472 J. Med. Chem. 2011, 54, 472–484
DOI: 10.1021/jm1011148

Journal of Medicinal Chemistry Article

Structure−Activity Relationship of 4(5)-Aryl-2-amino-1*H*-imidazoles, *N*1-Substituted 2-Aminoimidazoles and Imidazo[1,2-*a*]pyrimidinium Salts as Inhibitors of Biofilm Formation by *Salmonella* Typhimurium and *Pseudomonas aeruginosa*

Hans P. L. Steenackers,[†,§] Denis S. Ermolat'ev,[‡] Bharat Savaliya,[‡,∥] Ami De Weerdt,[†] David De Coster,[†] Anamik Shah,[∥] Erik V. Van der Eycken,[‡] Dirk E. De Vos,[§] Jozef Vanderleyden,[†] and Sigrid C. J. De Keersmaecker*[,†]

[†]*Centre of Microbial and Plant Genetics, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium,* [‡]*Laboratory for Organic & Microwave-Assisted Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200F, B-3001 Leuven, Belgium,* [§]*Centre for Surface Chemistry and Catalysis, Katholieke Universiteit Leuven, Kasteelpark Arenberg 23, B-3001 Leuven, Belgium, and* [∥]*Department of Chemistry, Saurashtra University, Rajkot-360 005, Gujarat, India*

Received May 19, 2010

A library of 112 4(5)-aryl-2-amino-1*H*-imidazoles, 4,5-diphenyl-2-amino-1*H*-imidazoles, and *N*1-substituted 4(5)-phenyl-2-aminoimidazoles was synthesized and tested for the antagonistic effect against biofilm formation by *Salmonella* Typhimurium and *Pseudomonas aeruginosa*. The substitution pattern of the 4(5)-phenyl group and the nature of the *N*1-substituent were found to have a major effect on the biofilm inhibitory activity. The most active compounds of this series were shown to inhibit the biofilm formation at low micromolar concentrations. Furthermore, the influence of 6 imidazo[1,2-*a*]pyrimidines and 18 imidazo[1,2-*a*]pyrimidinium salts on the biofilm formation was tested. These compounds are the chemical precursors of the 2-aminoimidazoles in our synthesis pathway. A good correlation was found between the activity of the imidazo[1,2-*a*]pyrimidinium salts and their corresponding 2-aminoimidazoles, supporting the hypothesis that the imidazo[1,2-*a*]pyrimidinium salts are possibly cleaved by cellular nucleophiles to form the active 2-aminoimidazoles. However, the imidazo[1,2-*a*]pyrimidines did not show any biofilm inhibitory activity, indicating that these molecules are not susceptible to *in situ* degradation to 2-aminoimidazoles. Finally, we demonstrated the lack of biofilm inhibitory activity of an array of 37 2*N*-substituted 2-aminopyrimidines, which are the chemical precursors of the imidazo[1,2-*a*]pyrimidinium salts in our synthesis pathway.

Introduction

Biofilms are defined as structured communities of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface.[1] Biofilms as compared with planktonic cells provide the bacterial cells with an array of advantages including the ability to resist challenges from predators, antibiotics, disinfectants, and host immune systems.[2−5] This causes serious problems and high associated costs in health care and industrial settings. In medicine, biofilms play a role in infectious diseases, both for specific conditions such as cystic fibrosis and periodontitis and in bloodstream and urinary tract infections as a result of indwelling medical devices.[3] According to the National Institutes of Health, more than 80% of microbial infections are related to biofilms.[6] This is particularly problematic given the fact that bacteria in biofilms can be up to 1000-fold more resistant to antibiotics.[7] Industry related biofilms can contribute, e.g., to contamination of installations in food industry, decreasing passage through pipelines by colonization of the interior of the pipes, mild steel corrosion, and enhanced resistance of vessels by initiating biofouling on the vessel hulls. The yearly economic loss caused by "biofouling" in the marine industry is estimated at $ 6.5 billion.[8]

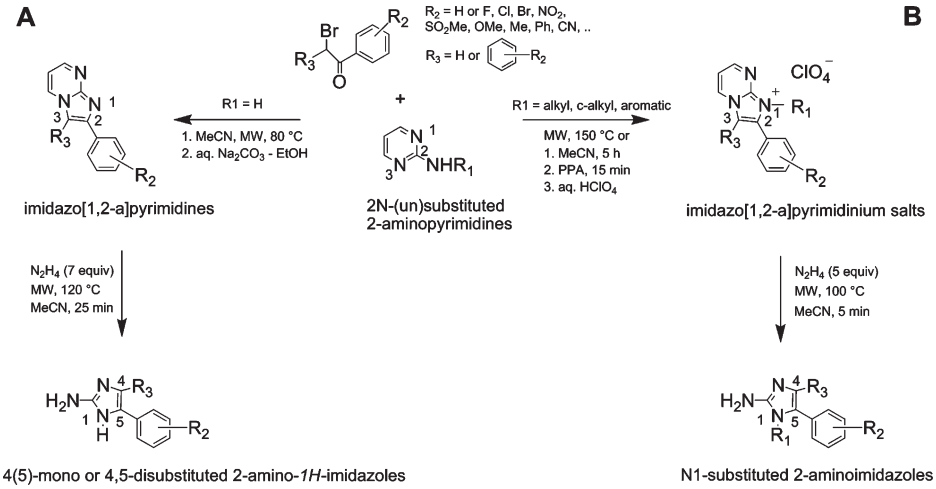
Given the extent of problems caused by biofilms, there has been a significant effort to develop small molecules that are able to inhibit biofilm formation.[9] Among the few molecular scaffolds that have been identified, the most intensively studied examples are (1) the halogenated furanones, which were originally isolated from the seaweed *Delisea pulchra*,[10,11,12] (2) analogues of the homoserine lactone signaling molecules,[13] and (3) analogues of the sponge-derived marine natural alkaloids oroidin and bromoageliferin.[14−21] As bacteria develop resistance against compounds with a microbiocidal activity, the development of compounds that specifically target the biofilm formation in a nontoxic manner is a valuable approach. These nontoxic biofilm inhibitors have the potential to be used in a preventive treatment of a wide diversity of industrial and medical surfaces. Furthermore, the potential to codose biofilm inhibitors and classical antibiotics for the treatment of biofilm infections is also an attractive option.[11]

Salmonella enterica serovar Typhimurium and *Pseudomonas aeruginosa* are two well-studied organisms in terms of biofilm formation. *Salmonella enterica* is worldwide one of the most important foodborne pathogens. *Salmonella* is able to form biofilms on different surfaces, ranging from abiotic surfaces (e.g., concrete, plastics, glass, polystyrene,...),[22,23] to biotic surfaces (gallstones,[24] plant surfaces,[25,26] and epithelial cell layers[27]). These biofilms are an important survival strategy in all stages of infection, from transmission to chronic infection. While severe nontyphoid *Salmonella* infections are commonly treated with fluoroquinolones and third-generation cephalosporins, there are alarming reports concerning the development of resistance against these antibiotics.[28] Alternative

*To whom correspondence should be addressed. Phone: 32-16321631. Fax: 32-16321966. E-mail: Sigrid.DeKeersmaecker@biw.kuleuven.be.

 Published on Web 12/20/2010

Scheme 1. Overview and Synthesis of the Different Classes of Compounds of Which the Antibiofilm Activity was Investigated in the Present Study[a]



[a] (A) Synthesis of 4(5)-mono and 4,5-disubstituted 2-amino-1*H*-imidazoles with aryl substituents from imidazo[1,2-*a*]pyrimidines.[32] (B) Synthesis of *N*1-substitued 2-aminoimidazoles from 2-aminopyrimidines via *N*1-substituted imidazo[1,2]pyrimidinium salts.[37,38]

anti-*Salmonella* strategies are thus urgently needed. Given the importance of biofilms in the spread of *Salmonella*, the development of *Salmonella* biofilm inhibitors seems a promising approach. *P. aeruginosa* is an opportunistic pathogen implicated in a myriad of infections. Patients with compromised host defenses, such as those infected with human immunodeficiency virus, burn patients, or those with cystic fibrosis (80% colonization rate of *P. aeruginosa*[29]) are particularly susceptible to *P. aeruginosa* infections.[30] There is evidence that biofilms are involved in chronic *P. aeruginosa* infections such as recurrent ear infections, chronical bacterial prostatitis, and lung infections in CF patients, the latter being extremely harmful as *P. aeruginosa* colonization and chronic lung infection is the major causative agent of morbidity and mortality in CF patients.[31] Moreover, *P. aeruginosa* can colonize as biofilms a variety of medical devices such as intravascular catheters and urinary catheters. Obviously, there is an urgent need of agents that can prevent or eradicate *P. aeruginosa* biofilms on infected tissues and on medical devices.

Recently, we have published a method for the microwave-assisted synthesis of substituted 2-amino-1*H*-imidazoles from imidazo[1,2-*a*]pyrimidines (Scheme 1, route A),[32] focusing on 4(5)-mono- or 4,5-disubstituted 2-amino-1*H*-imidazoles with aryl substituents. Some representatives of this class of imidazoles have been documented to inhibit plaque formation by *Streptococcus mutans* and biofilm formation by *Acinetobacter baumanii*.[33,34] Moreover, some representatives of the class of imidazo[1,2-*a*]pyrimidines, which are the chemical precursors of the 2-amino-1*H*-imidazoles in our synthesis pathway, have been shown to possess antibacterial and antifungal activity.[35,36] Therefore, we decided to initiate an in depth study on the structure−antibiofilm activity of both classes of compounds.

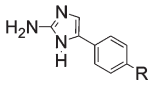
Additionally, we previously reported on a procedure for the synthesis of *N*1-substituted 2-aminoimidazoles from 2-aminopyrimidines via *N*1-substituted imidazo[1,2-*a*]pyrimidin-1-ium salts (Scheme 1, route B).[37,38] The availability of these *N*1-substituted 2-aminoimidazoles allowed us to study the effect of different kinds of *N*1-substituents on the antibiofilm activity of the 2-aminoimidazoles. We hypothesized that the imidazo-[1,2-*a*]pyrimidinium salts could also *in situ* (i.e., in the biofilm or in the cell) be degraded to 2-aminoimidazoles by attack of cellular nucleophiles. Therefore, we also studied the potential of the imidazo[1,2-*a*]pyrimidinium salts as biofilm inhibitors. Some of the 2-aminopyrimidines were previously shown to possess antibacterial and antifungal activity.[39,40] Therefore, we decided to investigate the influence of a broad array of 2*N*-substituted 2-aminopyrimidines on biofilm formation. Initially, the compounds were screened for antibiofilm activity against *S.* Typhimurium. In a second stage, the most active compounds were evaluated against *P. aeruginosa* biofilm formation.

