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1-(1*H*-Indol-3-yl)ethanamine Derivatives as Potent *Staphylococcus aureus* NorA Efflux Pump Inhibitors

Arnaud Hequet,[a] Olga N. Burchak,[b] Matthieu Jeanty,[b, e] Xavier Guinchard,[b, f] Emmanuelle Le Pihive,[c] Laure Maigre,[c] Pascale Bouhours,[a] Dominique Schneider,[c] Max Maurin,[c, d] Jean-Marc Paris,[a] Jean-Noël Denis,*[b] and Claude Jolivalt*[a]

The synthesis of 37 1-(1*H*-indol-3-yl)ethanamine derivatives, including 12 new compounds, was achieved through a series of simple and efficient chemical modifications. These indole derivatives displayed modest or no intrinsic anti-staphylococcal activity. By contrast, several of the compounds restored, in a concentration-dependent manner, the antibacterial activity of ciprofloxacin against *Staphylococcus aureus* strains that were resistant to fluoroquinolones due to overexpression of the NorA efflux pump. Structure–activity relationships studies revealed that the indolic aldonitrones halogenated at position 5 of the indole core were the most efficient inhibitors of the *S. aureus* NorA efflux pump. Among the compounds, (*Z*)-*N*-benzylidene-2-(*tert*-butoxycarbonylamino)-1-(5-iodo-1*H*-indol-3-yl)ethanamine oxide led to a fourfold decrease of the ciprofloxacin minimum inhibitory concentration against the SA-1199B strain when used at a concentration of 0.5 mg L^{-1}. To the best of our knowledge, this activity is the highest reported to date for an indolic NorA inhibitor. In addition, a new antibacterial compound, *tert*-butyl (2-(3-hydroxyureido)-2-(1*H*-indol-3-yl)ethyl)-carbamate, which is not toxic for human cells, was also found.

Introduction

The emergence and spread of pathogenic bacteria with multidrug resistance (MDR) to established classes of antibiotics is an ever-growing concern worldwide.[1] MDR bacteria are not only responsible for infections in hospital settings but also in the community, owing to their large spread in humans, animals, food processing, and various environments. This corresponds to serious public health concerns and significant economic losses, especially because of the prolonged hospitalization of patients and increases in treatment costs.[2] Among resistant bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen of particular concern because it is frequently involved in nosocomial infections.[3] For example, MRSA strains accounted for 44% of the healthcare-associated infections in 2008 in the European Union, with high associated mortality rates.[2b] MRSA is one of the examples of the consequences of improper use of antibiotics.[3c, 4] Indeed, the link between the large-scale use of fluoroquinolones, an important class of broad-spectrum antibiotics, and the emergence of MRSA and other MDR bacteria has been often reported in the literature.[4, 5]

Acquired resistances to fluoroquinolones may occur through four chromosomally and/or plasmid-mediated mechanisms: 1) alterations of type II topoisomerases (DNA gyrase and topoisomerase IV), the targets of fluoroquinolones, 2) extrusion of the antibiotic by overexpression of efflux pumps, and, less frequently, 3) DNA gyrase protection, and 4) decreased permeability of the bacterial membrane to these drugs.[6]

Overexpression of efflux pumps is frequently associated with MDR. Among the five known transporter families, classified according to their bioenergetic and structural characteristics,[7] two of them are involved in fluoroquinolone resistance in *S. aureus*: the major facilitator superfamily (MFS) and the multidrug and toxic compound extrusion (MATE) family.[6, 7b] Several representatives from the MFS family (such as the NorA, NorB, NorC, MdeA, SdrM, and QacA/B proteins) and one from the MATE family (MepA) have been described as fluoroquinolone exporters.[8] Most of these fluoroquinolone transporters confer an MDR phenotype to *S. aureus*, which restricts therapeutic options and favors dissemination of this pathogen.

Several approaches have been used to overcome antibiotic resistance: 1) a more rational use of antibiotics, 2) decrease in

[a] *Dr. A. Hequet, P. Bouhours, Dr. J.-M. Paris, Prof. Dr. C. Jolivalt*
Laboratoire Charles Friedel (LCF), CNRS UMR 7223, Chimie ParisTech
11 rue Pierre et Marie Curie, 75005 Paris (France)
E-mail: claude.jolivalt@upmc.fr

[b] *Dr. O. N. Burchak, Dr. M. Jeanty, Dr. X. Guinchard, Dr. J.-N. Denis*
Département de Chimie Moléculaire (SeRCO)
ICMG FR-2607, CNRS UMR 5250
Université Joseph Fourier, BP-53, 38041 Grenoble cedex 9 (France)
E-mail: jean-noel.denis@ujf-grenoble.fr

[c] *Dr. E. Le Pihive, Dr. L. Maigre, Prof. D. Schneider, Prof. M. Maurin*
Laboratoire Adaptation et Pathogénie des Micro-organismes
CNRS UMR 5163, Université Joseph Fourier Grenoble 1
Institut Jean Roget, Campus Santé
Domaine de la Merci, BP-170, 38042 Grenoble cedex 9 (France)

[d] *Prof. M. Maurin*
CHU de Grenoble, Université Joseph Fourier
BP-217, 38043 Grenoble cedex 9 (France)

[e] *Dr. M. Jeanty*
NovAliX, Centre de recherche Pharma
Campus de Maigremont, BP-615, 27106 Val-de-Reuil Cedex (France)

[f] *Dr. X. Guinchard*
Institut de Chimie des Substances Naturelles (ICSN)
Centre de Recherche CNRS
Avenue de la Terrasse, 91198 Gif-sur-Yvette (France)

the spread of MDR bacteria through better hygiene and a greater awareness of this public health problem by the general population, healthcare workers, food professionals, and farmers, and 3) the development of novel antibiotics.[9] Chemical modifications of known antibiotics have allowed the development of new compounds (for example, tigecycline).[10] Only two new classes of antibiotics, with original structures and modes of action, have been developed in the last three decades: the oxazolidinones (linezolid) and the cyclic lipopeptides (daptomycin). However, although their use is restricted to specific clinical situations, resistance mechanisms have already been reported for some of them.[9a,10] An alternative strategy to the discovery of new antibiotics consists of combining existing antibiotics with drugs potentiating their activity, such as inhibitors of resistance mechanisms (for example, inhibitors of antibiotic-inactivating or target-modifying enzymes and inhibitors of efflux pumps).[9b] The usefulness of such combinations has already been demonstrated by the previous development of the association of a β-lactam compound and a β-lactamase inhibitor (clavulanic acid, tazobactam, and sulbactam), although resistance mechanisms to β-lactamase inhibitors have now been reported.[9b,11] Clinical trials have evaluated the usefulness of an aerosolized formulation of a combination of ciprofloxacin and MC-601,205, an efflux pump inhibitor, in cystic fibrosis patients with pulmonary infections. Although these clinical trials have been stopped, ongoing efforts are being made[9b,11,12] in the field of efflux pump inhibitors (EPIs), which appear to be a promising way to restore the activity of antibiotics against MDR bacteria and/or to decrease the selection of bacteria harboring an MDR phenotype.[13]

