid (0.35−2 mL) was heated for 5−7 min on a steam bath and chilled to the room temperature, and then equimolar amounts of the appropriate 5-substituted-3(2H)-benzofuranone or 5-substitued 2-hydroxyacetophenone and concentrated sulfuric acid (0.65−3.5 mL) were added. The resulting mixture was allowed to stand overnight at room temperature and then was poured into crushed ice. The insoluble product was filtered off and purified by column chromatography (silica gel 60, 70−230 mesh; eluent chloroform) followed by recrystallization from ethyl acetate/ethanol 1:1 to yield 6−12%.

***(E)*-1-(2-Hydroxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (II.1):** mp 161−167 °C (lit.[17] mp 166 °C); ^{1}H NMR (200 MHz) δ 6.90 (d, J = 3.7 Hz, 1H), 7.00 (td, J = 8.5, 1.5, 1H), 7.03 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 3.7 Hz, 1H), 7.55 (td, J = 8.5 Hz, 1.5 Hz, 1H), 7.62 (d, J = 15.4 Hz, 1H), 7.85 (d, J = 15.4, 1H), 7.94 (dd, J = 8.5 Hz, 1.5 Hz, 1H), 12.56 (s, 1H); MS m/z 259 (M^+), 213 ($M^+ - NO_2$). Anal. ($C_{13}H_9NO_5$) C, H, N.

***(E)*-5-Methyl-1-(2-hydroxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (II.2):** mp 151−161 °C; ^{1}H NMR (200 MHz) δ 2.39 (s, 3H), 6.89 (d, J = 3.7 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H), 7.38 (d, J = 8.8 Hz, 1H), 7.41 (d, J = 3.7 Hz, 1H), 7.62 (d, J = 15.0 Hz, 1H), 7.72 (s, 1H), 7.85 (d, J = 15.0 Hz, 1H), 12.39 (s, 1H); MS m/z 273 (M^+), 227 ($M^+ - NO_2$). Anal. ($C_{14}H_{11}NO_5$) C, H, N.

***(E)*-5-Ethyl-1-(2-hydroxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (II.3):** mp 125−135 °C; ^{1}H NMR (200 MHz) δ 1.28 (t, J = 8.1 Hz, 3H), 2.69 (q, J = 8.1 Hz, 2H), 6.89 (d, J = 3.7 Hz, 1H), 6.98 (d, J = 8.1 Hz, 1H), 7.41 (dd, J = 8.1 Hz, 2.2 Hz, 1H), 7.41 (d, J = 3.7 Hz, 1H), 7.62 (d, J = 15.0 Hz, 1H), 7.71 (d, J = 2.2 Hz, 1H), 7.86 (d, J = 15.0 Hz, 1H), 12.41 (s, 1H); MS m/z 287 (M^+), 241 ($M^+ - NO_2$). Anal. ($C_{15}H_{13}NO_5$) C, H, N.

***(E)*-5-Chloro-1-(2-hydroxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (II.4):** mp 160−165 °C; ^{1}H NMR (200 MHz) δ 6.92 (d, J = 3.8 Hz, 1H), 7.02 (d, J = 8.9 Hz, 1H), 7.41 (d, J = 3.8 Hz, 1H), 7.49 (dd, J = 8.9 Hz, 2.6 Hz, 1H), 7.64 (d, J = 15.3 Hz, 1H), 7.74 (d, J = 15.3 Hz, 1H), 7.88 (d, J = 2.6 Hz, 1H), 12.44 (s, 1H); MS m/z 295 (M + 2), 293 (M^+), 247 ($M^+ - NO_2$). Anal. ($C_{13}H_8NO_5Cl$) C, H, N.

***(E)*-5-Nitro-1-(2-hydroxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (II.5):** mp 204−210 °C; ^{1}H NMR (200 MHz) δ 7.00 (d, J = 3.7 Hz, 1H), 7.16 (d, J = 9.5 Hz, 1H), 7.42 (d, J = 3.7 Hz, 1H), 7.73 (d, J = 15.0 Hz, 1H), 7.85 (d, J = 15.0 Hz, 1H), 8.42 (dd, J = 9.5 Hz, 2.5 Hz, 1H), 8.90 (d, J = 2.5 Hz, 1H), 13.21 (s, 1H); MS m/z 304 (M^+), 258 ($M^+ - NO_2$). Anal. ($C_{13}H_8N_2O_7$) C, H, N.