Results and Discussion

4(5)-Monosubstituted 2-amino-1*H*-imidazoles. The effect of the para-substituted 4(5)-phenyl-2-aminoimidazoles **1**−**10** (Table 1) on the biofilm formation of *S.* Typhimurium ATTC14028 was studied. The compounds were initially screened at 25 °C, mimicking conditions outside the host where biofilms are supporting survival and transmission of *Salmonella*. The basic compound **1** with a nonsubstituted phenyl group at the C4(5) position of the imidazole ring shows a moderate biofilm inhibitory activity (IC_{50} = 130 μM). Interestingly, substitution at the *para*-position of the phenyl ring with a chlorine, nitro, or phenyl group enhances the biofilm inhibitory activity about 10 times (IC_{50}s ∼ 15 μM). Substitution with a bromine, fluorine, methyl, or methoxy group does also improve biofilm inhibition moderately (IC_{50}s ∼ 45−120 μM), while substitution with a methanesulfonyl or nitrile function reduces biofilm inhibitory activity drastically. To validate that the compounds are true inhibitors of biofilm formation and not acting as bactericidal agents, growth curves of planktonic cells were measured at 25 °C in the presence of different concentrations of the most active compounds. Compounds **1**−**5** do not or only slightly slow down the planktonic growth at the IC_{50} for biofilm inhibition, indicating that the reduced biofilm formation is not a consequence of killing the bacteria (Table 1). Compound **9**, however, retards the bacterial growth substantially at the IC_{50} for biofilm inhibition, suggesting that the biofilm inhibition is at least partly due to killing the planktonic bacteria before biofilms could be established.

Salmonella can also form biofilms as a strategy to induce chronic infections[24,41,42] and even colonize host organisms. To mimic the conditions inside the host, we also determined the influence of the most active compounds **1**−**5** and **9** on *S.* Typhimurium biofilm formation at 37 °C. As depicted in Table 1, all the tested compounds inhibit biofilm formation

Table 1. Influence of *para*-Substituted 4(5)-Phenyl-2-amino-1*H*-imidazoles **1**−**10** on the Biofilm Formation and the Planktonic Growth of *S.* Typhimurium ATCC14028 at 25 and 37 °C



		25 °C									37 °C							
				effect on growth at[b]									effect on growth at[b]					
compd	R	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	400 μM	200 μM	150 μM	40 μM	25 μM	20 μM	10 μM	4 μM	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	400 μM	250 μM	150 μM	100 μM	50 μM	25 μM
1	H	130.2	112.6−150.7	+		−	−				−	146.4	67.7−316.5	−	−	−	−		
2	F	84.4	69.7−102.3		−		+				−	238.4	169.2−335.8						
3	Cl	16.0	14.3−17.9	o			−				−	30.1	20.5−44.0						
4	Br	47.9	36.6−62.8	o			−				−	54.3	38.5−76.6			−	−	−	−
5	NO_2	17.6	15.0−20.5	o			o	−		−		395.7	305.1−513.2	o					
6	SO_2Me	>800										nd[c]							
7	OMe	119.7	106.9−134.1									nd							
8	Me	77.7	70.4−85.8									nd							
9	Ph	17.3	15.9−18.8				o		+	−		348.9	277.9−437.9	o					
10	CN	316.4	224.4−446.1									nd							

[a] IC_{50}: concentration of inhibitor needed to inhibit biofilm formation by 50%. [b] o: the planktonic growth is completely or almost completely inhibited when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; +: the planktonic growth is retarded when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; −: the planktonic growth is not or only slightly affected when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; no symbol indicated: effect not determined. [c] nd: not determined.

Table 2. Influence of *para*-Substituted 4(5)-Phenyl-2-amino-1*H*-imidazoles **1**−**9** on the Biofilm Formation and the Planktonic Growth of *P. aeruginosa* at 25 and 37 °C

	25 °C							37 °C						
			effect on growth at[b]							effect on growth at[b]				
compd	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	100 μM	50 μM	40 μM	10 μM	5 μM	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	800 μM	400 μM	100 μM	40 μM	20 μM
1	72.6	38.7−136.2	−					22.7	8.5−61.1			−	−	−
2	15.0	8.6−26.2			−	−		~47.6				−	−	−
3	3.5	2.3−5.2					−	29.3	11.4−75.2					−
4	3.2	2.3−4.5			+	−	−	51.3	39.1−67.3			+	−	−
5	34.5	20.4−58.5	o	−				94.0	10.6−829.5			−	−	−
6	>800							nd[c]						
7	186.3	136.2−254.8						nd						
8	45.8	32.1−65.3						nd						
9	8.6	4.8−15.6			−	−		~364.6		o	+			

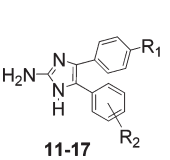
[a] IC_{50}: concentration of inhibitor needed to inhibit biofilm formation by 50%. [b] o: the planktonic growth is completely or almost completely inhibited when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; +: the planktonic growth is retarded when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; −: the planktonic growth is not or only slightly affected when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; no symbol indicated: effect not determined. [c] nd: not determined.

at 37 °C. Although the IC_{50} values at 37 °C are remarkably higher than those at 25 °C, this is still an interesting result, as to the best of our knowledge no *Salmonella* biofilm inhibitors acting both at room temperature and body temperature have been reported before. The halogenated furanones for instance are able to inhibit *Salmonella* biofilm formation at 16 °C and to a lesser extent at 25 °C, but they do not show activity at 37 °C.[11] Growth curve analysis at 37 °C revealed that compounds **1** and **4** have a broad concentration range with only biofilm inhibition and almost no effect on the planktonic growth. Compounds **5** and **9**, on the other hand, completely block the planktonic growth at the IC_{50} for biofilm inhibition, indicating that the biofilm inhibition in these cases is due to a bactericidal effect.

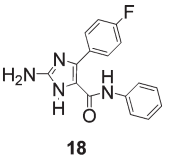
Next, the influence of compounds **1**−**9** on *P. aeruginosa* strain PA14 was investigated, both at 25 and 37 °C. As presented in Table 2, the biofilm formation at 25 °C is very sensitive to low (IC_{50}s <15 μM), nongrowth inhibiting concentrations of the compounds **2**−**4** and **9**, while compounds **1**, **5**, **7**, and **8** inhibit the biofilm formation at moderate concentrations (IC_{50}s ~ 50−200 μM). Also, at 37 °C, the biofilm formation is clearly affected at concentrations lower than 100 μM for all compounds, except for compound **9**, which has an IC_{50} higher than 300 μM (Table 2). No substantial effect on the planktonic growth of *Pseudomonas* was observed for compounds **1**−**5** at the IC_{50} for biofilm inhibition, indicating that the biofilm inhibition is not due to antibacterial properties of the compounds. Compound **9**, however, does reduce the planktonic growth at the biofilm-inhibiting concentrations.

4,5-Disubstituted 2-amino-1*H*-imidazoles. To determine whether the introduction of a second aryl group at the C−C double bond of the 2-aminoimidazole ring could enhance activity, we also synthesized a series of 4,5-disubstituted 2-amino-1*H*-imidazoles via our previously established procedure.[32] We screened these compounds against inhibition of *S.* Typhimurium ATCC14028 biofilm formation at 25 °C. Compounds **12**, **14**, **15**, and **17** were found to inhibit the biofilm formation at low concentrations (IC_{50}s ~20 μM) (Table 3), comparable with the active concentrations of the most promising *para*-substituted 4(5)-phenyl-2-amino-imidazoles. The other compounds are active at moderate concentrations (IC_{50} values of 50−200 μM). In general, each disubstituted compound was found to be more active than the least active of the two monosubstituted compounds

Table 3. Influence of 4,5-Disubstituted 2-Amino-1*H*-imidazoles **11**−**18** on the Biofilm Formation and the Planktonic Growth of *S.* Typhimurium ATCC14028 at 25 °C



11-17

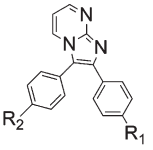


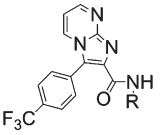
18

					effect on growth at[b]					
compd	Rl	R2	IC_{50}[a]	95% confidence interval for IC_{50}	400 μM	80 μM	40 μM	20 μM	10 μM	4 μM
11	H	4-OMe	77.1	60.6−98.0						
12	H	4-CF_3	29.2	22.7−37.6	o		−			−
13	H	4-Cl	46.9	35.2−62.5						
14	Cl	4-Me	12.9	10.7−15.6		o	o	o	+	−
15	Cl	4-CF_3	10.8	6.5−17.8		o	o	o	+	−
16	Cl	3,5-diF	71.8	50.2−102.5						
17	F	4-F	18.2	17.0−19.5		o	o	+	−	−
18			182.1	116.6−284.4	+		−			−

[a] IC_{50}: concentration of inhibitor needed to inhibit biofilm formation by 50%. [b] o: the planktonic growth is completely or almost completely inhibited when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; +: the planktonic growth is retarded when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; −: the planktonic growth is not or only slightly affected when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; no symbol indicated: effect not determined.

Scheme 2. Structures of the 2,3-Disubstituted Imidazo[1,2-*a*]pyrimidines **19**−**24**





Compound	R1	R2	Compound	R
19	H	H	23	Bn
20	H	4-OMe	24	Et
21	H	4-CF3		
22	H	4-F		

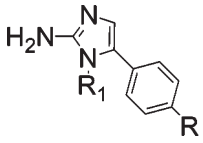
obtained by theoretically removing one substituent. To answer the question as to whether the tested compounds specifically interfere with biofilm formation or are bactericidal in nature, we determined the influence of the most active compounds on the planktonic growth of *Salmonella*. Compound **12** does only very slightly influence planktonic growth at the IC_{50} for biofilm inhibition, suggesting that biofilm inhibition is mediated by a biofilm specific mode of action. However, compound **17** slows down planktonic growth at the IC_{50} for biofilm inhibition, while compounds **14** and **15** block the growth almost completely.

2,3-Disubstituted Imidazo[1,2-*a*]pyrimidines. The effect of the 2,3-disubstituted imidazo[1,2-*a*]pyrimidines **19**−**24**, which are the chemical precursors of the 4,5-disubstituted 2-aminoimidazoles in our synthesis pathway (Scheme 2), against *Salmonella* biofilm formation, was assayed at 16, 25, and 37 °C. None of the compounds are able to reduce the biofilm formation at 400 μM, irrespective of the incubation temperature used (results not shown). In our chemical synthesis procedure, the 2-aminoimidazoles (containing active biofilm inhibitors, Tables 1, 2, and 3) are formed by cleavage of the imidazo[1,2-*a*]pyrimidines with a nucleophile such as hydrazine under conventional heating or microwave irradiation (Scheme 1).[32] Our rationale to test the antibiofilm effect of the 2,3-disubstituted imidazo[1,2-*a*]pyrimidines was based on the reported antibacterial and antifungal activity of some members of this class of compounds[35,36] but also on the hypothesis that the imidazo[1,2-*a*]pyrimidines could *in situ*, i.e. in the biofilm assay, be cleaved by cellular nucleophiles to form the active 2-aminoimidazoles. However, the lack of antibiofilm activity of the 2,3-disubstituted imidazo[1,2-*a*]pyrimidines means that this hypothesis cannot be substantiated for the tested 2,3-disubstituted imidazo[1,2-*a*]pyrimidines.