EPIs may act by two different types of mechanisms: 1) direct binding to the transporter, which leads to competitive or noncompetitive inhibition of the efflux of the pump's substrate, or 2) indirect action, through the collapse of the energy-driven efflux pump mechanisms of the bacterial cell membrane or through interference with the regulation system of the protein pump.[11,12,14]

To date, many structurally diverse compounds showing NorA and/or MepA EPI activity have been identified, including natural[15] and synthetic ones.[12,16] Reserpine is a reference and one of the oldest EPI compounds, but it is only effective at concentrations that are toxic for humans.[17] More recently, omeprazole, a series of pyrrolo[1,2-a]quinaxoline derivatives sharing structural analogies with omeprazole,[18] piperine,[16c] aryl and phenyl piperidine derivatives,[16a] taraquinar,[19] the synthetic acridone derivative GG918,[20] and 1,4-benzothiazine derivatives[21] have been shown to display inhibitory activities against *S. aureus* strains overexpressing the NorA efflux pump. Among heterocyclic compounds, the indolic scaffold is found in many compounds with biological activities,[22] including in NorA efflux pump inhibitors, such as the indole alkaloid reserpine, 5-nitro-2-phenyl-1*H*-indole (INF55),[23] and the more recently functionalized INF55.[24]

The structural diversity of the known NorA EPIs could result from the significant structural flexibility of this efflux pump, which allows the accommodation of diverse substrates in its active site. However, to the best of our knowledge, the lack of structural information on the NorA efflux pump makes it difficult to design new inhibitors from structure–activity relationship studies. The search for new EPIs has been mainly performed by chemical library screening or classical medicinal approaches, as illustrated in the case of the evolution from natural flavones to 2-(4-propoxyphenyl)quinoline derivatives[16b] or from 5′-methoxyhydnocarpin-D to the more active flavonolignans.[25] An alternative approach allowed German and coworkers to identify, in 2008,[16a] an ofloxacin-derived EPI, the affinity of which for the efflux pump was related to its quinolone scaffold.

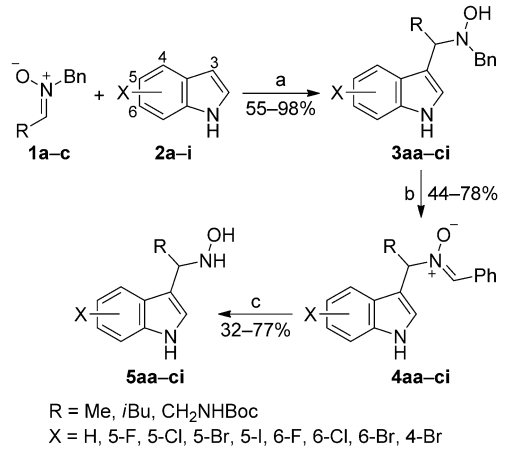
We recently described the evaluation of the antibacterial activity of series of indolic compounds against 29 bacterial strains.[26] From the four indolic sub-libraries tested, the aldonitrones displayed minimal inhibitory concentrations (MICs) greater than 128 mg L^{-1} for all of the tested Gram-negative and Gram-positive bacterial strains, including *S. aureus*. These compounds exhibited no antibacterial activity of their own, which meant that they were suitable candidates for efflux pump inhibition testing. We report herein the higher antibacterial activities of combinations of ciprofloxacin with these previously synthesized indolic derivatives,[26] relative to that of ciprofloxacin alone, against *S. aureus* strains resistant to fluoroquinolones due to overexpression of the NorA efflux pump. To enhance the solubility of the aldonitrones and improve their activity, an extended series of related compounds was designed, synthesized, and tested for biological activity in association with ciprofloxacin. In addition to MIC determination, we evaluated the accumulation of ethidium bromide (a NorA substrate) in *S. aureus* strains to confirm NorA efflux pump inhibition. The toxicity of the most active compounds was measured against three eukaryotic cell lines to evaluate their potential use as therapeutic agents.

Results and Discussion

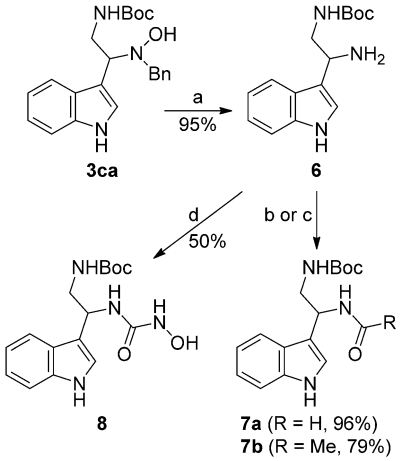
Chemistry

The syntheses of the 19 indolic aldonitrones of series **4** (**4aa**–**ci**) and 4 of the indolic hydroxyamines of series **3** (**3ca** and **3cd**) and **5** (**5ca** and **5cd**) were described in our previous publication.[26] The pathway to this type of heterocyclic derivatives is presented in Scheme 1. Thus, a coupling reaction between the three nitrones **1a**–**c** and the nine commercially available indoles **2a**–**i** in acidic conditions efficiently provided indolic *N*-hydroxyamines **3**. The latter compounds were then oxidized to form aldonitrones **4** with manganese dioxide in toluene at 100 °C. A hydroxyaminolysis of indolic nitrones **4** was performed to yield *N*-hydroxyamines **5** (Scheme 1).

