Structure−Activity Relationships of Phenyl-Furanyl-Rhodanines as Inhibitors of RNA Polymerase with Antibacterial Activity on Biofilms

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The dramatic rise of antibiotic-resistant bacteria over the past two decades has stressed the need for completely novel classes of antibacterial agents. Accordingly, recent advances in the study of prokaryotic transcription open new opportunities for such molecules. This paper reports the structure−activity relationships of a series of phenyl-furanyl-rhodanines (PFRs) as antibacterial inhibitors of RNA polymerase (RNAP). The molecules have been evaluated for their ability to inhibit transcription and affect growth of bacteria living in suspension or in a biofilm and for their propensity to interact with serum albumin, a critical parameter for antibacterial drug discovery. The most active of these molecules inhibit *Escherichia coli* RNAP transcription at concentrations of ≤ 10 μM and have promising activities against various Gram-positive pathogens including *Staphylococcus epidermidis* biofilms, a major cause of nosocomial infection.

Introduction

Antibiotic-resistant bacteria have become a major concern for public health professionals since bacterial resistance has dramatically increased during the last two decades.[1] Furthermore, antibacterial chemotherapy alone is ineffective to cure biofilm related infections, a major cause of nosocomial infections.[1] Indeed, bacteria growing as an adherent biofilm on catheters, orthopedic implants, and bones are inherently resistant to antibiotics and are poorly recognized by the host immune defenses.[2] Consequently, there is a growing need for novel classes of antibacterial agents able to address resistance and persistent infections because of biofilms.

Within that scope, RNA polymerase (RNAP[a]), the enzyme responsible for transcription, remains an attractive target for antibacterial drug discovery.[3] RNAP holoenzyme is a large protein composed of core subunits ($\alpha(2)\beta\beta'$) plus a σ factor that is required for specific promoter site recognition and the initiation of transcription. Several antibacterial molecules, mainly from natural origins, inhibit this enzyme with rather different mechanisms of action; however, some share cross-resistance.[4] Among these inhibitors, lipiarmycin, a narrow-spectrum antibacterial, has entered phase III clinical trials under the name of OPT-80 for the treatment of *Clostridium difficile* associated diarrhea.[5] Rifampicin (RIF) is still a first-line antibiotic for the treatment of tuberculosis, and RIF in association with antibiotics is frequently used to cure implant-associated infections because of staphylococcal biofilms.[6] The potential of RNAP as a target has also been recently highlighted by the discovery of three novel synthetic classes of inhibitors with antibacterial activity.[7−9]

We have previously described the biological activity of one of these new synthetic antibacterials: the phenyl-furanyl-rhodanines (PFRs).[8] Its representative molecule **1** (Figure 1) inhibits the core RNAP-σ^{70} factor assembly, affects transcription by the core polymerase and the holoenzyme in vitro, and inhibits RNA synthesis in *Staphylococcus epidermidis*. This molecule is bactericidal against pathogenic Gram-positive bacteria, for example, *Staphylococcus aureus* and *Bacillus anthracis*, but is not active against Gram-negative pathogens like *Escherichia coli* or *Pseudomonas aeruginosa*. Compound **2** (Figure 1), an analogue of **1**, shows interesting activity against *S. epidermidis* biofilms when compared to RIF.[10] Furthermore, these molecules are not toxic against various eukaryotic cells.

These previous results prompted us to undertake a structure−activity relationship (SAR) study to characterize structural determinants of this new class of RNAP inhibitors. We considered how modifications of the three different parts (A, B, and C) of the molecules affected their biochemical and antibacterial activities (Figure 1). Keeping in mind that these molecules may also act as systemic antibacterial drugs, we evaluated their binding to human serum albumin (HSA) by surface plasmon resonance (SPR). This parameter is critical since high free concentrations of antibiotics in plasma are needed to achieve multiples of the minimum inhibitory concentration (MIC) at the site of infection over a relatively short time period. This explains why most of the antibiotics in clinical use are more hydrophilic and have lower affinities for HSA than most of the commercial drugs.[11] In this paper, we report the synthesis of relevant analogues of compound **1** and our findings regarding their biological activity.

Results and Discussion

Synthesis of PFRs. Many compounds reported in this SAR study are commercially available. When synthesis was required, a two-reaction sequence of Suzuki−Miyaura coupling[12,13] and Knoevenagel condensation[14] was used (Scheme 1). Suzuki−Miyaura coupling was rather efficient when the reaction mixture was heated (reaction time < 19 h, 74−100% yields) and was much slower when performed at room temperature using Pd-

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[a] Abbreviations: PFR, phenyl-furanyl-rhodanine; RNAP, RNA polymerase; RIF, rifampicin; SAR, structure−activity relationships; HSA, human serum albumin; SPR, surface plasmon resonance; MIC, minimum inhibitory concentration; CFU, colony-forming unit; WRF, warfarin; MHB, Mueller-Hinton broth; CHO, Chinese hamster ovary; ATP, adenosine triphosphate; RU, resonance unit.

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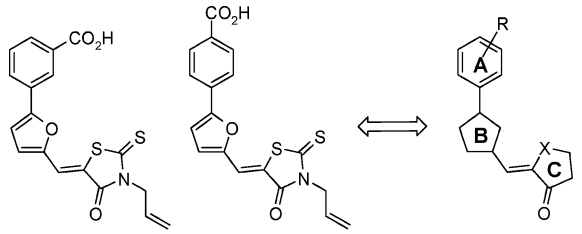
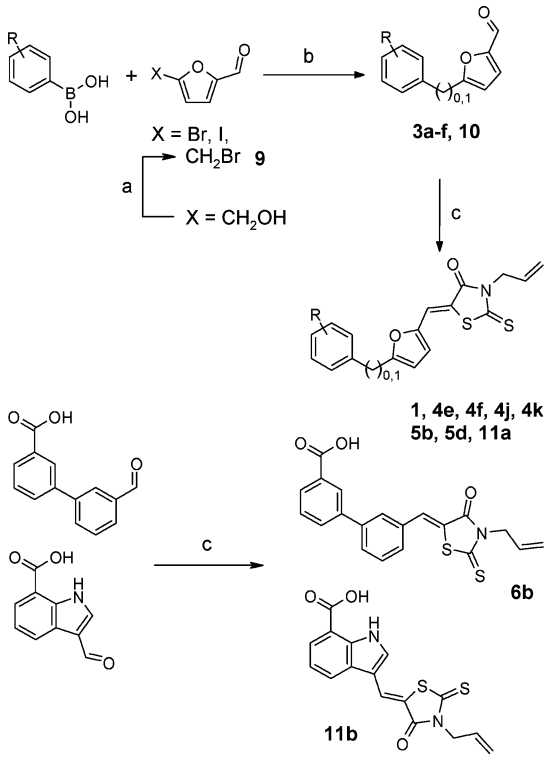


1 **2**

Figure 1. Compounds **1** and **2** and molecular template for the SAR study.

Scheme 1. Synthesis of PFRs[a]



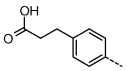
[a] (a) Me_3SiBr, $CHCl_3$, Δ; (b) cat. $(Ph_3P)_4Pd$, K_2CO_3, toluene/EtOH/H_2O, Δ, or cat. Pd−C, Na_3PO_4,12H_2O, EtOH/H_2O; (c) 3-allylrhodanine, ethylenediamine diacetate, MeOH, or allylrhodanine, piperidine, DMF.

carbon as catalyst (10 days, 41% yield). Suzuki−Miyaura coupling did not occur when the rhodanine moiety was present in the starting material, possibly because of sulfur poisoning of the catalyst.

Mild conditions were used for the Knoevenagel condensation. Ethylenediamine diacetate was used as the required base with methanol as solvent. These conditions gave the desired compounds in 35−75% yields. When solubility of the starting materials was poor in methanol, dimethylformamide was used with piperidine as the required base, but these conditions gave poor yields (8−22%).