***N*1-Substituted 2-Aminoimidazoles.** In an attempt to improve their biofilm inhibitory activity, we substituted the *N*1-position of some of the para-substituted 4(5)-phenyl-2-aminoimidazoles and some 4,5-disubstituted 2-aminoimidazoles with *n*-alkyl, cyclo-alkyl, and aromatic groups by using our previously established chemistry (Scheme 1).[37,38]

***n*-Alkyl Substituents.** In the first instance, a broad variety of alkyl groups, with lengths ranging from one carbon atom to 14 carbon atoms, were introduced at the *N*1-position of the 4(5)-monosubstituted 2-aminoimidazoles **1**, **3**, and **9**, resulting in compounds **25**−**38**, **39**−**51**, and **52**−**63**, respectively (Table 4). Each compound was assayed for the ability to inhibit *S.* Typhimurium ATCC14028 biofilm formation at 25 °C. As depicted in Figure 1, a clear correlation was found between the length of the alkyl substituent and the biofilm inhibitory activity. In general, compounds with a short alkyl chain substitution (C1, C2, C3) at the *N*1-position have a lower activity as compared to their respective unsubstituted counterparts. This effect is most pronounced for the methyl substituent, which causes a reduction in the activity of at

Table 4. Influence of *N*1-Alkylated 2-Aminoimidazoles **25**−**63** on the Biofilm Formation and the Planktonic Growth of *S.* Typhimurium ATCC14028 and *P. aeruginosa* PA14 at 25 °C



			S. Typhimurium							*P. aeruginosa*						
					effect on growth at[b]							effect on growth at[b]				
compd	R	R1	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	80 μM	40 μM	20 μM	10 μM	5 μM	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	80 μM	40 μM	20 μM	10 μM	5 μM
25	H	Me	431.5	261.6−711.7						95.2	67.2−134.7					
26	H	Et	206.0	168.7−251.5						20.7	9.7−44.2		−	−		
27	H	*i*-Pr	175.5	139.7−220.6						4.5	3.2−6.3				−	−
28	H	*n*-Bu	51.9	45.1−59.8						2.1	1.1−4.3				−	−
29	H	*n*-Pen	48.4	37.5−62.4	o	−	−	−	−	2.1	1.2−3.7	−	−			−
30	H	*n*-Hex	39.7	30.0−52.5						19.1	15.3−23.9		−	−		
31	H	*n*-Hept	12.2	9.9−15.1						6.8	4.0−11.5		−	−		−
32	H	*n*-Oct	11.9	10.2−13.8			+	−		18.5	8.4−40.7		−	−		
33	H	*n*-Non	∼6.0				o	+	−	8.4	6.0−11.7			+	−	−
34	H	*n*-Dec	∼6.1				o	o	−	15.0	9.3−24.2			−	−	
35	H	*n*-Und	7.2	5.8−8.8		o		+	−	16.3	10.5−25.2			−	−	
36	H	*n*-Dod	7.1	5.8−8.7			o	+	−	12.8	8.0−20.6	−	−		−	
37	H	*N*-Trd	6.0	5.4−6.6		o		o	−	27.3	20.5−36.3		−	−		
38	H	*N*-Tet	8.7	7.1−10.7	−	−	−	−		9.8	6.6−14.6	−	−		−	
39	Cl	Me	108.1	75.3−155.2						111.8	87.0−143.6					
40	Cl	Et	53.2	35.0−80.8	+	+	−	−	−	11.3	7.2−17.7			−	−	
41	Cl	*n*-Bu	57.8	34.7−96.5						16.7	11.1−26.0		−	−		
42	Cl	*n*-Pen	8.0	5.8−11.1		o	+	−	−	6.0	4.8−7.5			−	−	−
43	Cl	*n*-Hex	4.9	3.5−6.9				o	+	5.4	4.1−7. 1				−	−
44	Cl	*n*-Hept	9.5	5.4−16.8				o	−	2.6	2.2−3.2				+	−
45	Cl	*n*-Oct	∼5.9				o	o	+	4.0	3.0−5.2			o	+	−
46	Cl	*n*-Non	4.0	3.1−5.5			o	+	−	8.0	6.0−10.7			o	−	
47	Cl	*n*-Dec	4.1	2.4−7.0				o	−	22.3	16.3−30.6		−	−		
48	Cl	*n*-Und	4.1	2.9−5.6			o	+	−	44.0	29.6−65.4					
49	Cl	*n*-Dod	6.2	4.5−8.5			−	−	−	49.7	30.1−82.0					
50	Cl	*n*-Trd	19.5	11.1−34.5		−	−			33.9	15.0−76.4					
51	Cl	*n*-Tet	453.3	319.6−643.1						19.1	11.8−31.0	−	−			−
52	Ph	Et	14.0	13.0−15.1	o	o	−	−	−	∼95.1						
53	Ph	*n*-Bu	∼21.1			o	o			41.6	16.1−107.5					
54	Ph	*n*-Pen	9.2	7.1−11.8						73.9	57.5−94.9					
55	Ph	*n*-Hex	∼12.1				o	o		32.1	23.0−44.7					
56	Ph	*n*-Hept	9.3	6.9−12.4			+	−		25.2	15.6−40.9		+	+		
57	Ph	*n*-Oct	3.3	1.2−9.0		o	+		−	8.9	3.5−22.5			−	−	
58	Ph	*n*-Non	16.4	8.0−33.6	+	+	−			> 400						
59	Ph	*n*-Dec	5.7	3.2−10.3		−	−		−	> 400		−	−			−
60	Ph	*n*-Und	60.2	17.7−204.7						> 400						
61	Ph	*n*-Dod	166.6	100.7−275.5						> 400			−			−
62	Ph	*N*-Trd	> 800							> 400			−			−
63	Ph	*N*-Tet	> 800							> 400						

[a] IC_{50}: concentration of inhibitor needed to inhibit biofilm formation by 50%. [b] o: the planktonic growth is completely or almost completely inhibited when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; +: the planktonic growth is retarded when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; −: the planktonic growth is not or only slightly affected when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; no symbol indicated: effect not determined.

least 3 times. To a lesser extent, ethyl and isopropyl substituents also cause a reduction in activity. An exception to this rule is compound **52** (with an ethyl substituent), which is slightly more active than the unsubstituted molecule **9**. Substitution with a butyl group does not have an unequivocal effect. In the case of compound **28**, the activity is enhanced 3-fold, while in the other cases the activity is reduced. Remarkably, all the compounds substituted with an alkyl group with an intermediate length between 5 and 10 carbon atoms are very active biofilm inhibitors, with IC_{50} values in general below 12 μM. Compound **57**, substituted with an octyl group, is the most potent inhibitor, with an IC_{50} of 3.25 μM. The effect of substitution with a longer alkyl chain drastically depends on the nature of the substituent on the C5-position of the ring (phenyl, *p*-chlorophenyl, or biphenyl). Indeed, replacement of the *N*1-hydrogen of compound **1** (phenyl on C5 of the ring) by a long alkyl chain (C11−C14) raises the activity drastically, from an IC_{50} of 130 μM for compound **1** to values below 9 μM for the substituted compounds (**35**−**38**). In the case of compound **3** (*p*-chlorophenyl on C5 of the ring), substitution with an undecyl (**48**) and dodecyl (**49**) group results in very active compounds (IC_{50} values below 6.5 μM), while introduction of a tridecyl group (**50**) does not have a clear effect on the biofilm inhibitory activity and introduction of a tetradecyl (**51**) group drastically reduces the activity (IC_{50} = 453 μM). In the case

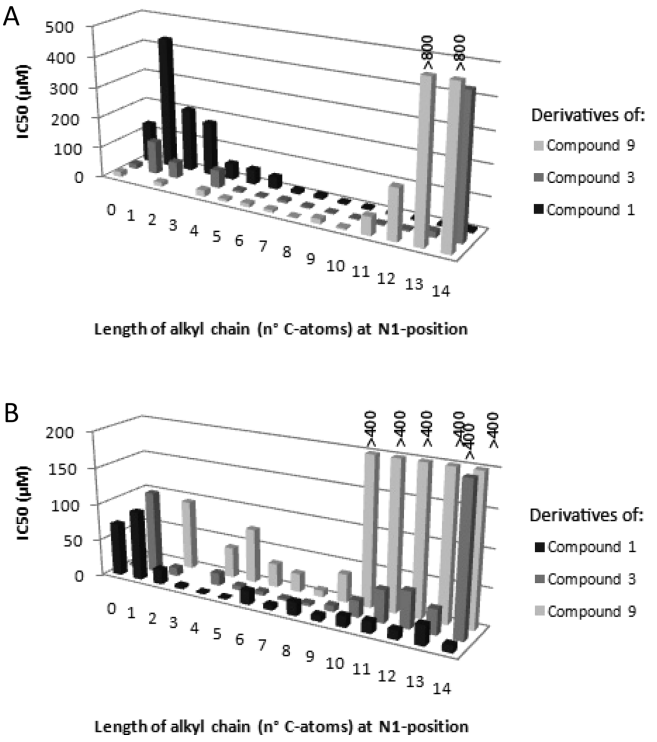


Figure 1. Effect of introduction of alkyl chains with different lengths (1−14 C-atoms; 0 means no alkyl substituent) at the *N*1-position of compound **1** (black bars), **3** (dark-gray bars), and **9** (light-gray bars) on the IC_{50} (μM) for inhibition of the biofilm formation of *S.* Typhimurium ATCC14028 (A) and *P. aeruginosa* PA14 (B) at 25 °C in TSB 1/20. All the substituents are *n*-alkyl groups, except for the alkyl group with 3 carbon atoms, which is an *iso*-propyl group.

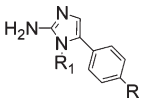
of compound **9** (biphenyl on C5 of the ring), however, substitution with all the long alkyl chains (C11−C14) (**60**−**63**) drastically reduces the activity, with the strongest reduction for the longest alkyl chains. Subsequently, we investigated the influence of some of the most active representatives of this class of compounds on the planktonic growth curve of *S.* Typhimurium at 25 °C. Most compounds only show a very small concentration range with a specific effect on biofilm formation and no effect on the planktonic growth (Table 4). Therefore, we cannot exclude the possibility that the biofilm inhibitory effect of these compounds (at the higher concentrations) is partially due to a reduction of the planktonic growth of the bacteria in the growth medium used in the setup to monitor biofilm formation. Interestingly, compounds **38**, **49**, and **59**, with long 3-alkyl chains, do not follow this general trend, as no effect on the growth was observed at 40 μM while the biofilm formation is inhibited at concentrations lower than 10 μM.