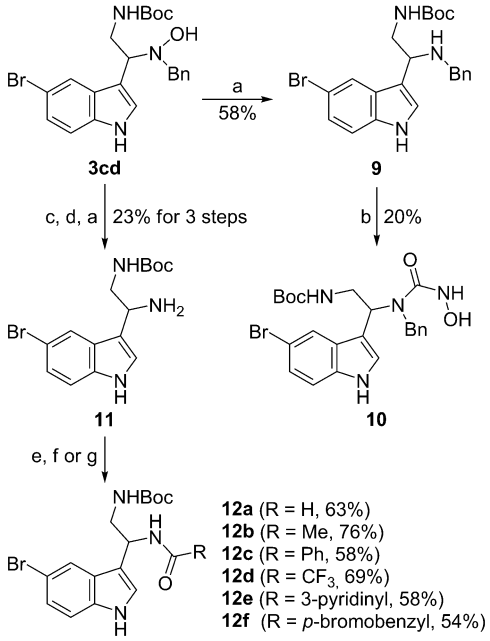
In an attempt to increase the solubility of the indolic compounds while keeping their antibacterial activity in combination with ciprofloxacin, four additional molecules were designed and synthesized by chemical modifications of compound **3ca**, an unhalogenated compound (Scheme 2). Simultaneous reduction and debenzylation of the *N*-benzyl-*N*-hydroxyamine function under mild conditions resulted in primary amine **6** with an excellent yield.[27] An indolic derivative of for-



Scheme 1. Synthesis of 3-substituted indoles. *Reagents and conditions*: a) HCl, MeOH, 0 °C, 2 h; b) MnO_2, toluene, 100 °C, 5 min; c) $NH_2OH{\cdot}HCl$, MeOH, RT, 1 h. Bn: benzyl; Boc: *tert*-butoxycarbonyl.



Scheme 2. Chemical modifications of the indolic derivative **3 ca**. *Reagents and conditions*: a) 40% $Pd(OH)_2$, H_2, AcOH, MeOH, RT, 40 h; b) TFEF, MTBE, DMF, 15 min , 0 °C then RT, 1 h; c) AcCl, Et_3N, CH_2Cl_2, 0 °C, 5 min; d) PhOCONHOH, DMSO, 50 °C, 2 h. TFEF: 2,2,2-trifluoroethylformate; MTBE: methyl *tert*-butyl ether; DMF: *N*,*N*-dimethylformamide; DMSO: dimethyl sulfoxide.



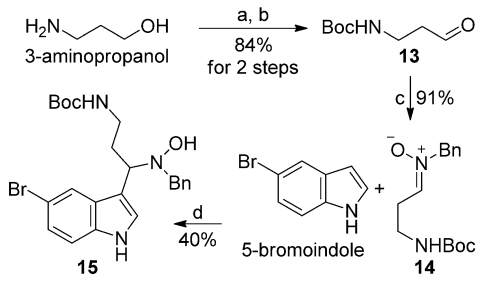
Scheme 3. Chemical modifications of the indolic derivative **3 cd**. *Reagents and conditions*: a) $TiCl_3$, $HCl_{(aq)}$, MeOH, RT, 30 min; b) PhOCONHOH, DMSO, 50 °C, 24 h; c) MnO_2, toluene, 100 °C, 5 min; d) $NH_2OH{\cdot}HCl$, MeOH, RT, 1 h; e) BtCHO, THF, 0 °C, 10 min then RT, 30 min (**12a**); f) RCOCl, Et_3N, CH_2Cl_2, 0 °C, 5 min (**12b**,**c**) or RT, 2 h (**12e**,**f**); g) TFAA, NEt_3, CH_2Cl_2, 15 min (**12d**). BtCHO: *N*-formylbenzotriazole; THF: tetrahydrofuran; TFAA: trifluoroacetic acid.

mamide, **7 a**, was obtained by treatment of amine **6** with trifluoroethyl formate.[28] Acylation of amine **6** with acetyl chloride resulted in acetamide **7 b**. Finally, treatment of amine **6** with phenyl *N*-hydroxycarbamate led to the corresponding *N*-hydroxycarbamide **8** with a modest yield.[29]

Another strategy to enhance the solubility of indoles was to modify the nitrone group while keeping the 5-bromoindole substitution, because these compounds were shown to be more biologically active than nonsubstituted ones.[26] Therefore, several chemical transformations of *N*-hydroxyamine **3 cd** were performed and resulted in nine indolic derivatives, of which eight are new (Scheme 3). Reduction of the *N*-hydroxyamine function of indole **3 cd** by $TiCl_3$ in the presence of HCl led to the secondary amine **9**. In contrast to the case with indolic compound **3 ca**, the use of $Pd(OH)_2$ for the reduction of the *N*-hydroxyamine into compound **11** was not possible because of debromination of the 5-bromoindole moiety under these conditions. Synthesis of primary amine **11** required a three-step sequence: oxidation to the aldonitrone with manganese dioxide, hydroxyaminolysis to the *N*-hydroxyamine, and ultimate reduction with titanium chloride in acidic medium.[30] The treatment of secondary amine **9** with phenyl *N*-hydroxycarbamate[29] led to the corresponding urea derivative **10** with a poor yield. Formylation of the primary amine **11** with *N*-formylbenzotriazole and acylation with the chlorides of several acids or trifluoroacetic anhydride resulted in the six heterocyclic amides **12 a**–**f** with good yields.

Finally, another part of *N*-hydroxyamine **3 cd** was modified, namely the CH_2NHBoc side chain was elongated (Scheme 4). For this purpose, 3-aminopropanol was converted into *N*-Boc-aminoaldehyde **13** by using *N*-Boc protection and oxidation steps. Condensation of aldehyde **13** with *N*-benzylhydroxyamine resulted in aldonitrone **14**. The reaction of the latter compound with 5-bromoindole led to indolic *N*-hydroxyamine **15** with a satisfactory yield.

The synthesis of 37 1-(1*H*-indol-3-yl)ethanamine derivatives was thus achieved through a series of simple and efficient chemical modifications. Amongst these compounds, 12 are new. It should be mentioned that all of the synthesized molecules have a chiral center and they have been tested as a racemic mixture. As a result of this work, several types of indolic compounds, such as primary and secondary amines, *N*-hy-



Scheme 4. Synthesis pathway of an analogue of the indolic derivative **3cd**. *Reagents and conditions*: a) Boc_2O, CH_2Cl_2, RT, 24 h; b) IBX, EtOAc, reflux, 3 h; c) BnNHOH, $MgSO_4$, CH_2Cl_2, RT, 3 h; d) HCl, MeOH, 0 °C, 6 h. IBX: 2-iodoxybenzoic acid.

droxyamines, aldonitrones, formamides, amides, and *N*-hydroxycarbamides, were elaborated.