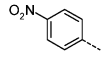
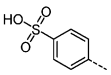
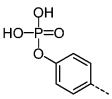
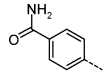
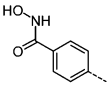
Synthesis of Carboxylic Acid Bioisosteres. The carboxylic acid moiety of **2** was replaced with various moieties that have been considered as bioisosteres of this function (Table 2).[15] Compounds **5b** and **5d** were obtained following the procedure described above. Analogues **5c** and **5e** were obtained starting from **4f** and **2**, respectively. Synthesis of compound **5c** is outlined in Scheme 2. Starting material **4f** was reacted with phosphorus oxychloride and then was hydrolyzed in water.[16] Compound **5c** was produced without further purification in poor yield (21%). Synthesis of compound **5e** is outlined in Scheme 3. Starting material **2** was reacted under mild conditions with

Table 1. SAR of Part A Derivatives of the Molecular Template

N°		Transcription inhibition at 10 μM (%)	*S. epidermidis*[a] MIC (μg/mL)	*E. coli tolC*[b] MIC (μg/mL)
1		102 ± 12	12.5	6.25
2		98 ± 9	3.13	12.5
4a		72 ± 11	≥100	≥100
4b		46 ± 14	≥100	3.13
4c		0 ± 5	≥100	≥100
4d		76 ± 10	≥100	12.5
4e		11 ± 12	≥100	≥100
4f		84 ± 5	12.5	3.13
4g		10 ± 19	≥100	≥100
4h		100 ± 13	6.25	25.0
4i		5 ± 10	≥100	≥100
4j		71 ± 16	≥100	≥100
4k		68 ± 7	25.0	12.5

[a] *S. epidermidis* CIP 105777. [b] *E. coli* tolC CGSC 5633.

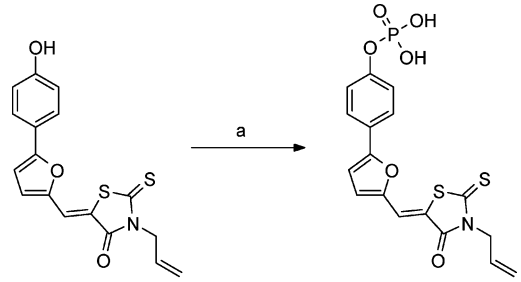
Table 2. SAR of Carboxylic Acid Bioisosters

N°		Transcription inhibition at 10 μM (%)	*S. epidermidis*[a] MIC (μg/mL)	*E. coli tolC*[b] MIC (μg/mL)
5a		3 ± 13	≥100	≥100
5b		9 ± 18	≥100	≥100
5c		0 ± 14	≥100	≥100
5d		102 ± 10	≥100	1.56
5e		100 ± 14	≥100	25.0

[a] *S. epidermidis* CIP 105777. [b] *E. coli* tolC CGSC 5633.

cyanuric chloride, hydroxylamine, *N*-methylmorpholine, and dimethylaminopyridine in catalytical amounts.[17] Compound **5e**

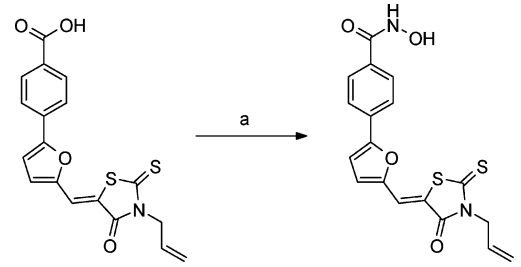
Scheme 2. Synthesis of Carboxylic Acid Bioisoster **5c** (Phosphoric Acid)[a]



4f **5c**

[a] (a) $POCl_3$, pyridine, 0 °C, 50 min, then H_2O, 0 °C to rt, 18 h.

Scheme 3. Synthesis of Carboxylic Acid Bioisoster **5e** (Hydroxamic Acid)[a]



2 **5e**

[a] (a) $NH_2OH{\cdot}HCl$, cyanuric chloride, NMM, cat. DMAP, THF, 0 °C to rt, 18 h.

was produced after purification by reverse-phase high-performance liquid chromatography (HPLC) in very poor yields (1%).

SAR Study. The compounds were tested for their ability to inhibit in vitro *E. coli* RNAP transcription and for their ability to inhibit bacterial growth of Gram-positive (*S. epidermidis*) and Gram-negative (*E. coli* tolC) species. Indeed, none of the molecules was able to inhibit the growth of the *E. coli* wild strain at concentrations up to 100 μg/mL (Supporting Information, Table S1). The *E. coli* tolC strain is a mutant deficient in multidrug efflux systems. This property allows for detection of the antibacterial activity of molecules that are able to penetrate *E. coli* but would be readily ejected from the bacteria. In addition, it allows for comparison of their MIC with data obtained in the in vitro transcription assay on the *E. coli* RNA polymerase. In parallel, the toxicity of the compounds was tested on eukaryotic cells, and none of these compounds affected their growth (Supporting Information, Table S1).

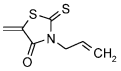
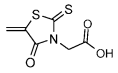
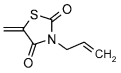
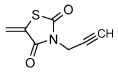
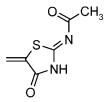
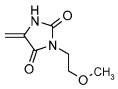
Modifications of part A of the molecular template stress the importance of the carboxylic acid moiety for inhibition of in vitro transcription (Table 1). In the absence of any functionality on the phenyl ring (**4b**), transcription inhibition was decreased 2-fold, but antibacterial activity remained good on *E. coli* tolC. Comparison of compounds **1**, **2**, and **4a** affords the conclusion that the position of the acid on the phenyl ring does not really matter with respect to the enzymatic activity, but the para- and meta-positions are required for antibacterial activity. Analogue **4k** illustrates that the carboxylic acid moiety can be distanced from the phenyl ring, only slightly affecting the in vitro transcription and antibacterial activities. Replacement of the carboxylic acid moiety by a weakly basic moiety (**4e**), or by a stronger electron-withdrawing group (**4c**), negatively affects the activity of these compounds in both the transcription and the antibacterial assays. However, replacement by a weaker electron-withdrawing group (**4d**) leads to a slight decrease of transcription

Table 3. SAR of Part B Derivatives of the Molecular Template

N°		Transcription inhibition at 10 μM (%)	*S. epidermidis*[a] MIC (μg/mL)	*E. coli tolC*[b] MIC (μg/mL)
1		97 ± 11	12.5	6.25
6a		100 ± 7	25.0	50.0
6b		26 ± 17	25.0	≥100

[a] *S. epidermidis* CIP 105777. [b] *E. coli tolC* CGSC 5633.

Table 4. SAR of Part C Derivatives of the Molecular Template

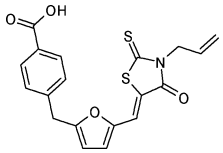
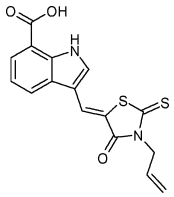
N°	Acid Position		Transcription inhibition at 10 μM (%)	*S. epidermidis*[a] MIC (μg/mL)	*E. coli tolC*[b] MIC (μg/mL)
1	p		100 ± 14	3.13	12.5
7a	p		82 ± 12	≥100	≥100
7b	m		89 ± 8	≥100	≥100
7c	m		17 ± 16	≥100	≥100
7d	p		75 ± 8	≥100	≥100
7e	p		100 ± 13	≥100	≥100
7f	p		98 ± 15	≥100	≥100
7g	p		55 ± 5	≥100	≥100
7h	m		85 ± 3	≥100	≥100
7i	p		30 ± 11	≥100	≥100
7j	m		72 ± 14	50.0	50.0

[a] *S. epidermidis* CIP 105777. [b] *E. coli* tolC CGSC 5633.

inhibition, while antibacterial activity is conserved on *E. coli* tolC. Comparison of molecules **4h** and **4j** with the previous molecules **4d** and **4e** reveals that the presence of the carboxylic acid moiety enhanced the activity on the RNAP. Furthermore, chlorine does not enhance or disfavor antibacterial activity, while the amine leads to loss of antibacterial activity. Biological activity is also lost when the acid is masked by a methyl ester function (**4g**) or by a lactone function (**4i**).

To this point of the discussion, the carboxylic acid was revealed to be the most efficient moiety for the inhibition of transcription. Further usual bioisosteres of this function were then tested to see if they may improve the inhibitory activities of lead compound **2** on RNAP and bacterial growth but also

Table 5. Biological Activity of Compounds **11a** and **11b**

compound	structure	transcription inhibition at 10 μM (%)	*S. epidermidis*[a] MIC (μg/mL)	*E. coli* tolC[b] MIC (μg/mL)
11a		63 ± 5	≥100	≥100
11b		95 ±9	25.0	≥100

[a] *S. epidermidis* CIP 105777. [b] *E. coli tolC* CGSC 5633.