As depicted in Table 4 and Figure 1, a similar structure −activity relationship was found for the inhibition of *P. aeruginosa* biofilm formation at 25 °C. Again, introduction of a short alkyl chain at the *N*1-position generally results in a decreased activity (except for compound **26**), while all the compounds with an intermediate alkyl chain length show very strong activities. The effect of substitution with a long alkyl chain is in this case also dependent on the nature of the C5-subsitutent. In the case of compound **1**, substitution with a long alkyl chain results in very active compounds (**34**−**38**), while these substitutions yield almost completely inactive compounds (**58**−**63**) in the case of compound **9**. The activity of compound **3** is also decreased after introduction of a long *N*1-alkyl chain but to a lesser extent than in the case of compound **9** (compounds **47**−**51**). Interestingly, growth curve analysis revealed that these compounds are much less toxic to *P. aeruginosa* than to *S.* Typhimurium. Most of the compounds tested show a broad concentration range with only biofilm inhibition and no effect on the planktonic growth (Table 4).

To consolidate the structure−activity relationship described above, we first synthesized some additional compounds with short alkyl substituents at the *N*1-position and compared their biofilm inhibitory activity with their unsubstituted counterparts (Table 5 and Figure 2). As expected, introduction of a methyl, ethyl, and *iso*-propyl group does in general markedly decrease the biofilm inhibitory activity, while introduction of a *n*-butyl group does not have an unequivocal effect. Because the introduction of a *n*-octyl side chain at the *N*1-position of compounds **1**, **3**, and **9** does result in very active inhibitors of both the *Salmonella* and *Pseudomonas* biofilm formation, we also decided to introduce a *n*-octyl chain at the *N*1-position of some other 4(5)-aryl-2-amino-1*H*-imidazoles and to evaluate the biofilm inhibitory activity of the substituted compounds (Table 6). All the compounds (**75**−**84**) are active inhibitors of both the *S.* Typhimurium and *P. aeruginosa* biofilm formation at 25 °C, except for compounds **82** and **83**. These compounds have very bulky C5-substituents, which could diminish the solubility of the compounds in the growth medium, reduce the uptake into the cell, or cause steric hindrance in the putative receptor binding pocket. Comparison of the activities of compounds **75**−**78** with their unsubstituted counterparts **2**, **5**, **6**, and **7** (Table 1 and 2) confirms that introduction of a *n*-octyl chain at the *N*1-position of the 4(5)-phenyl-2-amino-1*H*-imidazoles in most cases improves the biofilm inhibitory activity of the compounds. Growth curve analysis confirmed the previously described finding that the *N*1-alkylated 2-aminoimidazoles in general show a broad concentration range in which they specifically inhibit the biofilm formation of *P. aeruginosa*, without reducing the planktonic growth of the bacteria, while this concentration range is smaller in the case of *S.* Typhimurium. Compounds **81** and **84** are exceptions to this rule, as we found them to slow down the planktonic growth of *P. aeruginosa* at the IC_{50} for biofilm inhibition.

Cyclo-alkyl Substituents. To determine whether the introduction of an intermediate or long cyclo-alkyl chain could also enhance the activity of the compounds, a broad variety of cyclo-alkyl groups, with lengths ranging from 4 to 12 carbon atoms, were introduced at the *N*1-position of the 4(5)-monosubstituted 2-aminoimidazoles **1**, **3**, and **9** (Table 7). The compounds were tested against biofilm formation of *S.* Typhimurium and *P. aeruginosa* at 25 °C. As depicted in Figure 3, introduction of an intermediate cyclo-alkyl chain does improve the biofilm inhibitory activity of compound **1** against *S.* Typhimurium and even more drastically against *P. aeruginosa.* In the case of *Salmonella*, introduction of a cyclo-heptyl chain yields the most active compound (compound **89**), with an IC_{50} of 27 μM, as compared to 130 μM for compound **1**. In the case of *Pseudomonas*, cyclo-hexyl and cyclo-octyl are the best substituents as introduction of these groups improves the activity drastically from an IC_{50} of 73 μM for compound **1** to 5 μM for the substituted compounds. All compounds derived from **3** and **9** by introduction of an intermediate length side chain are very strong inhibitors of the *Salmonella* and *Pseudomonas* biofilm formation (Table 7), although their activity is not markedly better than the activity of the unsubstituted compounds. Introduction of a dodecyl side chain abolishes the

Table 5. Influence of 2-Aminoimidazoles **64**−**74** with Short Alkyl Substituents at the *N*1-Position on the Biofilm Formation and the Planktonic Growth of *S.* Typhimurium ATCC14028 at 25 °C



			S. Typhimurium		*P. aeruginosa*	
compd	R	Rl	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	IC_{50}[a](u.M)	95% confidence interval for IC_{50}
64	F	Et	290.4	221.1−381.5	67.8	39.32−116.9
65	F	*n*-Bu	56.2	45.20−69.89	55.7	47.57−65.14
66	Br	Et	126.1	67.65−235.1	60.5	53.71−68.21
67	Br	*n*-Bu	16.2	12.16−21.45	18.7	10.06−34.91
68	NO_2	Me	161.8	104.5−250.6	69.8	54.43−89.43
69	NO_2	Et	285.5	227.2−358.8	125.8	95.69−165.3
70	NO_2	*i*-Pr	359.7	311.9−414.9	> 800	
71	NO_2	*n*-Bu	114.3	92.12−141.9	372.6	218.2−636.3
72	Me	Me	62.3	48.56−79.85	31.7	25.89−38.79
73	Me	*i*-Pr	203.3	173.4−238.4	240.3	191.2−302.1
74	CN	Me	> 400		331.4	250.4−438.8

[a] IC_{50}: concentration of inhibitor needed to inhibit biofilm formation by 50%.

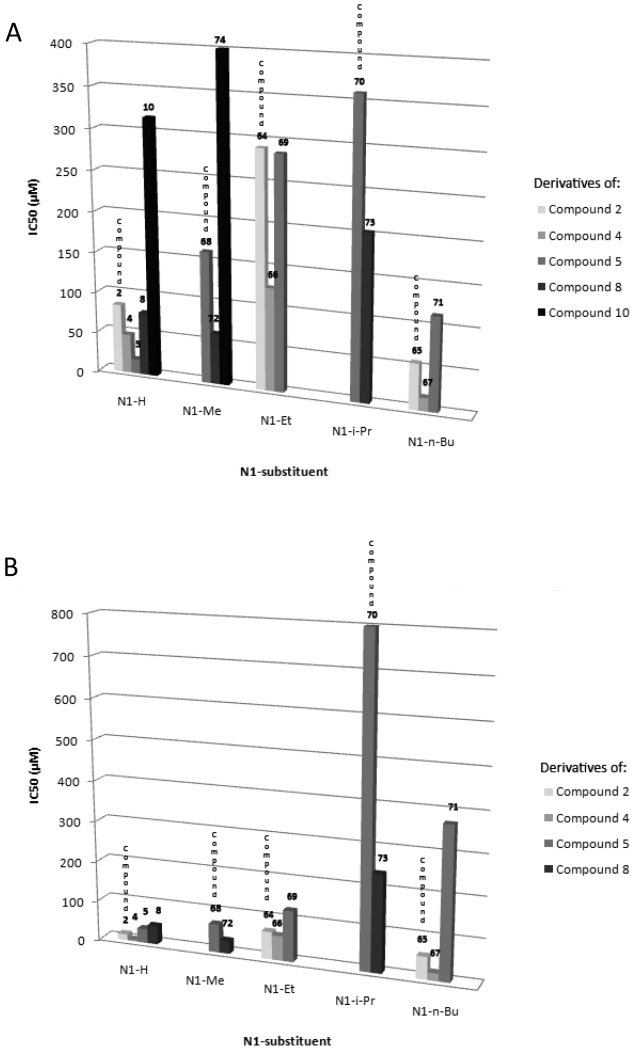
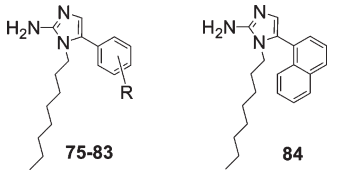


Figure 2. Effect of introduction of short alkyl chains (Me, Et, *i*-Pr, or *n*-Bu) at the *N*1-position of compounds **2**, **4**, **5**, **8**, and **10** on the IC_{50} (μM) for inhibition of the biofilm formation of *S.* Typhimurium ATCC14028 (A) and *P. aeruginosa* PA14 (B) at 25 °C in TSB 1/20.

activity of all three compounds against *P. aeruginosa* biofilm formation, while the effect of a cyclo-dodecyl substituent on the *Salmonella* biofilm inhibition depends on the nature of the C5-group. Indeed, compound **91**, derived from **1** by introduction of a cyclo-dodecyl group, is a very strong biofilm inhibitor, while compound **97**, derived from **3**, is a moderate biofilm inhibitor, and compound **103**, derived from **9**, does not have any activity. As in the case of the *n*-alkyl substituents, growth curve analysis revealed that most compounds with cyclo-alkyl substituents only possess a very small concentration range with a selective effect on the *Salmonella* biofilm formation and no effect on the planktonic growth. An exception is compound **91**, with a cyclo-dodecyl side chain, which does not affect the planktonic growth of *Salmonella* at 80 μM (the highest concentration tested), while the IC_{50} for biofilm inhibition is 15 μM. Interestingly, the concentration range between biofilm inhibition and growth inhibition is much broader in the case of *P. aeruginosa* (Table 7). We also tested the antibiofilm effect of *N*1-cyclohexyl 4,5-diphenyl-2-aminoimidazole **104**. This 1,4,5-trisubstituted compound shows a good effect against *S.* Typhimurium (IC_{50} = 24 μM) and *P. aeruginosa* (IC_{50} = 5 μM) biofilm formation, while only a slight *Salmonella* growth inhibition was observed at 80 μM.

Aromatic Substituents. Finally, we also tested the influence of substituting the *N*1-hydrogen with some aromatic groups on inhibition of the biofilm formation by *S.* Typhimurium and *P. aeruginosa*. As shown in Table 8, introduction of a phenyl group at the *N*1-position of the 4,5-disubstituted-2-amino-imidazole **14** (resulting in compound **105**) abolishes its biofilm inhibitory activity completely. Substitution of the *N*1-position of compound **1** with a benzyl group (resulting in **106**) enhances the activity against both *Salmonella* and *Pseudomonas* 2- to 3-fold. The same substitution also enhances the activity of compound **2** (compound **107**) against *Salmonella*, while it lowers the activity against *Pseudomonas*. Substitution of the *N*1-position of compounds **3** and **4** with a benzyl group (resulting in compounds **108** and **109**, respectively) on the other hand lowers the activity against both species. Substitution of the *N*1-hydrogen with a 3,4-dimethoxybenzyl group (**110**) decreases the activity of compound **1** against *Pseudomonas*, while no clear effect on the activity against *Salmonella* could be observed. Also substitution of the *N*1-hydrogen of compound **1** with a veratryl group (**111**) or a piperonyl group (**112**) does not have a substantial effect on the biofilm inhibitory activity.