Biological activity

In a previous work,[26] the indolic compounds from series **3** to **5** were tested for their antimicrobial activity against a panel of Gram-negative and Gram-positive bacterial strains representative of common human pathogens. The MIC values, that is, the minimal concentration allowing complete inhibition of bacterial growth as assessed by visual control and by absorbance measurement at 620 nm, showed that compounds from these sub-libraries were completely inactive when tested at 128 mg L^{-1} against Gram-positive and Gram-negative bacteria. The lack of intrinsic antibacterial activity of these compounds allowed us to test their activity as NorA efflux pump inhibitors.

The MIC results for aldonitrones **4aa–ci** against SA-1199B, a NorA-overexpressing strain, are summarized in Table 1. First, the antibacterial activity of each indolic compound alone was tested at a concentration range starting from the maximal concentration to ensure complete solubilization of the compound in Mueller–Hinton (MH) medium. Indeed, at the 128 mg L^{-1} concentration, all of the tested aldonitrones, except **4aa**, were found to be partly insoluble in MH medium, as assessed by visual control. The unhalogenated aldonitrones, that is, **4aa**, **4ba**, and **4ca**, were the more soluble compounds, with solubility obtained at concentrations of >128, >32, and >32 mg L^{-1}, respectively. For the other compounds in this series, the maximal concentration with complete solubilization was 16 mg L^{-1}. Therefore, the indolic compounds were tested at a concentration range of 16–0.015 mg L^{-1}, as recommended by the Clinical and Laboratory Standards Institute (CLSI) for water-insoluble compounds used in broth dilution susceptibility tests.

None of the aldonitrones showed intrinsic antibacterial activity against SA-1199B at the highest concentrations tested. By contrast, all of the compounds potentiated the antibacterial activity of ciprofloxacin (CIP) added at sub-inhibitory concentration. With the MIC value of ciprofloxacin against SA-1199B being 8 mg L^{-1}, three sub-inhibitory concentrations (MIC/2, MIC/4, and MIC/8) were tested. The less active compounds were those that were unhalogenated on the indole core and/or the aldonitrones with a short aliphatic chain (R: Me or *i*Bu), rather than the CH_2NHBoc side chain. Nitrones **4aa** and **4ba**, which cumulate these two flaws (that is, unsubstituted at the C5 position and with the methyl or isobutyl alkyl chain of the aldonitrone) were the least active, with MIC values of 32 and 16 mg L^{-1}, respectively, in the presence of 4 mg L^{-1} of CIP. The most active compounds were those that were substituted at

Table 1. Minimal inhibitory concentration of indolic derivatives alone or in the presence of a sub-inhibitory ciprofloxacin concentration against *S. aureus* 1199B and K2378 strains.[a]

Indoles	MIC [mg L^{-1}]						
	1199B				K2378		
[cipro.]:[b]	0	1	2	4	0	0.25	0.5
3ca	>32	32	16	8	–	–	–
3cd	16	8	4	2	16	–	4
4aa	>128	>128	64	32	–	–	–
4ab	>16	>16	16	4	–	–	–
4ac	>16	>16	8	4–2	–	–	–
4ad	>16	>16	8	4–2	–	–	–
4ae	>16	>16	16	4	–	–	–
4ba	>32	>32	>32	16	–	–	–
4bb	>16	>16	>16	8	–	–	–
4bc	>16	>16	8	4	–	–	–
4bd	>16	>16	4	2	–	–	–
4be	>16	>16	4	1	–	–	–
4ca	>32	>32	32	8	–	–	–
4cb	>16	>16	8	2	–	–	–
4cc	>16	8	2	0.25	>16	16	4
4cd	>16	4	2	2	>16	8	2
4ce	>16	2	0.5	0.5	>16	8	2
4cf	>16	>16	16	16	–	–	–
4cg	>16	16	8	8	–	–	–
4ch	>16	16	4	4	>16	8	2
4ci	>16	>16	16	4	–	–	–
5ca	>128	64	64	16	–	–	–
5cd	>32	16	8	2	32	–	8
6	>128	>128	>128	64	–	–	–
7a	>128	>128	>128	64	–	–	–
7b	>128	>128	>128	64	–	–	–
8	>128	32	8	0.5	>128	–	>128
9	8	8	8	1	8	–	8
10	>16	16	8	4	–	–	–
11	64	32	16	16	–	–	–
12a	>128	64	32	8	–	–	–
12b	>128	64	32	8	–	–	–
12c	>16	8	4	1	>16	–	2
12d	≥32	32	8	2	–	–	–
12e	>32	32	16	4	>32	–	8
12f	8	4	2	1	–	–	–
15	>16	16	8	2	–	–	–
reserpine	>32	–	4	–	>32	–	8

[a] At least two independent experiments were performed for each MIC measurement. Due to the experimental procedure, MIC values are reported to within a factor of 2. [b] Ciprofloxacin concentrations are given in mg L^{-1}.

the C5 position of the indole core with a halogen atom, namely **4bd**, **4be**, and **4cb–e**, with maximum MIC values of 2 mg L^{-1} in the presence of 4 mg L^{-1} of CIP. Halogenated compounds with a CH_2NHBoc side chain showed MIC values of 4 and 0.5 mg L^{-1} for **4ci** and **4ce**, respectively. The indolic derivatives of series **4ba–e** and **4ca–e** varied only with respect to the substituents at the indole C5 position, which allowed direct conclusions to be drawn regarding the importance of the 5-halogen group. The presence of a nitro group in that position had already been shown to lead to more active 2-phenyl-1*H*-indole derivatives, relative to the unsubstituted ones.[24a] In the present work, we confirm the importance of the presence of an electron-withdrawing group in the C5 position of the indole core.