General Procedure for the Preparation of 5-Substituted *(E)*-1-(2-Acetoxyphenyl)-3-(5-nitrofuryl)-2-propen-1-ones (III.1−III.5, *R*= *H, CH*$_3$, C$_2$H$_5$, Cl, and NO$_2$) (Scheme 2). A mixture of the appropriate compound **(II.1-II.5)** (0.66−0.77 mmol), acetic anhydride (4 mL, 30 mmol), and acetic acid (4 mL) was refluxed for 24 h, then the acetic acid and the excess of acetic anhydride were removed by distillation at reduced pressure. The solid residue was purified by column chromatography (silica gel, 60 mesh; eluent, chloroform) followed by recrystallization form ethyl acetate/ethanol 1:1 to yield 22−52%.

***(E)*-1-(2-Acetoxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (III.1):** mp 82−86 °C; ^{1}H NMR (300 MHz) δ 6.82 (d, J = 3.8 Hz, 1H), 7.19 (dd, J = 8.0 Hz, 1.5 Hz, 1H), 7.36 (d, J = 3.8 Hz, 1H), 7.38 (d, J = 15.5 Hz, 1H), 7.38 (td, J = 8.0 Hz, 1.5 Hz, 1H), 7.52 (d, J = 15.5 Hz, 1H), 7.59 (td, J = 8.0 Hz, 1.5 Hz, 1H), 7.80 (dd, J = 8.0 Hz, 1.5 Hz, 1H); MS m/z 301 (M^+), 259 ($M^+ - CH_2{=}C{=}O$), 213 ($M^+ - CH_2{=}C{=}O - NO_2$). Anal. ($C_{15}H_{11}NO_6$) C, H, N.

***(E)*-5-Methyl-1-(2-acetoxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (III.2):** mp 151−156 °C; ^{1}H NMR (300 MHz) δ 2.30 (s, 3H), 2.42 (s, 3H), 6.82 (d, J = 3.8 Hz, 1H), 7.06 (d, J = 8.5 Hz, 1H), 7.36 (d, J = 3.8 Hz, 1H), 7.38 (d, J = 15.0 Hz, 1H), 7.38 (dd, J = 8.5 Hz, 1.5 Hz, 1H), 7.47 (d, J = 15.0 Hz, 1H), 7.59 (d, J = 1.5 Hz, 1H); MS m/z 315 (M^+), 273 ($M^+ - CH_2{=}C{=}O$), 227 ($M^+ - CH_2{=}C{=}O - NO_2$). Anal. ($C_{16}H_{13}NO_6$) C, H, N.

***(E)*-5-Ethyl-1-(2-acetoxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (III.3):** mp 155 °C; ^{1}H NMR (300 MHz) δ 1.28 (t, J = 7.6, 3H), 2.31 (s, 3H), 2.73 (q, J = 7.6, 2H), 6.82 (d, J = 3.8 Hz, 1H), 7.09 (d, J = 8.3 Hz, 1H), 7.36 (d, J = 3.8 Hz, 1H), 7.38 (d, J = 16.0 Hz, 1H), 7.42 (dd, J = 8.3 Hz, 2.2 Hz, 1H), 7.48 (d, J = 16.0 Hz, 1H), 7.61 (d, J = 2.2 Hz, 1H); MS m/z 329 (M^+), 287 ($M^+ - CH_2{=}C{=}O$), 241 ($M^+ - CH_2{=}C{=}O - NO_2$). Anal. ($C_{17}H_{15}NO_6$) C, H, N.

***(E)*-5-Chloro-1-(2-acetoxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (III.4)**: mp 134−139 °C; ^{1}H NMR (200 MHz) δ 2.32 (s, 3H), 6.85 (d, J = 3.7 Hz, 1H), 7.15 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 3.8 Hz, 1H), 7.41 (s, 2H), 7.54 (dd, J = 8.7 Hz, 2.6 Hz, 1H), 7.75 (d, J = 2.6 Hz, 1H); MS m/z 335 (M^+), 293 ($M^+ - CH_2{=}C{=}O$), 247 ($M^+ - CH_2{=}C{=}O - NO_2$). Anal. ($C_{15}H_{10}NO_6Cl$) C, H, N.