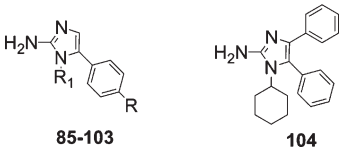
Table 6. Influence of *N*1-Octyl-2-aminoimidazoles **75**−**84** on the Biofilm Formation and the Planktonic Growth of *S.* Typhimurium ATCC14028 and *P. aeruginosa* PA14 at 25 °C



			S. Typhimurium							*P. aeruginosa*					
					effect on growth at[b]							effect on growth at[b]			
compd	R	R1	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	80 μM	40 μM	20 μM	10 μM	5 μM	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	40 μM	20 μM	10 μM	5 μM
75	4-F	*n*-Oct	~10.8				o	−		4.9	3.8−6.2		o	+	−
76	4-NO_2	*n*-Oct	35.4	25.2−49.7	+	+									
77	4-SO_2Me	*n*-Oct	~29.1			+	−			4.9	2.6−9.3		−		
78	4-OMe	*n*-Oct	8.5	7.8−9.3			+	−		1.2	0.9−1.7		−		
79	4-SMe	*n*-Oct	6.7	6.0−7.5				+	−	2.8	1.9−4.0		o	+	−
80	3,4-diCI	*n*-Oct	3.7	3.4−4.1				+	−						
81	3-Br	*n*-Oct	11.2	10.3−12.1			+	−		40.6	29.5−56.1	+	−		
82	4-(4′-NO_2Ph)	*n*-Oct	116.5	54.6−248.5						> 800					
83	4-(4′-biphenyl)	*n*-Oct	850.2	677.0−1068.0						> 800					
84			6.6	6.2−7.0				−	−	17.6	11.1−28.1		+	−	

[a] IC_{50}: concentration of inhibitor needed to inhibit biofilm formation by 50%. [b] o: the planktonic growth is completely or almost completely inhibited when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; +: the planktonic growth is retarded when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; −: the planktonic growth is not or only slightly affected when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; no symbol indicated: effect not determined.

Table 7. Influence of *N*1-Cyclo-alkyl-2-aminoimidazoles **85**−**104** on the Biofilm Formation and the Planktonic Growth of *S.* Typhimurium and *P. aeruginosa* PA14 at 25 °C



			S. Typhimurium							*P. aeruginosa*						
					effect on growth at[b]							effect on growth at[b]				
compd	R	R1	IC_{50}[a](μM)	95% confidence interval for IC_{50}	80 μM	40 μM	20 μM	10 μM	5 μM	IC_{50}[a](μM)	95% confidence interval for IC_{50}	80 μM	40 μM	20 μM	10 μM	5 μM
85	H	*c*-Pr	89.6	76.0−105.7						35.0	21.8−56.3					
86	H	*c*-Bu	116.3	91.5−147.9						19.9	14.4−27.6	−	−	−		
87	H	*c*-Pen	70.7	56.0−89.2						12.6	7.2−22.1			−		
88	H	*c*-Hex	85.2	54.1−134.3						4.6	2.8−7.68	−	−			−
89	H	*c*-Hep	26.9	16.5−43.7						14.2	9.7−20.9	−	−		−	
90	H	*c*-Oct	40.5	25.9−63.2	o		−	−		4.5	3.1−6.6	−	−			−
91	H	*c*-Dod	14.6	11.1−19.1	−	−		−		> 800						
92	Cl	*c*-Pr	54.8	47.0−64.0						13.0	7.0−24.2			−		
93	Cl	*c*-Bu	11.6	8.5−15.8	o	+		−		6.0	4.8−7.5	+	−			−
94	Cl	*c*-Hex	33.2	25.8−42.8						11.2	8.6−14.7		−	−		−
95	Cl	*c*-Hep	15.7	13.0−18.9						4.0	2.78−5.8			−	−	−
96	Cl	*c*-Oct	12.9	11.2−14.8		o	o	−		48.7	15.6−151.6					
97	Cl	*c*-Dod	120.2	70.3−205.4						> 800						
98	Ph	*c*-Bu	26.3	21.8−31.6						~24.5						
99	Ph	*c*-Pen	7.5	5.5−10.3	o	o	o	−	−	353.1	151.2−824.6					
100	Ph	*c*-Hex	~12.6			o	o	−		69.7	36.7−132.3					
101	Ph	*c*-Hep	~12.5			o	o	+		6.6	2.9−14.7					
102	Ph	*c*-Oct	−22.9				o			3.2	1.5−6.7		−	−	−	−
103	Ph	*c*-Dod	> 800							> 800						
104			23.9	17.49−32.60	−	−	−	−		5.0	1.5−16.5		−	−		

[a] IC_{50}: concentration of inhibitor needed to inhibit biofilm formation by 50%. [b] o: the planktonic growth is completely or almost completely inhibited when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; +: the planktonic growth is retarded when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; −: the planktonic growth is not or only slightly affected when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; no symbol indicated: effect not determined.

***N*1-Substituted Imidazo[1,2-*a*]pyrimidinium Perchlorate Salts.** In our chemical synthesis procedure, the *N*1-substituted 2-aminoimidazoles are formed by cleavage of the *N*1-substituted imidazo[1,2-*a*]pyrimidinium perchlorate salts with a nucleophile such as hydrazine, under conventional heating or microwave irradiation.[37,38] After cleavage, the *N*1-substituent of

2-aminoimidazole will be the same as the *N*1-substituent of the salt (Scheme 1). A possible mechanism for the pyrimidine ring cleavage of the salts upon reaction with amines involves a nucleophilic attack by a first molecule of amine at the *C*5-position, resulting in the formation of azabutadienes. 1,4-Addition of a second molecule of amine results in the formation of the 2-aminoimidazole.[38] As previously reported,[38] we found that the imidazo[1,2-*a*]pyrimidinium salts can also be cleaved to 2-aminoimidazoles at room temperature upon stirring with excess of secondary amine. Therefore, we hypothesized that these imidazo[1,2-*a*]pyrimidinium salts could be more susceptible to cleavage by cellular nucleophiles (e.g., proteins, metalloproteins, polyamines, amino acids,...) than the 4,5-disubstituted imidazo-[1,2-*a*]pyrimidines, leading to the *in situ* formation of the active 2-aminoimidazoles. To strengthen this hypothesis, we determined whether 1-ethyl-2-(4′-nitrophenyl)-imidazo[1,2-*a*]pyrimidin-1-ium perchlorate (compound **114** (Table 9)) could be degraded to the corresponding 2-aminoimidazole **69** under influence of 2 equiv of the amino acid analogue L-alanine ethyl ester hydrochloride at room temperature. We found the salt to be almost completely degraded to the imidazole after 12 h of reaction, indicating that the imidazo[1,2-*a*]pyrimidinium salts could indeed be cleaved by cellular nucleophiles within the time frame of our biofilm assay. To further substantiate our hypothesis, we synthesized a series of imidazo[1,2-*a*]pyrimidinium salts **112**−**130** with *N*1-alkyl substituents of different lengths and determined whether the structure−activity relationship found for these salts is in agreement with the previously described structure−activity relationship for the corresponding 2-aminoimidazoles. As depicted in Table 9, salts **113**−**117** (corresponding imidazoles **69**−**71**) with short *N*1-alkyl chains (C2−C4) have IC_{50} values higher than 400 μM both for the *Salmonella* and *Pseudomonas* biofilm inhibition at 25 °C, while compound **118** (corresponding imidazole **76**) with a *n*-octyl side chain is a good biofilm inhibitor, with IC_{50} values of 19 and 68 μM for *P. aeruginosa* and *S.* Typhimurium biofilm inhibition, respectively. Hence, we may conclude that the structure−activity relationship delineated for the *N*1-substituted 2-aminoimidazoles does also apply to the corresponding *N*1-substituted salts, which contributes to the plausibility of our hypothesis. As introduction of a *n*-octyl substituent at the *N*1-position of the 2-aminoimidazoles in general improves their biofilm inhibitory activity, we decided to synthesize a series of 2-aryl-imidazo[1,2-*a*]pyrimidinium perchlorate salts with *n*-octyl substituents at the *N*1-position. In agreement with the structure−activity relationship of the 2-aminoimidazoles, most of the *N*1-octyl substituted imidazo[1,2-*a*]pyrimidinium salts were found to be strong inhibitors of both the *S.* Typhimurium and *P. aeruginosa* biofilm formation, with IC_{50} values in general below 35 μM. However, in contrast to the almost inactive 2-aminoimidazoles **82** and **83**, the corresponding salts **127** and **130** are very active, with IC_{50} values below 10 μM. This could possibly be explained by a reduction of the planktonic growth of the bacteria in the growth medium used in the setup to

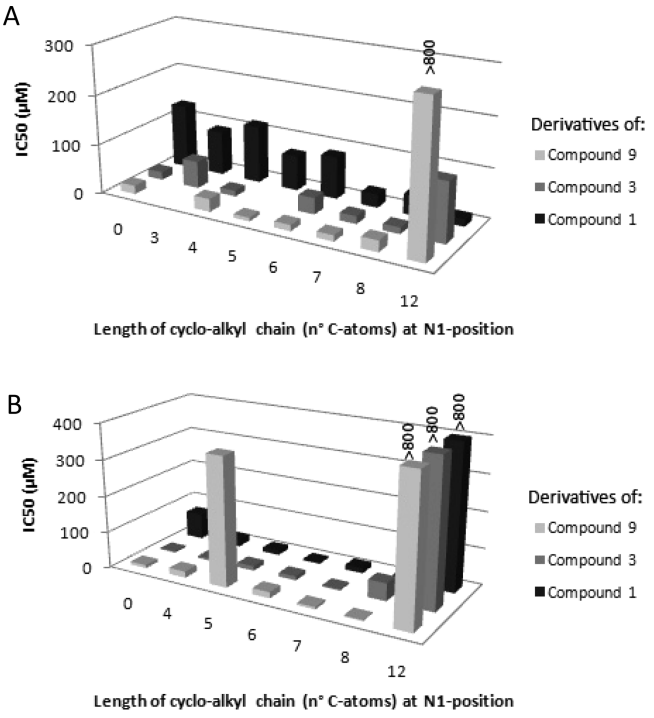
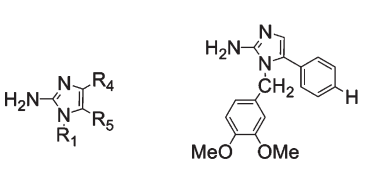


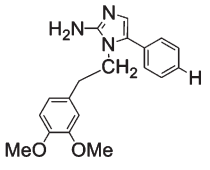
Figure 3. Effect of introduction of cyclo-alkyl chains with different lengths (1−12 C-atoms; 0 means no alkyl substituent) at the *N*1-position of compound **1** (black bars), **3** (dark-gray bars) and **9** (light-gray bars) on the IC_{50} (μM) for inhibition of the biofilm formation of *S.* Typhimurium ATCC14028 (A) and *P. aeruginosa* PA14 (B) at 25 °C.