The MIC values of the indolic compounds were dependent on the ciprofloxacin concentration used: for a given compound, the MIC values were higher when the CIP concentration was decreased from 4 mg L^{-1} to 1 mg L^{-1}. A similar ranking between the tested compounds was observed at CIP concentrations lower than 4 mg L^{-1}. However, in the presence of a CIP concentration that was an eighth of the MIC value (that is 1 mg L^{-1}), only three compounds, **4cc–e**, showed an MIC value lower than 16 mg L^{-1}. The lowest MIC value in this case, 2 mg L^{-1}, was observed for **4ce**. The 5-iodine derivative was thus able to significantly potentiate CIP antibacterial activity at a rather low concentration.

It was checked that the indolic derivatives did not induce any potentiation of the activity of CIP at the MIC/4 concentration against SA-1199 (data not shown), the parental strain of SA-1199B that is susceptible to CIP (MIC = 0.25 mg L^{-1}), to exclude any synergistic effect in association with ciprofloxacin, whereas positive results were obtained against the NorA-overexpressing strain SA-1199B, which is consistent with the hypothesis of NorA efflux pump inhibition as the mode of action. However, the SA-1199B strain is resistant to fluoroquinolones because of overexpression of NorA and also because of mutations in the DNA topoisomerase gene *grlA*.[23] Thus, to address the question of specific NorA efflux pump inhibition, four compounds (**4cc–e** and **4ch**) that were active against SA-1199B were further tested against K2378, an *S. aureus* strain that overexpresses NorA from a multicopy plasmid but has no mutations on DNA gyrase encoding genes.[21] We first checked that the MIC value of these compounds alone was >16 mg L^{-1} against K2378. The MIC values (Table 1) were then measured in the presence of MIC/4 and MIC/8 CIP concentrations, that is, 0.5 mg L^{-1} and 0.25 mg L^{-1}, respectively. All four compounds displayed an MIC value of 2–4 mg L^{-1} against the K2378 strain, which corroborated their potential activity as NorA efflux pump inhibitors.

To confirm the EPI properties of the most active indolic compounds among series **3** to **5**, we evaluated their ability to interfere with ethidium bromide (EtBr) efflux from SA-1199B. This test necessitates using EPIs at concentrations at which they have no antibacterial activity on their own, to exclude the possibility that any observed EtBr fluorescence increase could be related to cellular death and the subsequent collapse of the NorA pump proton driven activity rather than to active ethidium bromide efflux inhibition. For reserpine, a well-known NorA inhibitor, the MIC value of which is >32 mg L^{-1} against SA-1199B (Table 1), a concentration of 20 mg L^{-1} is considered relevant and is widely used in EtBr accumulation tests reported in the literature.[31] However, because of their low solubility, the aldonitrone compounds **4cc–e** and **4ch** could not be accurately prepared at a concentration higher than 16 mg L^{-1}. Therefore, it was decided to assay EtBr fluorescence in the presence of 5 mg L^{-1} of the tested compounds, that is, at a concentration lower than their maximum solubility and MIC value.

The effects of the indolic compounds on the EtBr efflux capability of SA-1199B, with reserpine as a reference, are shown on Figure 1 and in Table 2. All four of the tested aldonitrones allowed a significant EtBr fluorescence increase relative to the negative control. The efflux inhibition percentage calculated from the EtBr fluorescence increase (see the Experimental Section for calculation details) showed that the EtBr accumulation in the presence of the nitrones was two- to fivefold higher than that of reserpine at the same concentration (Table 2)

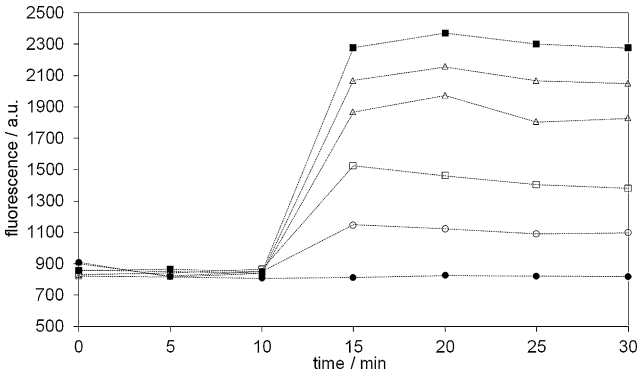


Figure 1. Effect of compounds **4cc** (△), **4cd** (▲), **4ce** (■), and **4ch** (□) on EtBr accumulation of SA-1199B. The tested compound was added after 10 min of bacterial incubation with EtBr. The positive control was reserpine (○) at 5 mg L^{-1} and the negative control (●) was the bacteria in the presence of 1.6% DMSO.

Table 2. Ethidium bromide fluorescence increase in the presence of indolic derivatives.[a]

Indoles	Fluorescence increase [%]			
	1199B		K2378	
	5 mg L^{-1}	20 mg L^{-1}	5 mg L^{-1}	20 mg L^{-1}
reserpine	31 ± 2	78 ± 6	45 ± 1	76 ± 8
4cc	116 ± 5	166	–	–
4cd	146 ± 6	163	68 ± 10	–
4ce	173 ± 5	180	56 ± 8	–
4ch	71 ± 2	153	71 ± 9	–
5cd	29 ± 1	–	27 ± 9	–
8	–	62 ± 1	–	7 ± 1
12c	156 ± 5	–	44 ± 19	–
15	98 ± 15	–	–	–

[a] Data represent the mean ± standard deviation of two or three independent experiments performed at least in duplicate.

when SA-1199B was used. The compound that was brominated at position 6 on the indole core, **4ch**, was twofold less efficient than the analogue substituted at position 5, **4cd**, which correlated well with the antibacterial effects of these compounds. Among the 5-substituted compounds, the iodine one, **4ce**, was the most active EPI, with a fivefold increase of EtBr accumulation relative to that with reserpine. However, both the chlorine and the bromine derivatives (**4cc** and **4cd**, respectively) also show significant effects on the EtBr accumulation at 5 mg L^{-1}. The chlorine compound **4cc** was almost fourfold more potent than reserpine at 5 mg L^{-1}. In the presence of *S. aureus* K2378, a resistant strain built to overexpress NorA, the ethidium bromide accumulation levels obtained for both reserpine and compound **4ch** were similar to those obtained against *S. aureus* 1199B, which corroborates the hypothesis of potential inhibition of the NorA pump as a mode of action for these compounds. However, the EtBr accumulation remained significant but lower for **4cc–e** relative to the results obtained with *S. aureus* 1199B, which would suggest that these products are not really NorA inhibitor specific.