***(E)*-5-Nitro-1-(2-acetoxyphenyl)-3-(5-nitrofuryl)-2-propen-1-one (III.5)**: mp 145−150 °C; ^{1}H NMR (200 MHz) δ 2.37 (s, 3H), 6.90 (d, J = 3.8 Hz, 1H), 7.38 (d, J = 3.8 Hz, 1H), 7.42 (d, J = 8.9 Hz, 1H), 7.45 (s, 2H), 8.44 (dd, J = 8.9 Hz, 2.8 Hz, 1H), 8.65 (d, J = 2.8 Hz, 1H); MS m/z 304 ($M^+ - CH_2{=}C{=}O$), 256 ($M^+ - CH_2{=}C{=}O - NO_2$). Anal. ($C_{15}H_{10}N_2O_8$) C, H, N.

Partition Coefficient Determinations. The log P values were determined by means of the reversed-phase high-performance liquid chromatography (RP-HPLC) method[19,20,21] using ODS or preferably *n*-octanol-coated ODS as the reverse stationary phase. The following ODS−Hypersil columns were employed: 17 × 4 mm, mean particle size 5 μm, from Hyperchrome-Bischoff part no 01461805 or ODS−Hypersil 20 × 4 mm, guard column, mean particle size 5 μm, from Hewllet-Packard. The columns were pretreated with three injections of 20 μL of pure *n*-octanol, and the chromatographic system was allowed to stabilize by standing overnight with a flow rate of one of each eluent, A, B, or C, adjusted to 0.3 mL/min. During the *n*-octanol injections the detector should be disconnected. As mobile phases one of the three following aqueous buffer solutions containing up to 50% v/v methanol were used, respectively: eluent A, *n*-octanol presaturated aqueous phosphate buffer (KH_2PO_4, 1.47 mM; K_2HPO_4, 10.6 mM; and NaCl, 140 mM; pH = 7.24; I = 0.2 M); eluent B, 25 mL of the previous aqueous phosphate buffer solution (eluent A), diluted to 500

mL with ultrapure water, was added to 500 mL of HPLC-grade methanol (approximate final concentrations: KH_2PO_4, 0.037 mM; K_2HPO_4, 0.22 mM; and NaCl, 3.5 mM); eluent C, 500 mL of aqueous phosphate buffer (KH_2PO_4, 3.4 g and concentrated phosphoric acid, 85% w/w, 1.7 mL, in ultrapure water), pH = 2.2, was added to 500 mL of HPLC-grade methanol (approximate final concentrations: KH_2PO_4, 25 mM; H_3PO_4, 25 mM).

All the measurements were made at room temperature, controlled and adjusted to (25 ± 3)°C. The linear equations that relate log k' to log P, for the chromatographic system employed, are determined by measuring log k' for standard substances of known log P value. Thiourea was used for the determinations of the dead-volume (V_o). The chromatographic system calibrations were done using the following substances as standard (El Tayar et al.[33] and Klein et al.[20]): benzamide (log P = 0.64), acetophenone (log P = 1.58), nitrobenzene (log P = 1.85), *p*-chlorophenol (log P = 2.4), benzophenone (log P = 3.2), *p*-dichlorobenzene (log P = 3.4), diphenylamine (log P = 3.4), diphenyl (log P = 4.0), and diphenyl ether (log P = 4.2). For each analyzed pure compound of sets I, II, and III, solutions in DMSO were previously prepared (2 mM) and about 5−10 μL of this solution was added respectively to 2 mL of each eluent A, B, or C to give 5−10 μM solutions, which were injected. No significant dependence of log P on compound concentration was observed for concentrations up to 10 μM. The log k' values and the capacity factors for the studied compounds were calculated from their retention times and are directly proportional to their log P values. Using eluent A, the log k' values for the compounds **I.5** and **II.2**−**II.4** could not be determined because of their high retention times. Moreover, using eluent A, some degree of instability was observed for the compounds of set II.

E Reduction (in Volts). The reduction behavior of the nitrofuran derivatives was studied in DMF by cyclic voltammetry.[24] The obtained cyclic voltammogram profiles for compounds of set I are different from those observed for sets II and III. For all the compounds the first reduction peak, in volts, was taken as the physicochemical parameter for QSAR analysis.