Table 8. Influence of 2-Aminoimidazoles **105**−**112** with Aromatic Substituents at the *N*1-Position on the Biofilm Formation of *S.* Typhimurium ATCC14028 at 25 °C

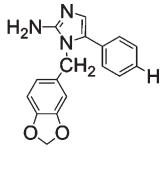


105-109

110



111

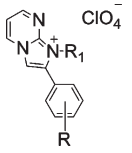


112

				S. Typhimurium		*P. aeruginosa*	
compd	R1	R4	R5	IC_{50}[a] (μM)	95% confidence interval for IC_{50}	IC_{50}[a] (μM)	95% confidence interval for IC_{50}
105	Ph	*p*-MePh	*p*-ClPh	> 400		> 800	
106	Bn	H	Ph	55.1	45.3−67.0	26.1	17.0−39.9
107	Bn	H	*p*-FPh	37.3	32.3−43.2	32.0	27.1−37.9
108	Bn	H	*p*-ClPh	28.5	22.0−36.9	14.8	11.9−18.5
109	Bn	H	*p*-BrPh	74.2	48.6−113.1	57.8	36.2−92.4
110	3,4-diMeOBn	H	H	155.8	109.8−221.1	159.3	115.0−220.6
111	veratryl	H	H	185.0	147.6−231.9	51.9	43.0−62.6
112	piperonyl	H	H	97.3	72.97−129.7	104.4	78.6−138.8

[a] IC_{50}: concentration of inhibitor needed to inhibit biofilm formation by 50%.

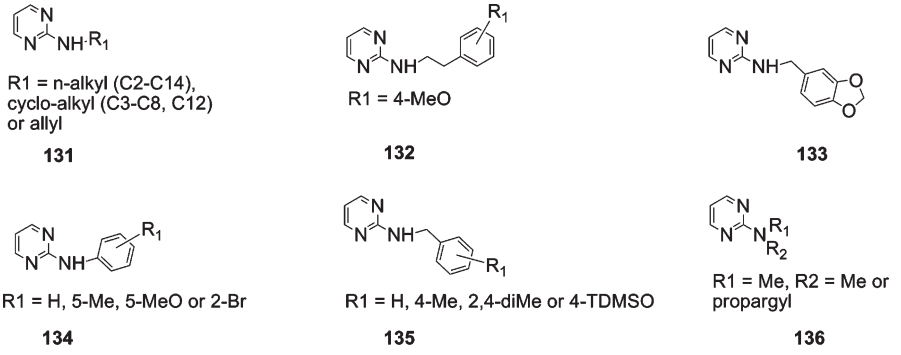
Table 9. Influence of *N*1-Substituted Imidazo[1,2-*a*]pyrimidinium Salts **113**−**130** on the Biofilm Formation and the Planktonic Growth of *S.* Typhimurium ATCC14028 and *P. aeruginosa* PA14 at 25°C



			S. Typhimurium						*P. aeruginosa*					
					effect on growth at[a]						effect on growth at[b]			
compd	R1	R	IC_{50} μM	95% confidence interval for IC_{50}	40 μM	20 μM	10 μM	5 μM	IC_{50} μM	95% confidence interval for IC_{50}	40 μM	20 μM	10 μM	5 μM
113	Me	3-NO_2	427.3	233.0−783.6					>800					
114	Et	4-NO_2	>800						>800					
115	*i*-Pr	4-NO_2	>800						>800					
116	*n*-Bu	4-NO_2	>400						>800					
117	*i*-Bu	4-NO_2	405.2	300.5−546.3					>800					
118	*n*-Oct	4-NO_2	63.6	54.1−74.7					18.7	12.6−27.8	+	−	−	
119	*c*-Hex	4-NO_2	561.0	277.9−1133.0					249.5	140.7−442.3				
120	*c*-Dod	4-NO_2	9.8	6.4−15.1		−	−		15.1	11.2−20.3	o	+	−	
121	Bn	4-NO_2	170.1	59.4−487.3					>800					
122	*n*-Oct	4-OMe	34.8	26.3−45.9	o	+			8.7	3.5−21.8	−	−	−	
123	*n*-Oct	4-F	35.7	27.9−45.7	−	−			19.5	14.1−26.9	+	−		
124	*n*-Oct	3.4-diF	3.8	3.2−4.7			+	−	12.7	10.7−15.1	o	+	+	
125	*n*-Oct	3-Br	7.3	6.0−8.9		+	-		7.0	4.5−10.9	+	-		
126	*n*-Oct	naphtyl	~6.0				+	−	6.8	2.8−16.9	o	+	+	−
127	*n*-Oct	4-(4′N0_2Ph)	1.5	1.2−1.8			o	+	1.0	0.4−3.0	o	o	o	o
128	*n*-Oct	4-SO_2Me	82.0	56.4−119.1					49.6	23.8−103.6				
129	*n*-Oct	4-SMe	20.7	17.4−24.5	o	+			9.3	7.0−12.3	+	−		
130	*n*-Oct	4-(4′-biphenyl)	5.0	4.1−6.2			+	−	nd[c]					

[a] IC_{50}: concentration of inhibitor needed to inhibit biofilm formation by 50%. [b] o: the planktonic growth is completely or almost completely inhibited when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; : the planktonic growth is retarded when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; −: the planktonic growth is not or only slightly affected when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor; no symbol indicated: effect not determined. [c] nd: not determined.

Scheme 3. Structures of the *N*2-Substituted 2-Aminopyrimidines **131**−**136**



monitor biofilm formation, as growth curve analysis revealed a toxic effect of salts **127** and **130**. Most of the other compounds tested show a small concentration range with only biofilm inhibition and no effect on the planktonic growth. To exclude the possibility that part of the toxic or biofilm inhibitory effect of the salts is due to the perchlorate anion, we tested the influence of different concentrations of sodium perchlorate on both the planktonic growth and biofilm formation of *Salmonella* and *Pseudomonas*. No effect was found at the highest concentration tested (500 μM).

2*N*-Substituted 2-Aminopyrimidines. Some members of the 2-aminopyrimidine class of compounds have previously been shown to possess antibacterial and antifungal activity.[39,40] These molecules are the precursors of the *N*1-substituted imidazo-[1,2-*a*]pyrimidinium salts in our synthesis pathway (Scheme 1). The availability of a broad array of 2-aminopyrimidines, substituted with *n*-alkyl, cyclo-alkyl, and aromatic groups at the *N*2-position, prompted us to investigate the potential of this class of compounds as biofilm inhibitors. The influence of compounds **131**−**136** (Scheme 3) on the biofilm formation of *S.* Typhimurium was tested at 25 °C. Remarkably, none of the compounds do have an effect on the biofilm formation at 400 μM, which was the highest concentration tested.

Conclusion

Recently, we published a method for the microwave-assisted synthesis of substituted 2-amino-1*H*-imidazoles from imidazo[1,2-*a*]pyrimidines, focusing on 4(5)-mono and 4,5-disubstituted 2-amino-1*H*-imidazoles with aryl substituents. Furthermore, we reported on a procedure for the synthesis of *N*1-substituted 2-aminoimidazoles from 2-aminopyrimidines via *N*1-substituted imidazo[1,2-*a*]pyrimidinium salts. In the present study, we investigated the effect of the different reaction

products (2-aminoimidazoles), intermediates (imidazopyrimidines, imidazo[1,2-*a*]pyrimidinium salts), and reagents (2-aminopyrimidines) on the biofilm formation of *S.* Typhimurium and *P. aeruginosa*. We found that our simplest 2-aminoimidazole structure, 4(5)-phenyl-2-amino-1*H*-imidazole **1**, showed a moderate biofilm inhibitory activity both against *S.* Typhimurium and *P. aeruginosa*. Substitution of the para-position of the 4(5)-phenyl ring with a chlorine, nitro, or phenyl group enhanced the activity against *Salmonella* biofilms (25 °C) about 10 times, while substitution with a bromine, fluorine, methyl, or methoxy group did improve the biofilm inhibition more moderately and substitution with a methanesulfonyl or nitrile function reduced the biofilm inhibitory activity drastically. In case of *Pseudomonas*, *para*-substitution with a chlorine, bromine or phenyl group increased the biofilm inhibitory (25 °C) activity about 20 times. The 4,5-disubstituted 2-amino-1*H*-imidazoles showed a high or moderate activity against *S.* Typhimurium biofilm formation, but they were in general highly toxic to *Salmonella*. We were able to delineate a relationship between the length of the *N*1-alkyl or *N*1-cyclo-alkyl chain of the *N*1-substituted 2-aminoimidazoles and their biofilm inhibitory activity. In general, introduction of a short alkyl chain at the *N*1-position resulted in a decreased activity against *S.* Typhimurium and *P. aeruginosa* biofilm formation, while all compounds with an intermediate alkyl or cyclo-alkyl chain length showed very strong activities in both test systems. The introduction of a long alkyl or cyclo-alkyl chain could either drastically enhance the activity or totally abolish the activity, depending on the nature of the C5-substitutent and the bacterial species tested. Growth curve analysis revealed that the *N*1-(cyclo-)alkylated 2-aminoimidazoles show a broad concentration range in which they specifically inhibit the biofilm formation of *P. aeruginosa*, without retarding the planktonic growth of the bacteria. However, these compounds were much more toxic to *S.* Typhimurium. Therefore, we cannot exclude the possibility that part of the inhibitory effect against *Salmonella* biofilms (at the higher concentrations) is due to a reduction of the planktonic growth of the bacteria in the growth medium surrounding the surface on which the biofilms are formed. To test the hypothesis that the 2,3-disubstituted imidazo[1,2-*a*]pyrimidines and the imidazo[1,2-*a*]pyrimidinium salts, which are the chemical precursors of the 2-aminoidazoles, could also *in situ* be cleaved by cellular nucleophiles to form the active 2-aminoimidazoles, we tested their influence on the biofilm formation. We found a good correlation between the activity of the imidazo[1,2-*a*]pyrimidinium salts and their corresponding 2-aminoimidazoles, supporting our hypothesis. The disubstituted imidazo[1,2-*a*]pyrimidines, however, did not show any biofilm inhibitory activity, indicating that these molecules are less susceptible to degradation by cellular nucleophiles. Finally, we tested the influence of a broad array of 2*N*-substituted 2-aminopyrimidines, which are the chemical precursors of the imidazo[1,2-*a*]pyrimidinium salts, and demonstrated their lack of activity against the biofilm formation of *S.* Typhimurium and *P. aeruginosa*, although some members of the class of 2-aminopyrimidines were previously reported to have antibacterial activity. In conclusion, the 2-aminoimidazoles and the imidazopyrimidinium salts of the present study are valuable candidates in the development of therapeutics and sanitizers for the combat of biofilm formation by *S.* Typhimurium, *P. aeruginosa*, and possibly other pathogenic bacteria.

Experimental Section

Chemistry. General Experimental Methods. Chemicals were purchased from commercial sources and used without further treatment. Melting points are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 75 with tetramethylsilane or solvent ($CDCl_3$, CD_3OD, DMSO-d_6) as internal standard (d ppm). The ion source temperature was 150−250 °C, as required. High-resolution EI-mass spectra were performed with a resolution of 10 000. The low-resolution spectra were obtained with a HP5989A MS instrument. For column chromatography, 70−230 mesh silica gel was used. The purity of the compounds was checked by HPLC. All compounds were obtained with a purity >95%.