In an attempt to increase the low solubility of the indolic compounds in series **3** to **5**, which prevented an accurate determination of their MIC values, four additional unhalogenated derivatives, **6** to **8**, were synthesized. It was previously observed in the present work that the replacement of the nitrone group in both **4ca** and **4cd** by a hydroxyamine (**3ca** and **3cd**, respectively) was not detrimental to the MIC value. Consequently, it was decided to replace the nitrone with an amine, **6**, and to an amide in a further step by formylation or acylaton of amine **6**. As expected, compounds **6** to **8** were all fully soluble at 128 mg L^{-1} and were inactive alone against both the ATCC 25923 (data not shown) and 1199B *S. aureus* strains (MIC > 128 mg L^{-1}), which allowed unambiguous interpretation of the results obtained in association with ciprofloxacin. However, the primary amine **6** and the two amides **7a** and **7b** were not active, with MIC > 128 mg L^{-1} in the presence of CIP at MIC/4 (Table 1).

The results obtained with compound **8** appeared to deviate from those of the same sub-library. Indeed, although the potency of derivative **8** was approximately the same as that of the parent compound **3ca** in the presence of 2 mg L^{-1} of CIP, the MIC value decreased to 0.5 mg L^{-1} when the ciprofloxacin concentration was increased twofold. Under the same conditions, the MIC value of **3ca** was 16-fold higher. However, **8** was completely inactive against the NorA-overexpressing SA-K2378, which strongly suggests that its mode of action is not related to the inhibition of the NorA efflux pump. As **8** significantly enhanced ethidium bromide accumulation in SA-1199B, a possible mode of action could be the inhibition of *S. aureus* efflux pumps other than NorA.

Due to the disappointing results we obtained with the unhalogenated compounds, it was decided to further enlarge the 5-bromoindole sub-library from the common **3cd–5cd** scaffold (5-Br substitution and carbamate side chain). Indeed, compounds **3cd–5cd** had been found to be active as antibacterial agents and/or as efflux inhibitors (Table 1). Substituent modifications at the side-chain nitrogen atom led to new derivatives **9–12** and **15**. The latter compound was similar to **3cd**, except for the length of the nitrogen side chain, with **15** including an additional methylene group. However, this structural modification induced no change in the biological results of **15** relative to those of parent compound **3cd**.

The nitrone group in **4cd** or *N*-hydroxyamine in **3cd** was replaced by a secondary amine in **9**. As already shown with compound **3cd**, compound **9** showed a significant antibacterial activity when tested alone against the fluoroquinolone-susceptible *S. aureus* ATCC 25923 strain (data not shown) and the fluoroquinolone-resistant SA-1199B strain (Table 1), with MIC values of 8 and 16 mg L^{-1} for **9** and **3cd**, respectively. The high toxicity of compound **9** observed against three cell lines is likely to be related to its antimicrobial activity (Table 3). When combined with ciprofloxacin at 4 mg L^{-1} against SA-1199B, the MIC values of compounds **3cd** and **9** decreased eightfold to 2 and 1 mg L^{-1}, respectively, which corresponded to a significant synergistic effect. Similar results were obtained against K2378 for **3cd**, whereas the MIC value of compound **9** against K2378 remained unchanged in the presence or absence of CIP (Table 1). However, because of the intrinsic activity of these compounds, no conclusion can be drawn about their potent EPI activity.

Table 3. Cell growth inhibition of normal (human lung fibroblast: MCR5) and cancer (human mouth carcinoma: KB; human colon tumor: HCT116) cell lines.[a]

Indole	Growth inhibition [%] at 10^{-5} M/10^{-6} M		
	KB	MRC5	HCT116
4cb	0	0	18
4cc	0	46	47
4cd	57/5	nd	75/14
4ce	56	26	39
4cf	0	8	0
4cg	0	0	0
4ch	42	30	21
4ci	88/0	92/0	87/5
8	0/0	0/0	0/1
9	100/0	100/0	100/0
10	33/5	5/8	25/11
12c	47/0	13/0	35/0
12d	41/0	10/0	0/0
12e	16/0	0/0	0/0
12f	84/0	60/0	88/0
15	4/0	16/0	42/11

[a] At least three independent experiments were performed for each measurement: data represent the average. nd: not determined.

Among the 5-Br derivatives **12**, the amides with the shortest substituents, namely H and Me groups, were fully soluble at 128 mg L^{-1}. The objective of increasing the solubility of the halogenated compounds was thus reached. Moreover, both indolic formamide **12a** and amide **12b** showed a high MIC value (> 128 mg L^{-1}), which allowed unambiguous interpretation of the results obtained in association with ciprofloxacin. Compounds **12c–f** were less soluble because of the aromatic or trifluoroacetyl substituent on the amide. Consequently, it can only be stated that the MIC values of **12c–e** were higher

than 16–32 mg L^{-1} when used alone against SA-1199B, without further precision. *p*-Bromobenzamide **12f**, the most active compound in the **12** series, showed significant antibacterial activity when used alone, which precluded any conclusion about its potential EPI activity. As with compound **9**, this antimicrobial activity was correlated to a high cellular toxicity in the same concentration range (Table 3). Two derivatives, **12d** and **12e**, were significantly active in the presence of ciprofloxacin. Compound **12e**, substituted with a nicotinic acid, displayed an intermediate MIC value of 4 mg L^{-1} in the presence of 4 mg L^{-1} of ciprofloxacin. Trifluoroacetamide **12d** was found to have an MIC value of only 2 mg L^{-1} at the same CIP concentration. Benzamide **12c** was one of the most active in the **12** series, with an MIC value as low as 1 mg L^{-1} in the presence of 4 mg L^{-1} of CIP. In the presence of 1 mg L^{-1} of CIP, **12c** exhibited an MIC value of 8 mg L^{-1}, which was one of the lowest observed in this work under the same conditions. Compound **12c** was also active against SA-K2378 in the presence of ciprofloxacin, with results similar to those obtained with the nitrone compound **4cd**, which showed that a charged group is not a requirement for NorA inhibitors. Indeed, it has been hypothesized that, although the three-dimensional structure of the NorA protein can only be predicted based on models,[32] inhibitors bind to the protein through both hydrophobic and electrostatic interactions, inside a large hydrophobic binding site on the protein. Aldonitrone derivatives **4aa–ci** are charged and present large hydrophobic areas and thus exhibit all of the known requisites to provide favorable interactions with NorA. However, when the nitrone group (in series **4**) was substituted by various polar ones, namely primary and secondary amines, *N*-hydroxyamines, or amides, the EPI activity was retained.