Microbiology. 50% Growth Inhibitory Activity, IC_{50}. The growth inhibitory activity was evaluated against *S aureus (*ATCC-25923 strain) and *C. crescentus* (NA1000 strain) by the sequential broth macrodilution test.[28] For cultivation of *S. aureus*, TSB (30 g of trypticase soy broth, Difco, dissolved in 100 mL of water) was used and the temperature of incubation was 37 °C, whereas *C. crescentus* was cultivated at 30 °C in PYE (1 g of peptone, Difco, and 2 g of yeast extract, Difco, dissolved in 1 L of water with 0.8 mM $MgSO_4$.and 0.5 mM $CaCl_2$). A suspension of bacteria was prepared as follows: from a recent, 24 h, culture of bacteria in the suitable medium, a small aliquot was taken and diluted into new medium, e.g., 100 μL of a 24 h culture of *S. aureus* was diluted in 2 mL of TSB. The new diluted suspension was then incubated for about 8 h. This new 8 h culture contains bacteria in an exponential growth phase, and contains about 3×10^8 bacteria/mL, for an absorbance of about 0.43 observed at 600 nm. This 8-h culture was then diluted 1500 times with medium (or proportional to the absorbance when the absorbance is different from 0.43) to obtain about 2×10^5 bacteria/mL. A sequence of tubes containing 1 mL of solutions of the nitrofurans in culture media with 2-fold decreasing concentration was prepared, and then 1 mL of the suspension of prepared bacteria (2×10^5 bacteria/mL) was added to each tube. The tubes were incubated at a suitable temperature for 20 h (*C. crescentus*) or for 12 h (*S. aureus*), and then the proliferation of the bacteria was measured by the absorbance at 600 nm. The log($1/IC_{50}$), values, the logarithm of the concentration leading to 50% decrease in growth, were taken as the biological parameter. The values were expressed in μmol/mL.

Nitrofuran Reduction Mediated by *C. crescentus* Extracts.[8] *C. crescentus* cell extracts were prepared as described by Gomes and Shapiro,[29] with modifications. The cells present in 500 mL of a 16-h culture of *C. crescentus* (absorbance of 1.15 at 600 nm) were harvested by centrifugation at 5000*g* for 15 min. The culture medium was discarded, and the cells were washed with phosphate buffer, pH 7.0, and resuspended in 15 mL of phosphate buffer (50 mM, pH 7.0) containing protease inhibitors (25 mM bezamidine and 150 μg/mL phenyl methyl sulfonyl fluoride). The cells were then disrupted in a French press cell (11 000 psi, three passages), and after centrifugation (10 000*g*, for 15 min) the resulting supernatant was tested for nitroreductase activity. The total protein content of the aqueous extract was determined by the Bradford method.[34]

Assay of Nitroreductase Activity. Nitroreductase activity was determined for each compound of set I through the observation of the rate of disappearance of the compound (nitrofuran derivative) at 422 nm, the λ_{max} of the compound where R=H. To a quartz cell, optical length 4 cm, were added 4 mL of 50 mM phosphate buffer, pH = 7.0, 50 μL of NADPH solution 40 mg/mL in phosphate buffer, 50 μL of *C. crescentus* extracts (total protein concentration of the extract was 1.7 mg/mL), and different volumes of each tested compound of set I, in DMSO (2 mg/mL) to obtain initial concentrations of nitrofuran in the range of 1−10 μM. The absorbance of the nitrofuran was determined for 15 min, in a Beckman DU70 spectrometer, and 10 points per minute were read and the first 5 min were used to measure the initial velocity of nitrofuran derivative disappearance. The profile of the initial velocity curve of nitrofuran consumption versus nitrofuran concentration was analyzed, and the maximum velocity for each compound (V_{max} in μM/min) was taken as a biological parameter.

QSAR Analyses. QSAR models were derived by multiple regression analyses that were performed using the BILIN program[35] to determine the coefficients of the correlation equations. In addition to the *F*-test, the cross-validation test was applied to evaluate the significance of the correlations. In all the equations in this paper, the numbers in parentheses represent the 95% confidence intervals of the coefficients; n is the number of points on which the equation is based, r is the correlation coefficient, s is the standard deviation, F is the Fisher significance test value, and Q^2 and s-PRESS are the squared correlation coefficient and the standard deviation, respectively, associated with the cross-validation procedure.

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Supporting Information Available: Table listing biological and chemical descriptors used in QSAR analysis for compounds of sets I, II, and III. This material is available free of charge via the Internet at http://pubs.acs.org.

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