Microwave Irradiation Experiments. A multimode Milestone MicroSYNTH microwave reactor was used in the standard configuration as delivered, including proprietary software. All experiments were carried out in sealed microwave process vials (15, 30, and 50 mL). After completion of the reaction, the vial was cooled down to 25 °C via air jet cooling before opening. Reaction temperatures were monitored by an IR sensor on the outside wall of the reaction vial and a fiber-optic sensor inside the reaction vial.

4(5)-Monosubstituted 2-Amino-1*H*-imidazoles and 4,5-Disubstituted 2-Amino-1*H*-imidazoles. The 4(5)-Monosubstituted 2-amino-1*H*-imidazoles and 4,5-disubstituted 2-amino-1*H*-imidazoles were synthesized according to our previously established protocols.[32]

2,3-Disubstituted Imidazo[1,2-*a*]pyrimidines. The 2,3-disubstituted imidazo[1,2-*a*]pyrimidines were synthesized by using our previously described protocols.[43]

***N*1-Substituted 2-Aminoimidazoles and Imidazo[1,2-*a*]pyrimidinium Salts.** The *N*1-substituted 2-aminoimidazoles and imidazo-[1,2-*a*]pyrimidinium salts were synthesized following our previously published methods.[37,38] A general procedure is described below:

(1) To a solution of substituted 2-aminopyrimidine (6 mmol) and α-bromoketone (7.2 mmol, 1.2 equiv) in acetonitrile (12 mL) was added 4-dimethylaminopyridine (6 mg, 0.05 mmol). After stirring at 85 °C for 5 h, the reaction mixture was diluted with acetone (20 mL) and the precipitate was filtered and washed with acetone (2 × 20 mL) and ether (2 × 20 mL) and dried over P_2O_5 to give a white solid (2,3-dihydro-2-hydroxyimidazo[1,2-*a*]pyrimidin-1-ium bromide).

(2) A mixture of 84% polyphosphoric acid (3 g) and 2,3-dihydro-2-hydroxyimidazo[1,2-*a*]pyrimidin-1-ium bromide (3 mmol) was heated in 50 mL beaker upon intensive stirring at 150 °C for 15 min. After cooling to the room temperature, the resulting viscous mass was dissolved in 30 mL of water and 1 mL (10 mmol) of 70% $HClO_4$ was added dropwise upon mild stirring. The white precipitate was washed with distilled water (3 × 10 mL) and ether (2 × 10 mL) until a neutral reaction of the pH paper and then dried over P_2O_5 to give salt (imidazo[1,2-*a*]pyrimidin-1-ium perchlorate) as fine white crystals.

(3) Perchlorate salt (1 mmol) was dissolved in acetonitrile (5 mL) and hydrazine hydrate (0.15 mL, 3 mmol of a 64% aqueous solution, 3 equiv) was added, and the mixture was irradiated in the sealed reaction tube for 10 min at a ceiling temperature of 100 °C at 150 W maximum power. After cooling down, hydrazine hydrate was evaporated with toluene (3 × 20 mL). The resulting residue was purified by column chromatography (silica gel; MeOH-DCM 1:9 v/v with 5% of 6 M NH3 in MeOH) to afford 2-aminoimidazole as bright-yellow needles.

The analytical data of the new compounds are listed as Supporting Information.

Substituted 2-Aminopyrimidines. General procedure for the preparation of substituted 2-aminopyrimidines: in a 50 mL microwave vial were successively dissolved in EtOH (20 mL) 2-chloropyrimidine (3.43 g, 30 mmol), primary amine (39 mmol, 1.3 equiv), and triethylamine (6.2 mL, 45 mmol, 1.5 equiv). The reaction tube was sealed and irradiated in the cavity of a microwave reactor at a ceiling temperature of 120 °C at 80 W maximum power for 5 min. After the reaction mixture was cooled with an air flow for 15 min, it was diluted with water (100 mL), extracted with CH_2Cl_2 (2 × 150 mL), and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was subjected to silica gel flash

chromatography (from 0% to 5% MeOH−CH_2Cl_2) to afford substituted 2-aminopyrimidine.

The analytical data of the new compounds are listed as Supporting Information.

Reaction of Imidazo[1,2-*a*]pyrimidin-1-ium Salt with Amino Acid Derivative. 1-Ethyl-2-(4′-nitrophenyl)-imidazo[1,2-*a*]pyrimidin-1-ium perchlorate (185 mg, 0.5 mmol, 1 equiv) was dissolved in methanol (3 mL), and triethylamine was added (210 μL, 1.5 mmol, 3 equiv). Then L-alanine ethyl ester hydrochloride (155 mg, 1 mmol, 2 equiv) was added and the reaction mixture was allowed to stir for 12 h. After reaction completion (as judged by TLC), satd NH_4Cl solution (30 mL) was added and the mixture was extracted by DCM (2 × 50 mL). The combined organic extracts were washed with water (100 mL) and brine (50 mL). After drying over anhydrous $MgSO_4$ and evaporating the solvent, the resulting residue was subjected to flash chromatography using MeOH−DCM mixture (1:9) as an eluent to afford 1-ethyl-5-(4′-nitrophenyl)-2-aminoimidazole as red crystals in 83% yield (97 mg).

Biological Assays. Static Peg Assay for Prevention of *Salmonella* Typhimurium and *Pseudomonas aeruginosa* Biofilm Formation.[11] The device used for biofilm formation is a platform carrying 96 polystyrene pegs (Nunc no. 445497) that fits as a microtiter plate lid with a peg hanging into each microtiter plate well (Nunc no. 269789).[44] Two-fold serial dilutions of the compounds in 100 μL of liquid broth per well were prepared in the microtiter plate (two or three repeats per compound). For the *S.* Typhimurium biofilm experiments at 25 °C, Tryptic Soy Broth diluted 1/20 (TSB 1/20; BD Biosciences) was used, while Colonization factor antigen (CFA)[45] broth was used for the experiments at 37 °C. For the *P. aeruginosa* biofilm experiments at 25 °C, TSB 1/20 was used, while Luria−Bertani medium[46] without salt (LBNS) was used for the experiments at 37 °C. Subsequently, an overnight culture of *S.* Typhimurium ATCC14028 (grown in Luria−Bertani medium[46]) or *P. aeruginosa* (grown in TSB) was diluted 1:100 into the respective liquid broth and 100 μL (~10^6 cells) was added to each well of the microtiter plate, resulting in a total amount of 200 μL of medium per well. The pegged lid was placed on the microtiter plate, and the plate was incubated for 24 h at 25 or 37 °C without shaking. During this incubation period, biofilms were formed on the surface of the pegs. After 24 h, the optical density at 600 nm (OD_{600}) was measured for the planktonic cells in the microtiter plate using a VERSAmax microtiter plate reader (Molecular Devices). This gives a first indication of the effect of the compounds on the planktonic growth. For quantification of biofilm formation, the pegs were washed once in 200 μL of phosphate buffered saline (PBS). The remaining attached bacteria were stained for 30 min with 200 μL of 0.1% (w/v) crystal violet in an 2-propanol/methanol/PBS solution (v/v 1:1:18). Excess stain was rinsed off by placing the pegs in a 96-well plate filled with 200 μL of distilled water per well. After the pegs were air-dried (30 min), the dye bound to the adherent cells was extracted with 30% glacial acetic acid (200 μL). The OD_{570} of each well was measured using a VERSAmax microtiter plate reader (Molecular Devices). The IC_{50} value for each compound was determined from the concentration gradient by using the GraphPad software of Prism.

Bioscreen Assay for Measuring *Salmonella* Typhimurium and *P. aeruginosa* Gowth Inhibition. The Bioscreen device (Oy Growth Curves AB Ltd.) was used for measuring the influence of the chemical compounds on the planktonic growth of *S.* Typhimurium and *P. aeruginosa*. The Bioscreen is a computer-controlled incubator/reader/shaker that uses 10 × 10 well microtiter plates and measures light absorbance of each well at a specified wavelength in function of time. An overnight culture of *S.* Typhimurium ATCC14028 (grown up in LB medium) or *P. aeruginosa* (grown up in TSB) was diluted 1:200 in liquid broth. For the *S.* Typhimurium bioscreen experiments at 25 °C, TSB 1/20 was used, while CFA broth was used for the experiments at 37 °C. For the *P. aeruginosa* experiments at 25 °C, TSB 1/20 was used, while LBNS was used for the experiments at 37 °C. 300 μL of the diluted overnight culture was added to each well of the 10 × 10 well microtiter plate. Subsequently, serial dilutions of the chemical compounds were prepared in DMSO or EtOH. Three μL of each diluted stock solution was added to the wells (containing the 300 μL of bacterial culture) in 3-fold. As a control, 3 μL of the appropriate solvent was also added to the plate in 3- or 4-fold. The microtiter plate was incubated in the Bioscreen device at 25 or 37 °C for at least 24 h, with continuous medium shaking. The absorbance of each well was measured at 600 nm each 15 min. Excel was used to generate the growth curves for the treated wells and the untreated control wells.

The effect of each compound concentration on the planktonic growth was classified into one of the following categories:

(1) The planktonic growth is not or only slightly affected, indicated by the symbol "−".
(2) The planktonic growth is retarded, indicated by the symbol "+".
(3) The planktonic growth is completely or almost completely inhibited, indicated by the symbol "o".

The following criterium was used to decide between the first and the second category: If the absorbance (measured at 600 nm) of the bacterial culture treated with the compound is at least 0.5 (for *Salmonella*) or 0.8 (for *Pseudomonas*) units lower than the absorbance of the untreated culture during 4 consecutive hours, then the effect on the planktonic growth is classified in category 2.

Acknowledgment. This work was supported by the Industrial Research Fund of KU Leuven (KP/06/014), the Research Council of KU Leuven (CoE EF/05/007 SymBioSys), the FWO (Fund for Scientific Research—Flanders (Belgium)) and by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT—Vlaanderen) through scholarships to H.S. J.V. and D.D.V. are grateful for support in the frame of the IAP program Functional Supramolecular Systems. E.D.S. is grateful to the KU Leuven for obtaining a postdoctoral fellowship and S.B. to Erasmus Mundus External Cooperation Window Lot 15 India (EMECW15) for obtaining a scholarship.