Three compounds, **5cd**, **12c**, and **15**, within the 5-Br series and among those with the highest MIC values in the absence of CIP and the lowest MIC values in the presence of CIP were tested for their ability to allow EtBr accumulation in *S. aureus* strains (Table 2). All three compounds induced an efflux inhibition of EtBr when tested with SA-1199B, with the most efficient one being benzamide **12c**, which increased EtBr fluorescence fivefold relative to reserpine.

To assess the potential of the indolic compounds as therapeutic antibacterial drugs, their toxicity against several eukaryotic lines was evaluated (Table 3). All of the compounds were tested at the same concentrations, expressed in molar. As their molecular weight varied from 334 g mol^{-1} (for **8**) to 551 g mol^{-1} (for **12f**), the corresponding mass concentrations varied from 3–5.5 mg L^{-1}, respectively, according to the units usually used to express MIC values. Among the nitrone derivatives, the fluorine and chlorine substituents at position 6 (**4cf** and **4cg**, respectively) showed no toxicity at 10^{-5} M, namely ~4 mg L^{-1}. It should be noticed that the MIC value of chlorine compound **4cg** was 8 mg L^{-1} in the presence of 2 mg L^{-1} of CIP, that is, the compound could be used to have a therapeutic effect against bacteria without being toxic toward mammalian cells.

The iodine and bromine derivatives were slightly toxic at 10^{-5} M as a result of the weaker carbon/halogen bond strength, relative to that of the fluorine and chlorine substituents, leading to the formation of radicals inducing cellular toxicity. Among the 5-bromo derivatives, **12c** and **4cd**, between which the only structural difference is the replacement of the nitrone function in **4cd** by an amide in **12c**, showed similar toxicity against eukaryotic cells.

Conclusions

The present work shows the potent NorA efflux pump inhibition of a new family of indole compounds. The most efficient were the indolic aldonitrones **4cc–ce**, halogenated at position 5 of the indole core. Both chlorine and bromine derivatives showed MIC values as low as 2 mg L^{-1} in combination with sub-inhibitory concentrations of CIP (MIC/4); these values are similar to that observed for the best indolic structure known to date, that is, a 5-nitro-2-phenylindole called INF55,[24a] which is capable of producing a fourfold increase in *S. aureus* susceptibility to ciprofloxacin when co-administered with the antibiotic at a concentration of 1.5 mg L^{-1}. Moreover, the efficiency of this lead was even increased with the 5-iodine aldonitrone **4ce**, which showed a lower MIC value of 0.5 mg L^{-1}. In addition, it was shown that the EPI activity of these derivatives was superior to that shown for the reference compound reserpine against both SA-1199B and K2378. These results show a significant potentiating effect of the indolic compounds. However, from the standpoint of the potential use of these compounds in clinical situations, the iodine substituent is rather labile and its release has been reported to be responsible for the toxicity of iodine-containing compounds. Therefore, additional compounds should be synthesized with a chlorine substituent, which is known to be less labile than iodine.

Replacement of the charged nitrone group by an amide one in the 5-Br derivative **12c** led to unchanged MIC values in the presence of CIP at MIC/4, as well as a similar EtBr efflux inhibition that reached fivefold the value observed for the reference compound reserpine, whereas the toxicity was similar to that of the parent nitrone **4cd**.

Within the amide series, *tert*-butyl (2-(5-bromo-1*H*-indol-3-yl)-2-(nicotinamido)ethyl)carbamate **12e** and *tert*-butyl (2-(5-bromo-1*H*-indol-3-yl)-2-(2,2,2-trifluoroacetamido)ethyl)carbamate **12d** showed no to very low toxicity at 10^{-5} M, a concentration similar or even higher than the MIC values in the presence of CIP (MIC/4).

In addition, a new indole hydroxycarbamate, **8**, was identified with significant antibacterial activity against SA-1199B in combination with ciprofloxacin. However, the compound was inactive when tested against the NorA-overexpressing strain K2378 and was shown to be unable to inhibit EtBr efflux, which suggests that this compound was not a NorA efflux pump inhibitor. The mode of action of this compound and the reason for its selective activity remains to be investigated. One cellular target of compound **8** could be an efflux pump other than NorA but involved in fluoroquinolone resistance.

Experimental Section

Chemistry

General: Purchased reagents and solvents were used without purification except those indicated below. Tetrahydrofuran and methyl *tert*-butyl ether were heated at reflux over sodium benzophenone and then distilled. CH_2Cl_2, *N*,*N*-dimethylformamide, MeOH, and Et_3N were dried by heating at reflux over CaH_2 and then distilled. Acetyl chloride and benzoyl chloride were distilled over $CaCl_2$. Reactions were monitored by thin layer chromatography with commercial aluminum-backed silica gel plates (Merck G 60 F_{254}). TLC spots were viewed under ultraviolet light and by heating the plate after treatment with either a 0.5% solution of ninhydrin in 3% ethanolic acetic acid or a 2% solution of potassium permanganate in 7% aqueous Na_2CO_3. *N*-Hydroxyamines were detected with a 1% triphenyltetrazolium chloride in EtOH (red color). Product purification by gravity column chromatography was performed by using Macherey–Nagel silica gel 60 (70–230 mesh). Infrared spectra were recorded on a Nicolet Impact-400 Fourier transform infrared spectrometer or on a Nicolet Magna-550 Fourier transform infrared spectrometer equipped with an attenuated total reflection (ATR) device and the data are reported in reciprocal centimeters (cm^{-1}). ^1H NMR and ^{13}C NMR spectroscopy was performed on a Bruker Advance300 spectrometer. Chemical shifts for ^1H spectra are values downfield from the internal solvent signal (CD_3OD: $\delta = 3.31$ ppm; [D_6]DMSO: $\delta = 2.5$ ppm) or from tetramethylsilane in $CDCl_3$ ($\delta = 0.00$ ppm) and are reported as follows: chemical shifts (δ) in parts per million (ppm), multiplicity, coupling constants (J) in Hertz (Hz), and integration. Low-resolution mass spectra were recorded on a ThermoFinnigan PolarisQ ion-trap spectrometer by using direct chemical ionization (DCI; ammonia/isobutane, 63:37) or on a Bruker Esquire 3000 plus (ESI). High-resolution mass spectra were recorded on a Bruker maXis mass spectrometer by the "Fédération de Recherche" ICOA/CBM (FR2708) platform, Orleans, France. Elemental analyses were performed at the "Service d'Analyse Elémentaire" of the Département de Chimie Moléculaire, Université Joseph Fourier (Grenoble, France).