Table 6. Antibacterial Spectrum of PFRs

	MIC (μg/mL)							
	S. aureus		*S. epidermidis*			*S. pneumoniae*	*C. difficile*	*B. anthracis*
N°	CIP 76.25[a]	49589[b]	RP62A	40004	48155	CIP 103566	DSM 1296	9131
1	12.5	25.0	12.5	25.0	25.0	50.0	12.5	6.25
2	≥100	≥100	3.13	6.25	3.13	≥100	≥100	3.13
4b	≥100	≥100	≥100	nd[c]	nd	≥100	≥100	0.78
4f	6.25	25.0	12.5	12.5	12.5	25.0	50.0	6.25
4h	50.0	50.0	6.25	6.25	12.5	12.5	12.5	3.13
4k	25.0	25.0	25.0	25.0	12.5	≥100	50.0	12.5
5d	≥100	≥100	≥100	nd	nd	≥100	≥100	12.5
5e	≥100	≥100	≥100	nd	nd	25.0	≥100	50.0
6a	50.0	25.0	25.0	50.0	25.0	25.0	6.25	6.25
6b	≥100	25.0	25.0	50.0	50.0	≥100	12.5	12.5
11b	50.0	50.0	25.0	25.0	25.0	50.0	25.0	12.5

[a] Reference strain. [b] RIF-resistant multiresistant strain. [c] Not determined.

if they may decrease HSA binding (Table 2). Nitrobenzene (**5a**), sulfonic acid (**5b**), and phosphoric acid (**5c**) derivatives are neither biochemically active nor antibacterial. Benzamide (**5d**) and hydroxamic acid (**5e**) inhibit in vitro transcription as efficiently as lead compounds **1** and **2**. None of these bioisosters are antibacterial against *S. epidermidis*, but **5e** and, more interestingly, **5d** show activity against *E. coli* tolC. Furthermore, these compounds present clear advantages regarding HSA binding (see below).

Modifications of part B of the molecular template show that biological activity is enhanced by a furan (Table 3). Comparison of molecules **1**, **6a**, and **6b** afforded the conclusion that the molecules require a five-membered planar heterocycle to be optimally biochemically active. However, **6b** proves to be antibacterial against *S. epidermidis* and not against the permeable *E. coli* tolC strain. Antibacterial activity is also observed on *B. anthracis* and *S. aureus* (Table 6). A possible explanation may be that this compound does not affect transcription of *E. coli* but does affect transcription of other microorganisms. This property remains unclear since, in this study, the transcription assay was performed with purified RNAP from *E. coli*. Furan and pyrrole display comparable activities regarding holoenzyme dissociation and transcription inhibition, but furan seems to slightly favor antibacterial activity (Table 6). Nevertheless, pyrrole seems advantageous when bacteria are grown as a biofilm (see below).

Modifications of part C of the molecular template underlined the importance of the allylrhodanine for antibacterial activity (Table 4). With the exception of compounds **7c** and **7i**, all the compounds are able to inhibit transcription in vitro. Strikingly, only the lead compounds **1** and **2**, bearing the allylrhodanine moiety, prove to be antibacterial agents. Comparison of compounds **2**, **7a**, **7b**, and **7d** highlights the importance of the thioxo-function and the N-allyl chain in this heterocycle for antibacterial activity. We felt concerned that the antibacterial activity of our series of molecules was only due to the allylrhodanine and not to its ability to inhibit bacterial transcription. Indeed, some rhodanine derivatives related to our template have been previously reported as antibacterial agents.[18,19] However, scrutiny of other molecules related to our template indicates that its biological activity is not limited to only the allylrhodanine or the presence of specific fragments connected randomly (Supporting Information, Table S2).

Analogues **11a** and **11b** reveal how parts A and B of the molecular template can be connected (Table 5). The merge of the two fragments (**11b**) does not disfavor biochemical activity, while distancing (**11a**) disfavors it. Antibacterial activity is lost completely with compound **11a**.

Spectrum of Antibacterial Activity. The most interesting PFRs of this series have a broad spectrum of activity on relevant Gram-positive pathogens (Table 6). A multiresistant *S. aureus* clinical isolate also resistant to RIF is still affected by compounds active against a *S. aureus* reference strain. The molecules are weakly active against *Streptococcus pneumoniae* and *C. difficile*. Nevertheless, the MIC of **6a** against *C. difficile* is not much higher than that of vancomycin, a second-line antibiotic for the treatment of intestinal infections caused by this pathogen.[5] Very encouraging activities are observed on *B. anthracis*; the best activity is observed with **4b,** with an MIC of 0.78 μg/mL. With respect to this pathogen, a previous report stated that **4h** was a good inhibitor of anthrax lethal factor, and some analogues were active in an animal infection model.[20]

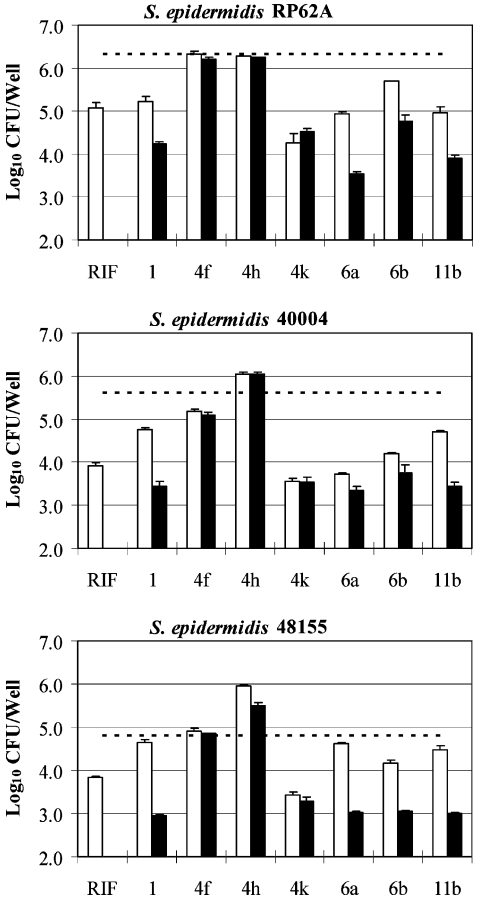


Figure 2. Amount of bacteria measured by ATP-bioluminescence within 24 h biofilms of *S. epidermidis* RP62A, 40004, and 48155 after 24 h exposure to RIF (10 μg/mL) and PFRs. The mean amounts of bacteria (dotted line) within 24 h biofilms were 6.32 $\pm$ 0.05, 5.62 $\pm$ 0.08, and 4.80 $\pm$ 0.09 Log_{10} CFU/well for strains RP62A, 40004, and 48155, respectively. White bars: antibacterial concentration at 4 times the MIC; black bars: antibacterial concentration at 8 times the MIC.

However, we show that this compound does not only target the toxin but also affects the growth of *B. anthracis* with a good efficacy (MIC of 3.13 μg/mL). This dual effect on the growth of *B. anthracis* and its virulence factor could explain the results achieved in vivo.[20]

Regarding SAR, a striking aspect of this series of antibacterials is highlighted by **1** and **2**. These molecules present rather different patterns of antibacterial activity. However, this is despite their very close chemical structure and very close behavior on RNAP in vitro. Such discrepancies between the inhibitory effect on the purified target and on bacteria are frequently reported.[21−23] They are likely to reflect the inability of many molecules to reach their target and to reflect how bacteria can select against small molecules present outside the cell.

Activity on Biofilms. Since some compounds display antibacterial activity against the *S. epidermidis* RP62A strain under planktonic conditions (i.e., suspended bacteria), they were challenged against 24 h old biofilms of the same strain. Two additional *S. epidermidis* clinical isolates (strains 40004 and 48155) obtained from patients with device-related staphylococcal infections were also investigated. The MICs of the investigated compounds do not vary significantly between the different *S. epidermidis* strains (Table 6). Biofilms were grown for 24 h and were then challenged with the antibacteria for 24 h at concentrations of 4 times and 8 times the MIC (Figure 2). RIF was tested at 10 μg/mL, a concentration much higher than its MIC (0.003−0.006 μg/mL), but high concentrations are often encountered in the literature when RIF and other antibiotics are used against staphylococcal biofilms.[24,25] Furthermore, we have previously observed that very high concentrations of RIF do not significantly improve its antibacterial activity, and a limit is almost reached at a concentration of 8 times the MIC.[10]

Among the investigated compounds, only **4f** and **4h** do not affect *S. epidermidis* biofilms at all. The other compounds **1**, **4k**, **6a**, **6b**, and **11b** display good activities on biofilms at a concentration of 8 times the MIC and are able to decrease the amount of bacteria by 2−3 Log_{10} CFU/well. Interestingly, compound **4k** displays similar activities on the three strains at 4 times and 8 times the MIC. These results stress the potential role of distancing the carboxylic acid moiety from the phenyl ring with respect to biofilms. The best activity against the *S. epidermidis* RP62A, 40004, and 48155 strains is observed with **6a** at a concentration of 8 times MIC, with a decrease in levels of 2.8 Log_{10} CFU/well, 2.3 Log_{10} CFU/well, and 1.8 Log_{10} CFU/well, respectively. This latter compound is not the most efficient under planktonic conditions in terms of its MIC.