Supporting Information Available: Analytical data of *N*1-substituted 2-aminoimidazoles and imidazo[1,2-*a*]pyrimidinium salts and substituted 2-aminopyrimidines. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Costerton, J. W.; Stewart, P. S.; Greenberg, E. P. Bacterial biofilms: a common cause of persistent infections. *Science* **1999**, *284*, 1318–1322.
(2) Stewart, P. S.; Costerton, J. W. Antibiotic resistance of bacteria in biofilms. *Lancet* **2001**, *358*, 135–138.
(3) Donlan, R. M.; Costerton, J. W. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.* **2002**, *15*, 167–193.
(4) Mah, T. F.; Pitts, B.; Pellock, B.; Walker, G. C.; Stewart, P. S.; O'Toole, G. A. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. *Nature* **2003**, *426*, 306–310.
(5) Matz, C.; McDougald, D.; Moreno, A. M.; Yung, P. Y.; Yildiz, F. H.; Kjelleberg, S. Biofilm formation and phenotypic variation enhance predation-driven persistence of *Vibrio cholerae*. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 16819–16824.
(6) Davies, D. Understanding biofilm resistance to antibacterial agents. *Nature Rev. Drug Discovery* **2003**, *2*, 114–122.
(7) Olson, M. E.; Ceri, H.; Morck, D. W.; Buret, A. G.; Read, R. R. Biofilm bacteria: formation and comparative susceptibility to antibiotics. *Can. J. Vet. Res.* **2002**, *66*, 86–92.
(8) Blanke, S. R.; Yang, R. Identification of novel genes essential for bacterial survival during environmental biofouling. *Environ. Inst. Houston* **2002**.
(9) Musk, D. J., Jr.; Hergenrother, P. J. Chemical countermeasures for the control of bacterial biofilms: effective compounds and promising targets. *Curr. Med. Chem.* **2006**, *13*, 2163–2177.

(10) De Nys, R.; Givskov, M.; Kumar, N.; Kjelleberg, S.; Steinberg, P. D. Furanones. *Prog. Mol. Subcell. Biol.* **2006**, 42.
(11) Janssens, J. C.; Steenackers, H.; Robijns, S.; Gellens, E.; Levin, J.; Zhao, H.; Hermans, K.; De Coster, D.; Verhoeven, T. L.; Marchal, K.; Vanderleyden, J.; De Vos, D. E.; De Keersmaecker, S. C. Brominated furanones inhibit biofilm formation by *Salmonella enterica* serovar Typhimurium. *Appl. Environ. Microbiol.* **2008**, *74*, 6639–6648.
(12) Steenackers, H. P.; Levin, J.; Janssens, J. C.; De Weerdt, A.; Balzarini, J.; Vanderleyden, J.; De Vos, D. E.; De Keersmaecker, S. C. Structure-activity relationship of brominated 3-alkyl-5-methylene-2(5H)-furanones and alkylmaleic anhydrides as inhibitors of Salmonella biofilm formation and quorum sensing regulated bioluminescence in Vibrio harveyi. *Bioorg. Med. Chem.* **2010**, *18*, 5224–5233.
(13) Geske, G. D.; Wezeman, R. J.; Siegel, A. P.; Blackwell, H. E. Small molecule inhibitors of bacterial quorum sensing and biofilm formation. *J. Am. Chem. Soc.* **2005**, *127*, 12762–12763.
(14) Huigens, R. W.; Richards, J. J.; Parise, G.; Ballard, T. E.; Zeng, W.; Deora, R.; Melander, C. Inhibition of *Pseudomonas aeruginosa* biofilm formation with Bromoageliferin analogues. *J. Am. Chem. Soc.* **2007**, *129*, 6966–6967.
(15) Richards, J. J.; Ballard, T. E.; Melander, C. Inhibition and dispersion of *Pseudomonas aeruginosa* biofilms with reverse amide 2-aminoimidazole oroidin analogues. *Org. Biomol. Chem.* **2008**, *6*, 1356–1363.
(16) Richards, J. J.; Huigens, R. W.; Ballard, T. E.; Basso, A.; Cavanagh, J.; Melander, C. Inhibition and dispersion of proteobacterial biofilms. *Chem. Commun.* **2008**, 1698–1700.
(17) Huigens, R. W.; Ma, L.; Gambino, C.; Moeller, P. D.; Basso, A.; Cavanagh, J.; Wozniak, D. J.; Melander, C. Control of bacterial biofilms with marine alkaloid derivatives. *Mol. Biosyst.* **2008**, *4*, 614–621.
(18) Richards, J. J.; Ballard, T. E.; Huigens, R. W.; Melander, C. Synthesis and screening of an oroidin library against *Pseudomonas aeruginosa* biofilms. *ChemBioChem* **2008**, *9*, 1267–1279.
(19) Rogers, S. A.; Melander, C. Construction and screening of a 2-aminoimidazole library identifies a small molecule capable of inhibiting and dispersing bacterial biofilms across order, class, and phylum. *Angew. Chem., Int. Ed. Engl.* **2008**, *47*, 5229–5231.
(20) Ballard, T. E.; Richards, J. J.; Wolfe, A. L.; Melander, C. Synthesis and antibiofilm activity of a second-generation reverse-amide oroidin library: a structure−activity relationship study. *Chemistry* **2008**, *14*, 10745–10761.
(21) Sullivan, J. D.; Giles, R. L. 2-Aminoimidazoles from Leucetta sponges: Synthesis and Biology of an Important Pharmacophore. *Curr. Bioact. Compd.* **2009**, *5*, 39–78.
(22) Romling, U.; Sierralta, W. D.; Eriksson, K.; Normark, S. Multicellular and aggregative behaviour of *Salmonella* Typhimurium strains is controlled by mutations in the agfD promoter. *Mol. Microbiol.* **1998**, *28*, 249–264.
(23) Latasa, C.; Roux, A.; Toledo-Arana, A.; Ghigo, J. M.; Gamazo, C.; Penades, J. R.; Lasa, I. BapA, a large secreted protein required for biofilm formation and host colonization of *Salmonella enterica* serovar Enteritidis. *Mol. Microbiol.* **2005**, *58*, 1322–1339.
(24) Prouty, A. M.; Schwesinger, W. H.; Gunn, J. S. Biofilm formation and interaction with the surfaces of gallstones by *Salmonella* spp. *Infect. Immun.* **2002**, *70*, 2640–2649.
(25) Barak, J. D.; Whitehand, L. C.; Charkowski, A. O. Differences in attachment of *Salmonella enterica* serovars and *Escherichia coli* O157:H7 to alfalfa sprouts. *Appl. Environ. Microbiol.* **2002**, *68*, 4758–4763.
(26) Brandl, M. T.; Mandrell, R. E. Fitness of *Salmonella enterica* serovar Thompson in the cilantro phyllosphere. *Appl. Environ. Microbiol.* **2002**, *68*, 3614–3621.
(27) Boddicker, J. D.; Ledeboer, N. A.; Jagnow, J.; Jones, B. D.; Clegg, S. Differential binding to and biofilm formation on, HEp-2 cells by *Salmonella enterica* serovar Typhimurium is dependent upon allelic variation in the fimH gene of the fim gene cluster. *Mol. Microbiol.* **2002**, *45*, 1255–1265.
(28) Parry, C. M.; Threlfall, E. J. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. *Curr. Opin. Infect. Dis.* **2008**, *21*, 531–538.
(29) Govan, J. R.; Deretic, V. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol. Rev.* **1996**, *60*, 539–574.
(30) Wagner, V. E.; Iglewski, B. H. *P. aeruginosa* Biofilms in CF Infection. *Clin. Rev. Allergy Immunol.* **2008**, *35*, 124–134.
(31) Gomez, M. I.; Prince, A. Opportunistic infections in lung disease: *Pseudomonas* infections in cystic fibrosis. *Curr. Opin. Pharmacol.* **2007**, *7*, 244–251.
(32) Ermolat'ev, D. S.; Svidritsky, E. P.; Babaev, E. V.; Van der Eycken, E. Microwave-assisted synthesis of substituted 2-amino-1*H*-imidazoles from imidazo[1,2-*a*]pyrimidines. *Tetrahedron Lett.* **2009**, *50*, 5218–5220.
(33) Cavalleri, B.; Volpe, G.; Arioli, V.; Parenti, F. Synthesis and biological activity of some 2-aminoimidazoles. *Arzneim. Forsch.* **1977**, *27*, 1889–1895.
(34) Bunders, C. A.; Richards, J. J.; Melander, C. Identification of aryl 2-aminoimidazoles as biofilm inhibitors in Gram-negative bacteria. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3797–3800.
(35) Rival, Y.; Grassy, G.; Taudou, A.; Ecalle, R. Antifungal activity in vitro of some imidazo[1,2-*a*]pyrimidine derivatives. *J. Med. Chem.* **1991**, *26*, 13–18.
(36) Rival, Y.; Grassy, G.; Michel, G. Synthesis and Antibacterial Activity of Some Imidazo[1,2-*a*]pyrimidine Derivatives. *Chem. Pharm. Bull.* **1992**, *40*, 1170–1176.
(37) Ermolat'ev, D. S.; Babaev, E. V.; Van der Eycken, E. V. Efficient one-pot, two-step, microwave-assisted procedure for the synthesis of polysubstituted 2-aminoimidazoles. *Org. Lett.* **2006**, *8*, 5781–5784.
(38) Ermolat'ev, D. S.; Van der Eycken, E. V. A divergent synthesis of substituted 2-aminoimidazoles from 2-aminopyrimidines. *J. Org. Chem.* **2008**, *73*, 6691–6697.
(39) Ghannoum, M.; Abu Elteen, K.; el-Rayyes, N. R. Antimicrobial activity of some 2-aminopyrimidines. *Microbios* **1989**, *60*, 23–33.
(40) Fioravanti, R.; Biava, M.; Porretta, G. C.; Landolfi, C.; simonetti, N.; Villa, A.; Conte, E.; Porta-Puglia, A. Research on antibacterial and antifungal agents. XI. Synthesis and antimicrobial activity of *N*-heteroaryl benzylamines and their Schiff bases. *Eur. J. Med. Chem.* **1995**, *30*, 123–132.
(41) Davey, M. E.; O'Toole, G A. Microbial biofilms: from ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 847–867.
(42) Costerton, J. W.; Lewandowski, Z.; Caldwell, D. E.; Korber, D. R.; Lappin-Scott, H. M. Microbial biofilms. *Annu. Rev. Microbiol.* **1995**, *49*, 711–745.
(43) Ermolat'ev, D. S.; Giménez, V. N.; Babaev, E. V.; Van der Eycken, E. Efficient Pd(0)-mediated microwave-assisted arylation of 2-substituted imidazo[1,2-*a*]pyrimidines. *J. Comb. Chem.* **2006**, *8*, 659–663.
(44) De Keersmaecker, S. C.; Varszegi, C.; van Boxel, N.; Habel, L. W.; Metzger, K.; Daniels, R.; Marchal, K.; De Vos, D.; Vanderleyden, J. Chemical synthesis of (*S*)-4,5-dihydroxy-2,3-pentanedione, a bacterial signal molecule precursor, and validation of its activity in *Salmonella* Typhimurium. *J. Biol. Chem.* **2005**, *280*, 19563–19568.
(45) Humphries, A.; Deridder, S.; Baumler, A. J. *Salmonella enterica* serotype Typhimurium fimbrial proteins serve as antigens during infection of mice. *Infect. Immun.* **2005**, *73*, 5329–5338.
(46) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.