HSA Binding and Antibacterial Activity. Some of the molecules described in this paper possess interesting antibacterial activities. However, like most of the antibacterial molecules identified after the screening of large synthetic libraries,[9,26,27] they are very likely to interact with serum proteins because of their physicochemical properties.[28] The binding of the molecules to serum proteins, essentially to HSA, reduces their free concentrations in plasma and thus decreases their antibacterial efficiency. The common but rather indirect approach to evaluate the propensity of a molecule to bind to HSA is the determination of its MIC in the presence of this protein. SPR technology is a complementary approach, since it measures accurately the binding of a molecule to HSA. In a previous report, Frostell-Karlsson et al. used this technique in an attempt to predict HSA binding levels of drug compounds.[29] Their results demonstrate that it is possible to classify compounds as low (binding < 80%), medium (binding of 80−90%), and high binders (binding > 90%) of HSA, according to their SPR biosensor response. To have a further selection criterion for lead optimization, we have used the same method to truly evaluate and rank the binding of PFRs to HSA (Figure 3). As expected, HSA bindings, measured by SPR, of the lead compounds **1** and **2** are very high, with biosensor responses 10-fold higher than that of warfarin (WRF), a high binder of HSA.[29] All analogues also show high response values, with the exception of two compounds (**4f**, **5d**) that display biosensor responses comparable to WRF. Replacement of the carboxylic acid moiety (**2**) by a hydroxyl (**4f**) or an amide moiety (**5d**) decreases HSA binding by 10-fold. Comparatively, substitution by a hydroxamic acid moiety (**5e**) decreases its HSA binding by almost 5-fold but decreases its activity on the enzyme. Despite having no antibacterial activity, the distancing of the benzoic acid from the furan (**11a**) decreases HSA binding by 6-fold. Encouragingly, the lowest binding values were not far from those of the clinically used antibiotic RIF.

Antibacterial compounds were then evaluated against *E. coli* tolC in the presence of HSA to assess the relevance of the SPR method (Figure 3). All the very high binders of HSA (**1**, **2**, **4h**, and **4k**) lose their antibacterial activity in the presence of a concentration as low as 1% HSA (w/v). MICs of compounds **4b** (not evaluated in SPR because of poor solubility) and **5d** increase by more than 10-fold to reach 25 μg/mL. The hydroxamic acid is an interesting moiety since the MIC of compound **5e** only increases by 2-fold. Surprisingly, **4f**, which ranks as one of the lowest HSA binders, loses its antibacterial activity in presence of the serum protein. A possible explanation

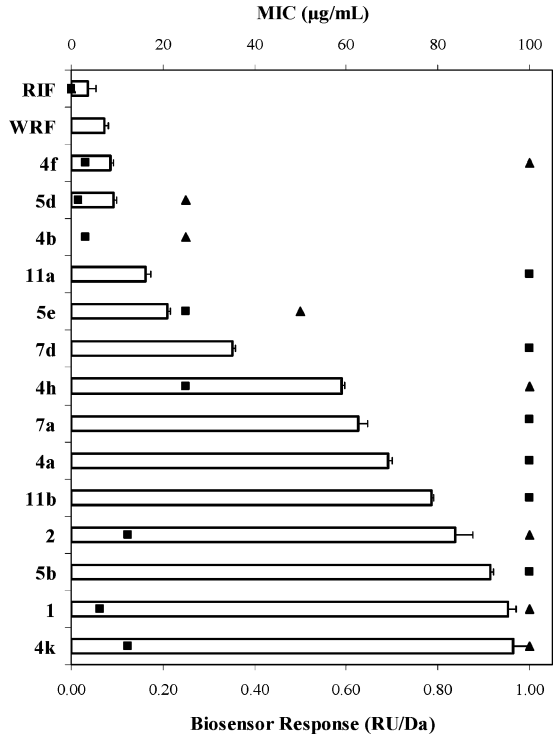


Figure 3. Ranking of HSA binding levels of PFRs according to biosensor response and influence of HSA on antibacterial activity. Rifampicin (RIF), a low binder of HSA (73% binding), and warfarin (WRF), a high binder of HSA (98% binding), were used as reference molecules to assess protein binding.[29] Compound **4b** was not tested because of its poor solubility in the SPR buffer. White bars: biosensor response ± standard error; black square: *E. coli* tolC MIC in the absence of HSA; black triangle: *E. coli* tolC MIC in the presence of 1% (w/v) HSA.

would be that some PFRs bind to some sites of HSA that may be unaccessible subsequent to NH_2-tethering of the protein to the biosensor chip. These putative sites would be different for the major drug binding sites according to Frostell-Karlsson et al.'s conclusions.[29]

Despite this limitation, SPR results are rather informative, and it appears that amide and hydroxamic acid moieties should be valuable for development of this series with respect to attenuation of binding to serum proteins.

Conclusion

The tested compounds inhibited bacterial RNAP in the micromolar range with an interesting spectrum of activity against clinically relevant Gram-positive pathogens and biofilms. Mapping of the binding site of the PFRs on the core RNAP would be a milestone that could lead to understanding their mechanism of action and a more rational design of improved ligands. Not surprisingly, from an antibacterial provided by the screening of a large synthetic library, the original molecule interacts too strongly with HSA. However, it is possible to significantly decrease this binding by slight modifications of the benzoic acid moiety.

SAR studies on antibiotics are often difficult to achieve. Many molecules with good activity on the purified target and bacteria turn out to be inactive after modification either because these modifications alter their interaction with the target or because permeability limitations are difficult to assess. We conclude from this study that the PFR skeleton can be modified without losing its antibacterial properties and that it is a promising template for development of new drugs to fight infection.

Experimental Section

Chemistry. All reagents were purchased from commercial suppliers and were used as provided. Purification by reverse-phase HPLC was performed on a Waters SymmetryPrep C18 column (19 × 150 mm, 7 µM). Analytical LC-MS was performed on a Waters Alliance 2690 HPLC coupled to a Micromass Platform II spectrometer (electrospray ionization mode, ESI+ or ESI−). All analyses were carried out using a RP-18, 3.5 µm, 2.1 × 30 mm reverse-phase column. A flow rate of 600 µL/min and a gradient of 0−100% B over 5 min was used; eluent-A: water/0.1% TFA, eluent-B: acetonitrile/0.1% TFA. Proton nuclear magnetic resonance (^{1}H NMR) analyses were performed by Laboratoire des Mesures Physiques of University of Montpellier-2 (Montpellier, France). ^{1}H NMR spectra recorded on a Bruker DRX spectrometer (400 MHz) or on a Bruker AC spectrometer (250 MHz) are given in parts per million (ppm) and are referenced to an internal standard of tetramethylsilane (TMS, δ 0.00 ppm). Peak multiplicity is reported as s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet), and br s (broad singlet). Elemental analyses were performed by Spectropole of Marseille (Marseille, France).

3-(5-Formyl-furan-2-yl)-benzoic Acid (3a). A solution of K_2CO_3 (476 mg, 3.44 mmol) in water (3 mL) was added to a mixture of 3-carboxyphenylboronic acid (316 mg, 1.90 mmol) and 5-bromo-2-furaldehyde (301 mg, 1.72 mmol) in toluene/ethanol (20 mL). $(Ph_3P)_4Pd$ (20 mg, 0.02 mmol) was added, and the reaction mixture was stirred at 90 °C for 7 h. The reaction mixture was cooled to room temperature and was diluted with water (15 mL); the pH was then adjusted to 1 by addition of a solution of HCl (6 N). The diluted reaction mixture was extracted with CH_2Cl_2 (3 × 100 mL); the combined organic fractions were washed with brine (1 × 10 mL), were dried over $MgSO_4$, and were concentrated under reduced pressure to afford 367 mg of **3a** in quantitative yield. MS: 217 ($[M + H]^+$).

3-(5-Formyl-furan-2-yl)-aniline (3b). A solution of K_2CO_3 (486 mg, 3.52 mmol) in water (3 mL) was added to a mixture of 3-aminobenzeneboronic acid monohydrate (300 mg, 1.94 mmol) and 5-bromo-2-furaldehyde (308 mg, 1.76 mmol) in toluene/ethanol (20 mL). $(Ph_3P)_4Pd$ (20 mg, 0.02 mmol) was added, and the reaction mixture was stirred at 90 °C for 7 h. The reaction mixture was cooled to room temperature and was diluted with water (15 mL). The diluted reaction mixture was extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic fractions were washed with brine (1 × 10 mL), were dried over $MgSO_4$, and were concentrated under reduced pressure to afford 215 mg of **3b**. The product was used without further purification.

4-(5-Formyl-furan-2-yl)-phenol (3c). A solution of K_2CO_3 (301 mg, 2.18 mmol) in water (3 mL) was added to a mixture of 4-hydroxyphenylboronic acid (200 mg, 1.45 mmol) and 5-iodo-2-furaldehyde (386 mg, 1.74 mmol) in toluene/ethanol (20 mL). $(Ph_3P)_4Pd$ (15 mg, 0.01 mmol) was added, and the reaction mixture was stirred at 90 °C for 19 h. The reaction mixture was carried out using the method described for the synthesis of **3a** to afford 214 mg of **3c** with a 78% yield. MS: 189 ($[M + H]^+$).

3-Amino-5-(5-formyl-furan-2-yl)-benzoic Acid (3d). A solution of K_2CO_3 (86 mg, 0.62 mmol) in water (1 mL) was added to a mixture of 3-amino-5-carboxyphenylboronic acid (75 mg, 0.41 mmol) and 5-iodo-2-furaldehyde (110 mg, 0.50 mmol) in toluene/ethanol (10 mL). $(Ph_3P)_4Pd$ (4 mg, 3 nmol) was added, and the reaction mixture was stirred at 90 °C for 19 h. The reaction mixture was cooled to room temperature and was diluted with water (10 mL), and the pH was adjusted to 1 by addition of a solution of HCl (6 N). The product was precipitated after addition of CH_2Cl_2 (40 mL). The solid was collected by filtration and was washed with cold methanol (3 × 5 mL) and cold ether (3 × 5 mL) to afford 70 mg of **3d** in a 74% yield. MS: 232 ($[M + H]^+$).

3-[4-(5-Formyl-furan-2-yl)-phenyl]-propionic Acid (3e). 10% Pd-carbon (1 mg) was added to a solution of Na_3PO_4,$12H_2O$ (261 mg, 0.69 mmol), 4-(2-carboxyethyl)benzeneboronic acid (67 mg, 0.34 mmol), and 5-bromo-2-furaldehyde (60 mg, 0.34 mmol) in water/ethanol (4:1, 7 mL) under a nitrogen atmosphere. The reaction

mixture was stirred at room temperature for 10 days. The reaction mixture was filtered through a Celite bed, and pH was adjusted to 1 by addition of a solution of HCl (6 N). The mixture was extracted with CH_2Cl_2 (3 × 25 mL), and the combined organic fractions were washed with brine (1 × 5 mL), were dried over $MgSO_4$, and were concentrated under reduced pressure to afford 34 mg of **3e** in a 41% yield. MS: 487 ($[2M - H]^-$).

4-(5-Formyl-furan-2-yl)-benzamide (3f). A solution of K_2CO_3 (129 mg, 0.93 mmol) in water (1 mL) was added to a mixture of 4-aminocarbonylphenylboronic acid (100 mg, 0.62 mmol) and 5-iodo-2-furaldehyde (165 mg, 0.75 mmol) in toluene/ethanol (10 mL). $(Ph_3P)_4Pd$ (4 mg, 3 nmol) was added, and the reaction mixture was stirred at 90 °C for 17 h. The reaction mixture was carried out using the method described for the synthesis of **3b** to afford 114 mg of **3f** in an 86% yield. MS: 216 ($[M + H]^+$).

3-(5-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-furan-2-yl)-benzoic Acid (1). 3-Allylrhodanine (24 mg, 0.14 mmol) was added to a solution of **3a** (30 mg, 0.14 mmol) and ethylenediamine diacetate (75 mg, 0.42 mmol) in methanol (3 mL). The reaction mixture was stirred at room temperature for 18 h, and the product was precipitated. The solid was collected by filtration and was washed with water (3 × 5 mL) to afford 33 mg of **1** in a 64% yield as a red powder. ^{1}H NMR (400 MHz, DMSO): δ 4.65 (2H, d, J = 3.7 Hz), 5.14 (1H, d, J = 17.2 Hz), 5.19 (1H, d, J = 10.4 Hz), 5.86 (1H, m), 7.40 (2H, dd, J = 5.8, 6.0 Hz), 7.60 (1H, dd, $J_1 = J_2$ = 7.7 Hz), 7.71 (1H, s), 7.97 (2H, m), 8.39 (1H, d, J = 1.2 Hz); MS: 370 ($[M - H]^-$); Anal. ($C_{18}H_{13}NO_4S_2$): C, H, N, S.

3-(5-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-furan-2-yl)-aniline (4e). 3-Allylrhodanine (23 mg, 0.13 mmol) was added to a solution of **3b** (25 mg, 0.13 mmol) along with ethylenediamine diacetate (72 mg, 0.40 mmol) in methanol (3 mL); the reaction was then continued as described for the synthesis of **1** to afford 30 mg of **4e** in a 68% yield. ^{1}H NMR (400 MHz, DMSO): δ 4.65 (2H, d, J = 5.2 Hz), 5.13 (1H, d, J = 17.2 Hz), 5.19 (1H, d, J = 10.4 Hz), 5.40 (2H, s), 5.86 (1H, m), 6.63 (1H, d, J = 7.7 Hz), 7.01 (3H, m), 7.13 (1H, d, J = 3.7 Hz), 7.19 (1H, dd, $J_1 = J_2$ = 8.0 Hz), 7.36 (1H, d, J = 3.7 Hz), 7.68 (1H, s); MS: 343 ($[M + H]^+$); Anal. ($C_{17}H_{14}N_2O_2S_2$): C, H, N, S.

4-(5-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-furan-2-yl)-phenol (4f). 3-Allylrhodanine (46 mg, 0.27 mmol) was added to a solution of **3c** (50 mg, 0.27 mmol) along with ethylenediamine diacetate (48 mg, 0.27 mmol) in methanol (3 mL). The reaction mixture was stirred at room temperature for 17 h. The pH of the reaction mixture was adjusted to 1 by addition of a solution of HCl (6 N). The reaction mixture was extracted with CH_2Cl_2 (3 × 25 mL), and the combined organic fractions were washed with brine (1 × 5 mL), were dried over $MgSO_4$, and were concentrated under reduced pressure to afford 69 mg of **4f** in a 75% yield. ^{1}H NMR (400 MHz, DMSO): δ 4.64 (2H, d, J = 3.7 Hz), 5.13 (1H, d, J = 17.2 Hz), 5.19 (1H, d, J = 10.4 Hz), 5.84 (1H, m), 6.95 (2H, d, J = 6.7 Hz), 7.12 (1H, d, J = 3.7 Hz), 7.35 (1H, d, J = 3.7 Hz), 7.65 (1H, s), 7.71 (2H, d, J = 6.7 Hz), 10.08 (1H, s); MS: 344 ($[M + H]^+$); Anal. ($C_{17}H_{13}NO_3S_2$): C, H, N, S.

3-Amino-5-(5-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-furan-2-yl)-benzoic Acid (4j). 3-Allylrhodanine (19 mg, 0.11 mmol) was added to a solution of **3d** (25 mg, 0.11 mmol) and piperidine (11 μL, 0.11 mmol) in dimethylformamide (3 mL). The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with water/acetonitrile (3:2, 0.1% TFA, 30 mL) and was purified by reverse-phase HPLC with a linear gradient of 40−90% acetonitrile (0.1% TFA) over 45 min at 10 mL/min. Relevant fractions were combined, and the solvent was removed by freeze-drying to afford 3 mg of **4j** in an 8% yield. MS: 387 ($[M + H]^+$).

3-[4-(5-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-furan-2-yl)-pheny l]-propionic Acid (4k). 3-Allylrhodanine (14 mg, 0.08 mmol) was added to a solution of **3e** (20 mg, 0.08 mmol) and ethylenediamine diacetate (30 mg, 0.16 mmol) in methanol (3 mL); the reaction was continued as described for the synthesis of **4f**. The product was further washed with methanol (2 × 5 mL) to afford 11 mg of **4k** in a 35% yield. ^{1}H NMR (250 MHz, DMSO): δ 2.58 (2H, t, J = 7.8 Hz), δ 2.87 (2H, t, J = 7.8 Hz), 4.65 (2H, d, J = 4.8 Hz), 5.13 (1H, d, J = 17.5 Hz), 5.19 (1H, d, J = 10.6 Hz), 5.82 (1H, m), 7.30 (1H, d, J = 3.7 Hz), 7.38 (1H, d, J = 3.8 Hz), 7.41 (2H, d, J = 8.4 Hz), 7.70 (1H, s), 7.78 (1H, d, J = 8.2 Hz); MS: 398 ($[M + H]^+$); anal. ($C_{20}H_{17}NO_4S_2$): C, H, N, S.

4-(5-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-furan-2-yl)-benzenesulfonic Acid (5b). 3-Allylrhodanine (17 mg, 0.10 mmol) was added to a solution of 4-(5-formyl-2-furyl)-benzenesulfonic acid (25 mg, 0.10 mmol) and piperidine (10 μL, 0.10 mmol) in dimethylformamide (3 mL); the reaction was continued as described for the synthesis of **4j** to afford 9 mg of **5b** in a 22% yield. ^{1}H NMR (400 MHz, DMSO): δ 4.66 (2H, d, J = 5.2 Hz), 5.14 (1H, d, J = 17.2 Hz), 5.19 (1H, d, J = 10.4 Hz), 5.85 (1H, m), 7.36 (1H, d, J = 3.7 Hz), 7.40 (1H, d, J = 3.7 Hz), 7.48 (1H, s), 7.71 (1H, s), 7.75 (2H, d, J = 8.4 Hz), 7.83 (2H, d, J = 8.31 Hz); MS: 408 ($[M + H]^+$).

Phosphoric Acid Mono-(4-[5-(3-allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl)-furan-2-yl]-phenyl) Ester (5c). Phosphorus oxychloride (135 μL, 0.15 mmol) was added dropwise to a solution of **4f** in pyridine (5 mL) at 0 °C. The reaction mixture was stirred for 50 min, and water (30 mL) was added. The reaction mixture was allowed to warm to room temperature and was stirred for 18 h. The reaction mixture was then extracted with CH_2Cl_2 (3 × 100 mL), and the organic fractions were combined and concentrated under reduced pressure to afford 13.1 mg of **5c** in a 21% yield. ^{1}H NMR (400 MHz, DMSO): δ 4.65 (2H, d, J = 5.2 Hz), 5.14 (1H, d, J = 17.2 Hz), 5.19 (1H, d, J = 10.4 Hz), 5.85 (1H, m), 7.28 (1H, d, J = 3.7 Hz), 7.39 (1H, d, J = 3.4 Hz), 7.59 (2H, dd, J = 7.6, 5.9 Hz), 7.70 (1H, s), 7.86 (2H, d, J = 8.6 Hz); MS: 424 ($[M + H]^+$); anal. ($C_{17}H_{14}NO_6PS_2$): C, H, N, S.

4-(5-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-furan-2-yl)-benzamide (5d). 3-Allylrhodanine (24 mg, 0.14 mmol) was added to a solution of **3f** (30 mg, 0.14 mmol) and piperidine (15 μL, 0.14 mmol) in dimethylformamide (3 mL); the reaction was continued as described for the synthesis of **4j** to afford 6 mg of **5d** in a 12% yield. ^{1}H NMR (250 MHz, DMSO): δ 4.64 (2H, d, J = 5.4 Hz), 5.12 (1H, d, J = 17.2 Hz), 5.18 (1H, d, J = 10.8 Hz), 5.83 (1H, m), 7.40 (1H, d, J = 4.4 Hz), 7.46 (1H, d, J = 3.1 Hz), 7.71 (1H, s), 7.95 (2H, d, J = 7.5 Hz), 8.03 (2H, d, J = 9.3 Hz); MS: 371 ($[M + H]^+$).

4-(5-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-furan-2-yl)-*N*-hydroxybenzamide (5e). A solution of cyanuric chloride (149 mg, 0.81 mmol) in tetrahydrofurane (3 mL) was added dropwise to a solution of **2** (200 mg, 0.54 mmol), *N*-methylmorpholine (89 μL, 0.81 mmol), dimethylaminopyridine (0.7 mg, 5 nmol), and hydroxylamine hydrochloride salt (112 mg, 1.62 mmol) in tetrahydrofurane (12 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred for 18 h. The reaction mixture was diluted with water (15 mL) and was extracted with CH_2Cl_2 (3 × 100 mL). The organic fractions were combined, were washed with brine (1 × 15 mL), were dried over $MgSO_4$, and were concentrated under reduced pressure. The product was diluted with water/acetonitrile (7:3, 0.1% TFA, 30 mL) and was purified by reverse-phase HPLC with a linear gradient of 30−90% acetonitrile (0.1% TFA) over 55 min at 10 mL/min. Relevant fractions were combined, and the solvent was removed by freeze-drying to afford 5 mg of **5e** in a 1% yield. ^{1}H NMR (400 MHz, DMSO): δ 4.66 (2H, d, J = 5.1 Hz), 5.14 (1H, d, J = 17.2 Hz), 5.20 (1H, d, J = 10.5 Hz), 5.85 (1H, m), 7.41 (1H, d, J = 3.7 Hz), 7.46 (1H, d, J = 3.7 Hz), 7.73 (1H, s), 7.92 (4H, s), 9.14 (1H, br s), 11.32 (1H, s); MS: 387 ($[M + H]^+$).

3′-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-biphenyl-3-carboxylic Acid (6b). 3-Allylrhodanine (31 mg, 0.18 mmol) was added to a solution of 3′-formyl-(1,1′-biphenyl)-3-carboxylic acid (40 mg, 0.18 mmol) and ethylenediamine diacetate (64 mg, 0.35 mmol) in methanol (3 mL); the reaction was continued as described for the synthesis of **1** to afford 37 mg of **6b** in a 54% yield. ^{1}H NMR (400 MHz, DMSO): δ 4.67 (2H, d, J = 5.1 Hz), 5.16 (1H, d, J = 18.7 Hz), 5.20 (1H, d, J = 11.7 Hz), 5.85 (1H, m), 7.58 (2H, dd, $J_1 = J_2$ = 7.7 Hz), 7.65 (2H, m), 7.85 (1H, d, J = 7.3

Hz), 7.89 (1H, d, $J = 7.8$ Hz), 7.97 (1H, s), 7.99 (1H, s), 8.30 (1H, s); MS: 380 ($[M - H]^-$); anal. ($C_{20}H_{15}NO_3S_2$): C, H, N, S.

5-Bromomethyl-2-furaldehyde (9). A solution of 5-hydroxymethyl-2-furaldehyde (150 mg, 1.19 mmol) in $CHCl_3$ (2 mL) was added dropwise to a solution of Me_3SiBr (462 μL, 3.57 mmol) in $CHCl_3$ (8 mL) under an argon atmosphere.[30] The reaction mixture was stirred at 45 °C for 6 h. The reaction mixture was then cooled to room temperature and was diluted with saturated $NaHCO_3$ solution (6 mL). The reaction mixture was extracted with CH_2Cl_2 (3×50 mL), and the combined organic fractions were washed with brine (1×10 mL), were dried over $MgSO_4$, were concentrated under reduced pressure, and were purified by flash column chromatography to provide 198 mg of **9** in an 88% yield as a brown oil. Thin layer chromatography: retention factor 0.6 (CH_2Cl_2).

4-(5-Formyl-furan-2-ylmethyl)-benzoic Acid (10). A solution of K_2CO_3 (92 mg, 0.66 mmol) in water (1 mL) was added to a mixture of 4-carboxyphenylboronic acid (50 mg, 0.37 mmol) and **9** (63 mg, 0.33 mmol) in toluene/ethanol (10 mL). $(Ph_3P)_4Pd$ (4 mg, 3 nmol) was added, and the reaction mixture was stirred at 90 °C for 7 h. The reaction mixture was cooled to room temperature and was diluted with water (5 mL), and the pH was adjusted to 1 by addition of a solution of HCl (6 N). The diluted reaction mixture was extracted with CH_2Cl_2 (3×100 mL), and the combined organic fractions were washed with brine (1×10 mL), were dried over $MgSO_4$, and were concentrated under reduced pressure to afford 45 mg of **10** in a 59% yield. TLC: Rf 0.7 (ethyl acetate).

4-(5-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-furan-2-ylmethyl)-benzoic Acid (11a). 3-Allylrhodanine (17 mg, 0.10 mmol) was added to a solution of **8** (22 mg, 0.10 mmol) and ethylenediamine diacetate (52 mg, 0.29 mmol) in methanol (3 mL). The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then diluted with water/acetonitrile (3:1, 0.1% TFA, 40 mL) and was purified by reverse-phase HPLC with a linear gradient of 15−100% acetonitrile (0.1% TFA) over 60 min at 10 mL/min. Relevant fractions were combined, and the solvent was removed by freeze-drying to afford 1 mg of **11a** in a 4% yield. MS: 386 ($[M + H]^+$).

2-[3-Allyl-4-oxo-2-thioxo-thiazolidinylidenemethyl]-1H-indole-7-carboxylic Acid (11b). 3-Allylrhodanine (23 mg, 0.13 mmol) was added to a solution of 3-formyl-1H-indole-7-carboxylic acid (25 mg, 0.13 mmol) and ethylenediamine diacetate (71 mg, 0.40 mmol) in methanol (3 mL); the reaction was continued as described for the synthesis of **1** to afford 25 mg of a mixture of **11b** in a 56% yield. ^{1}H NMR (400 MHz, DMSO): δ 4.65 (2H, d, $J = 5.2$ Hz), 5.13 (1H, d, $J = 17.2$ Hz), 5.19 (1H, d, $J = 10.4$ Hz), 5.86 (1H, m), 7.27 (1H, dd, $J_1 = J_2 = 7.6$ Hz), 7.73 (1H, s), 7.81 (1H, d, $J = 7.4$ Hz), 8.12 (1H, d, $J = 7.9$ Hz), 8.14 (1H, s); MS: 345 ($[M + H]^+$); anal. ($C_{16}H_{12}N_2O_3S_2$): C, H, N, S.

Transcription Inhibition Assay. Transcription inhibition was evaluated using a commercial RNAP inhibitor screening kit (Kool NC-45, Epicentre Biotechnologies, Madison, WI). This screening assay is based on the Rolling Circle Transcription technology and relies on the property that some small circular, single-stranded DNAs mimic an open promoter and are efficiently transcribed by RNAP. This assay does not rely on σ factors and therefore was performed without any.

MIC Determinations and Bacterial Strains. The MICs were determined as recommended by the CLSI.[31] Compounds predissolved in DMSO were tested at final concentrations (prepared from serial 2-fold dilutions) ranging from 100 to 0.4 μg/mL. The MIC was defined as the lowest concentration which yielded no visible growth. MICs were evaluated for *S. aureus* CIP 76.25, *S. epidermidis* RP62A CIP 105777, *E. coli* CIP 76.24, *E. coli* tolC CGSC 5633, *C. difficile* DSM 1296, and *S. pneumoniae* CIP 103566. The multiresistant *S. aureus* strain 49589 (see Supporting Information, Table S3 for antibiotics susceptibility) and *S. epidermidis* strains 40004 and 48155 were clinical isolates obtained from University Hospital of Nîmes, France. The nonpathogenic *B. anthracis* strain 9131 was obtained from Institut Pasteur, France. *C. difficile* was grown in Wilkins-Chalgren broth under anaerobic conditions (GENbag anaer, bioMérieux, Marcy l'Etoile, France). The test medium for *S. pneumoniae* was Mueller-Hinton broth (MHB) supplemented with 0.5% (v/v) of sheep blood. The inoculum was 5×10^5 CFU/mL for every microorganism. The inoculated microplates were incubated at 37 °C for 18 h before being read.

Cytotoxicity. Chinese hamster ovary (CHO) cells were grown in 96-well plates in RPMI medium supplemented with 5% fetal calf serum. The cells were incubated for 24 h at 37 °C in the absence of serum and in the presence of the compounds, and the cytotoxicity was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide at a final concentration of 300 μg/mL. After 3 h incubation at 37 °C in the dark, acidified isopropanol (10 μL isopropanol, 0.4 M HCl) was added and mixed in the well. The plates were incubated at room temperature for 1 h in the dark. The solution optical density in the 96-well plate was measured at 595 nm using a spectrophotometer.

***S. epidermidis* Biofilms.** The wells of a black 96-well polystyrene microtiter plate (Greiner bio-one, Frickenhausen, Germany) were filled with 100 μL aliquots of *S. epidermidis* inoculum (10^7 CFU/mL) in MHB; the plate was then incubated for 24 h at 37 °C. Each well was rinsed three times with 200 μL sterile water to remove nonadherent bacteria. Subsequently, 100 μL MHB containing the desired antibiotic concentration was added to each well, and the plate was incubated at 37 °C for 24 h without shaking. After the challenge, the plate was washed three times with 200 μL sterile water, and 10 μL of DMSO was added to each well to lyse the bacteria. The plate was shaken for 3 min, and 90 μL of ATP-counting bioluminescence buffer (25 mM Hepes, 24 mM $MgCl_2$, 2 mM ethylenediamine tetra-acetate, 2 mM dithiothreitol, 60 μg/mL bovine serum albumine, 20 μg/mL luciferase, 0.6 mM D-luciferin, pH 7.25) was added to each well before being read by a luminometer (Chameleon, Hidex, Turku, Finland).[32] All of the experiments were done in triplicate.

To establish the correlation between luminescence values and the amount of biofilm-embedded cells, bacteria of a 24 h biofilm grown in a well of the microtiter plate were resuspended in sterile water by sonication using a Branson 450 Sonifier with a microtip (4×2 s, 10% of the maximal amplitude). The bacterial suspension was serially diluted in sterile water; 10 μL of this serial dilution was drawn from the wells to be measured by bioluminescence while the number of bacteria in the wells was estimated by classical plate counting. Measures by bioluminescence and by plate counting afforded establishment of the following correlation equations:

S. epidermidis RP62A

$$\mathrm{Log}_{10}(\mathrm{CFU/well}) = 0.90 \times \mathrm{Log}_{10}(\mathrm{luminescence}) + 0.85 \qquad R^2 = 0.9994$$

S. epidermidis 40004

$$\mathrm{Log}_{10}(\mathrm{CFU/well}) = 0.93 \times \mathrm{Log}_{10}(\mathrm{luminescence}) + 0.83 \qquad R^2 = 0.9911$$

S. epidermidis 48155

$$\mathrm{Log}_{10}(\mathrm{CFU/well}) = 0.94 \times \mathrm{Log}_{10}(\mathrm{luminescence}) + 0.91 \qquad R^2 = 0.9982$$

The same procedure was applied with a 48 h biofilm, and no significant differences were observed between the correlation equations (Supporting Information, Table S4). Thus, 24 h correlation equations were used in this study to assess the amount of biofilm-embedded bacteria.

HSA Binding Ranking by SPR. SPR experiments were performed at 25 °C using a BIACORE 3000 apparatus (Biacore AB, Uppsala Sweden). HSA (Sigma St. Louis, MO) diluted to 40 μg/mL in 10 mM acetate buffer, pH 5.2, was immobilized on CM5 sensor chips using amine-coupling chemistry. Immobilization levels were between 9000 and 11 000 RU (resonant unit). The surface was blocked with 1 M ethanol amine, pH 8.0, and was washed by three 30 s pulses of 50 mM NaOH to remove noncovalently bound HSA.

Drugs were prepared as 10 mM stock solutions in 100% DMSO. Drugs were diluted in PBS to make a final DMSO concentration of 5%. Binding studies were measured in phosphate buffer saline containing 5% DMSO with a running buffer flow rate of 30 μL/min. For ranking of drug compound binding to HSA, duplicate injections of drugs at 50 μM were done during 60 s over the HSA and reference surface. The surfaces were washed with running buffer until complete regeneration was reached. For cleaning the flow system, a bypass wash was performed with DMSO 50% and DMSO 5% between each injection. Buffer blanks were injected for double referencing[33] before each drug injection, and binding responses were corrected for DMSO bulk differences via calibration curves (eight DMSO solutions between 4.5 and 5.8% DMSO).[29]

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Supporting Information Available: Antibacterial activity on *E. coli*, cytotoxicity on CHO cells; biological activity of some allylrhodanine derivatives; antibiotic susceptibility of the multiresistant *S. aureus* clinical isolate 49589; correlation equations between luminescence values and the amount of 48 h old biofilm-embedded bacteria; elemental analyses; HPLC purity. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Rice, L. B. Unmet medical needs in antibacterial therapy. *Biochem. Pharmacol.* **2006**, *71*, 991−995.
(2) Costerton, J. W.; Stewart, P. S.; Greenberg, E. P. Bacterial biofilms: a common cause of persistent infections. *Science* **1999**, *284*, 1318−1322.
(3) Villain-Guillot, P.; Bastide, L.; Gualtieri, M.; Leonetti, J. P. Progress in targeting bacterial transcription. *Drug Discovery Today* **2007**, *12*, 200−208.
(4) O'Neill, A.; Oliva, B.; Storey, C.; Hoyle, A.; Fishwick, C.; Chopra, I. RNA polymerase inhibitors with activity against rifampin-resistant mutants of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2000**, *44*, 3163−3166.
(5) Finegold, S. M.; Molitoris, D.; Vaisanen, M. L.; Song, Y.; Liu, C.; Bolanos, M. In vitro activities of OPT-80 and comparator drugs against intestinal bacteria. *Antimicrob. Agents Chemother.* **2004**, *48*, 4898−4902.
(6) Trampuz, A.; Zimmerli, W. Antimicrobial agents in orthopaedic surgery: Prophylaxis and treatment. *Drugs* **2006**, *66*, 1089−1105.
(7) Artsimovitch, I.; Chu, C.; Lynch, A. S.; Landick, R. A new class of bacterial RNA polymerase inhibitor affects nucleotide addition. *Science* **2003**, *302*, 650−654.
(8) André, E.; Bastide, L.; Michaux-Charachon, S.; Gouby, A.; Villain-Guillot, P.; Latouche, J.; Bouchet, A.; Gualtieri, M.; Leonetti, J. P. Novel synthetic molecules targeting the bacterial RNA polymerase assembly. *J. Antimicrob. Chemother.* **2006**, *57*, 245−251.
(9) Arhin, F.; Belanger, O.; Ciblat, S.; Dehbi, M.; Delorme, D.; Dietrich, E.; Dixit, D.; Lafontaine, Y.; Lehoux, D.; Liu, J.; McKay, G. A.; Moeck, G.; Reddy, R.; Rose, Y.; Srikumar, R.; Tanaka, K. S.; Williams, D. M.; Gros, P.; Pelletier, J.; Parr, T. R., Jr.; Far, A. R. A new class of small molecule RNA polymerase inhibitors with activity against Rifampicin-resistant *Staphylococcus aureus*. *Bioorg. Med. Chem.* **2006**, *14*, 5812−5832.
(10) Gualtieri, M.; Bastide, L.; Villain-Guillot, P.; Michaux-Charachon, S.; Latouche, J.; Leonetti, J. P. In vitro activity of a new antibacterial rhodanine derivative against *Staphylococcus epidermidis* biofilms. *J. Antimicrob. Chemother.* **2006**, *58*, 778−783.
(11) Kratochwil, N. A.; Huber, W.; Muller, F.; Kansy, M.; Gerber, P. R. Predicting plasma protein binding of drugs: a new approach. *Biochem. Pharmacol.* **2002**, *64*, 1355−1374.
(12) Wiesner, J.; Mitsch, A.; Jomaa, H.; Schlitzer, M. Structure-activity relationships of novel anti-malarial agents. Part 7: N-(3-benzoyl-4-tolylacetylaminophenyl)-3-(5-aryl-2-furyl)acrylic acid amides with polar moieties. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2159−2161.
(13) Zhang, G. Ligand-free Suzuki−Miyaura reaction catalysed by Pd/C at room temperature. *J. Chem. Res.* **2004**, 593−595.
(14) Howard, M. H.; Cenizal, T.; Gutteridge, S.; Hanna, W. S.; Tao, Y.; Totrov, M.; Wittenbach, V. A.; Zheng, Y. J. A novel class of inhibitors of peptide deformylase discovered through high-throughput screening and virtual ligand screening. *J. Med. Chem.* **2004**, *47*, 6669−6672.
(15) Wermuth, C. G. Molecular variations based on isosteric replacements. In *The Practice of Medicinal Chemistry*, 2nd ed.; Wermuth, C. G., Ed.; Academic Press: London, UK, 2003; pp 189−214.
(16) Toya, M.; Takagi, M.; Kondo, T.; Nakata, H.; Isobe, M.; Goto, T. Improved synthetic methods of firefly luciferin derivatives for use in bioluminescent analysis of hydrolytic enzymes; carboxylic esterase and alkaline phosphatase. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2604−2610.
(17) Giacomelli, G.; Porcheddu, A.; Salaris, M. Simple one-flask method for the preparation of hydroxamic acids. *Org. Lett.* **2003**, *5*, 2715−2717.
(18) Zervosen, A.; Lu, W. P.; Chen, Z.; White, R. E.; Demuth, T. P., Jr.; Frere, J. M. Interactions between penicillin-binding proteins (PBPs) and two novel classes of PBP inhibitors, arylalkylidene rhodanines and arylalkylidene iminothiazolidin-4-ones. *Antimicrob. Agents Chemother.* **2004**, *48*, 961−969.
(19) Grant, E. B.; Guiadeen, D.; Baum, E. Z.; Foleno, B. D.; Jin, H.; Montenegro, D. A.; Nelson, E. A.; Bush, K.; Hlasta, D. J. The synthesis and SAR of rhodanines as novel class C beta-lactamase inhibitors. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2179−2182.
(20) Forino, M.; Johnson, S.; Wong, T. Y.; Rozanov, D. V.; Savinov, A. Y.; Li, W.; Fattorusso, R.; Becattini, B.; Orry, A. J.; Jung, D.; Abagyan, R. A.; Smith, J. W.; Alibek, K.; Liddington, R. C.; Strongin, A. Y.; Pellecchia, M. Efficient synthetic inhibitors of anthrax lethal factor. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9499−9504.
(21) Nie, Z.; Perretta, C.; Lu, J.; Su, Y.; Margosiak, S.; Gajiwala, K. S.; Cortez, J.; Nikulin, V.; Yager, K. M.; Appelt, K.; Chu, S. Structure-based design, synthesis, and study of potent inhibitors of beta-ketoacyl-acyl carrier protein synthase III as potential antimicrobial agents. *J. Med. Chem.* **2005**, *48*, 1596−1609.
(22) Tedder, M. E.; Nie, Z.; Margosiak, S.; Chu, S.; Feher, V. A.; Almassy, R.; Appelt, K.; Yager, K. M. Structure-based design, synthesis, and antimicrobial activity of purine derived SAH/MTA nucleosidase inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3165−3168.
(23) Molteni, V.; He, X.; Nabakka, J.; Yang, K.; Kreusch, A.; Gordon, P.; Bursulaya, B.; Warner, I.; Shin, T.; Biorac, T.; Ryder, N. S.; Goldberg, R.; Doughty, J.; He, Y. Identification of novel potent bicyclic peptide deformylase inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1477−1481.
(24) Cerca, N.; Martins, S.; Cerca, F.; Jefferson, K. K.; Pier, G. B.; Oliveira, R.; Azeredo, J. Comparative assessment of antibiotic susceptibility of coagulase-negative *staphylococci* in biofilm versus planktonic culture as assessed by bacterial enumeration or rapid XTT colorimetry. *J. Antimicrob. Chemother.* **2005**, *56*, 331−336.
(25) Lee, J. Y.; Ko, K. S.; Peck, K. R.; Oh, W. S.; Song, J. H. In vitro evaluation of the antibiotic lock technique (ALT) for the treatment of catheter-related infections caused by *staphylococci*. *J. Antimicrob. Chemother.* **2006**, *57*, 1110−1115.
(26) Li, Z.; Francisco, G. D.; Hu, W.; Labthavikul, P.; Petersen, P. J.; Severin, A.; Singh, G.; Yang, Y.; Rasmussen, B. A.; Lin, Y. I.; Skotnicki, J. S.; Mansour, T. S. 2-Phenyl-5,6-dihydro-2H-thieno[3,2-c]pyrazol-3-ol derivatives as new inhibitors of bacterial cell wall biosynthesis. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2591−2594.
(27) Payne, D. J.; Miller, W. H.; Berry, V.; Brosky, J.; Burgess, W. J.; Chen, E.; DeWolf, W. E., Jr.; Fosberry, A. P.; Greenwood, R.; Head, M. S.; Heerding, D. A.; Janson, C. A.; Jaworski, D. D.; Keller, P. M.; Manley, P. J.; Moore, T. D.; Newlander, K. A.; Pearson, S.; Polizzi, B. J.; Qiu, X.; Rittenhouse, S. F.; Slater-Radosti, C.; Salyers, K. L.; Seefeld, M. A.; Smyth, M. G.; Takata, D. T.; Uzinskas, I. N.; Vaidya, K.; Wallis, N. G.; Winram, S. B.; Yuan, C. C.; Huffman, W. F. Discovery of a novel and potent class of FabI-directed antibacterial agents. *Antimicrob. Agents Chemother.* **2002**, *46*, 3118−3124.
(28) Kragh-Hansen, U.; Chuang, V. T.; Otagiri, M. Practical aspects of the ligand-binding and enzymatic properties of human serum albumin. *Biol. Pharm. Bull.* **2002**, *25*, 695−704.
(29) Frostell-Karlsson, A.; Remaeus, A.; Roos, H.; Andersson, K.; Borg, P.; Hamalainen, M.; Karlsson, R. Biosensor analysis of the interaction between immobilized human serum albumin and drug compounds for prediction of human serum albumin binding levels. *J. Med. Chem.* **2000**, *43*, 1986−1992.
(30) Komla, S.; Rigal, L.; Gaset, A. Synthesis of 5-(bromomethyl)- and of 5-(chloromethyl)-2-furancarboxaldehyde. *Carbohydr. Res.* **1989**, *187*, 15−23.

(31) National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 6th ed.; Approved Standard M07-A6; National Committee for Clinical Laboratory Standards: Villanova, PA, 2003.
(32) Gracia, E.; Fernandez, A.; Conchello, P.; Alabart, J. L.; Perez, M.; Amorena, B. In vitro development of *Staphylococcus aureus* biofilms using slime-producing variants and ATP-bioluminescence for automated bacterial quantification. *Luminescence* **1999**, *14*, 23−31.
(33) Myszka, D. G. Improving biosensor analysis. *J. Mol. Recognit.* **1999**, *12*, 279−284.

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