The preparation of indolic compounds **3ca**, **3cd**, **4aa**–**4ci**, **5ca**, and **5cd** was previously described.[26]

***tert*-Butyl (2-amino-2-(1*H*-indol-3-yl)ethyl)carbamate (6)**:[30] The Pearlman catalyst ($Pd(OH)_2$, 152 mg, 40% w/w) was added to a stirred solution of indolic *N*-hydroxyamine **3ca** (381 mg, 1.0 mmol) in a mixture of MeOH (20 mL) and acetic acid (0.7 mL). The argon atmosphere was replaced by hydrogen. The resulting mixture was stirred at room temperature for 40 h. It was then filtered through Celite. The resulting filtrate was treated with an aqueous solution of NaOH (6 N) until pH 12 was reached. The MeOH was then evaporated in vacuo. The resulting aqueous mixture was extracted with EtOAc (3×20 mL). The combined organic layers were washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Pure compound **6** was obtained as a white solid (260 mg, 95%): mp 145–146 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.44$ (s, 9H), 1.61 (br s, 2H), 3.33–3.43 (m, 1H), 3.53–3.61 (m, 1H), 4.40 (dd, $J_1 = 7.2$, $J_2 = 5.6$ Hz, 1H), 4.90 (br s, 1H), 7.09–7.23 (m, 3H), 7.36 (dd, $J_1 = 8.0$, $J_2 = 0.8$ Hz, 1H), 7.70 (d, $J = 7.7$ Hz, 1H), 8.33 ppm (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 28.4$, 47.5, 48.7, 79.2, 111.3, 117.8, 119.3, 119.6, 121.0, 122.3, 126.0, 136.6, 156.7 ppm; IR (neat): $\tilde{\nu} = 3404$, 3260, 2970, 2920, 1690, 1575, 1440, 1365, 1295, 1250, 1155, 980, 740 cm^{-1}; MS (ESI^+): *m/z* (%): 298 (19) $[M+Na]^+$, 276 (10) $[M+H]^+$, 203 (100); elemental analysis: calcd for $C_{15}H_{21}N_3O_2$: C 65.43, H 7.69, N 15.26; found: C 65.22, H 7.69, N 15.19.

***tert*-Butyl (2-formamido-2-(1*H*-indol-3-yl)ethyl)carbamate (7a)**: TFEF (34 μL, 0.35 mmol) was added to a solution of indolic amine **6** (88 mg, 0.32 mmol) in a mixture of dry MTBE (15 mL) and DMF (2 mL) at 0 °C. The resulting mixture was stirred for 15 min at 0 °C and then it was allowed to reach room temperature. After 1 h, MTBE (30 mL) was added to the mixture. The organic solution was then washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Pure compound **7a** was obtained as a white solid (93 mg, 96%): ^1H NMR (300 MHz, CD_3OD): $\delta = 1.42$ (s, 9H), 3.20–3.31 (m, 1H), 3.37–3.59 (m, 1H), 5.48 (dd, $J_1 = 8.4$, $J_2 = 5.5$ Hz, 1H), 6.58 (t, $J = 6.5$ Hz, 1H), 7.00–7.14 (m, 2H), 7.23 (s, 1H), 7.35 (d, $J = 8.1$ Hz, 1H), 7.63 (d, $J = 7.9$ Hz, 1H), 8.13 ppm (d, $J = 0.8$ Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 28.8$, 45.6, 46.6, 79.8, 112.5, 119.7, 120.1, 120.3, 122.8, 123.0, 123.0, 127.4, 162.4, 163.4 ppm; IR (neat): $\tilde{\nu} = 3415$, 3305, 3055, 2980, 2930, 2860, 1690, 1655, 1540, 1515, 1435, 1375, 1280, 1155, 735 cm^{-1}; MS (ESI^+): *m/z* (%): 326 (100) $[M+Na]^+$; HRMS (ESI^+): *m/z* calcd for $C_{16}H_{21}N_3NaO_3$ $[M+Na]^+$: 326.1475; found: 326.1475; *m/z* calcd for $C_{16}H_{22}N_3O_3$ $[M+H]^+$: 304.1656; found: 304.1656.

***tert*-Butyl (2-acetamido-2-(1*H*-indol-3-yl)ethyl)carbamate (7b)**: Acetyl chloride (23 μL, 0.6 mmol) was added to a solution of indolic amine **6** (138 mg, 0.5 mmol) and Et_3N (101 mg, 1.0 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C. The resulting mixture was stirred for 5 min at 0 °C and then the CH_2Cl_2 was evaporated. Diethyl ether (50 mL) was added to the mixture, and the resulting organic solution was washed with an aqueous solution of HCl (1 M), a saturated aqueous solution of $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography with EtOAc/pentane (10:90–99:1) yielded pure compound **7b** as a white solid (125 mg, 79%): ^1H NMR (300 MHz, CD_3OD): $\delta = 1.42$ (s, 9H), 1.97 (s, 3H), 3.48 (dd, $J_1 = 13.6$, $J_2 = 8.5$ Hz, 1H), 3.59 (dd, $J_1 = 13.6$, $J_2 = 5.5$ Hz, 1H), 5.40 (dd, $J_1 = 8.5$, $J_2 = 5.5$ Hz, 1H), 6.99–7.13 (m, 2H), 7.22 (s, 1H), 7.34 (dt, $J_1 = 8.0$, $J_2 = 1.0$ Hz, 1H), 7.62 ppm (dt, $J_1 = 7.5$, $J_2 = 1.2$ Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 22.9$, 28.8, 45.6, 48.0, 80.2, 112.4, 114.9, 119.8, 120.1, 122.7, 123.1, 127.6, 138.2, 158.8, 172.9 ppm; IR (neat): $\tilde{\nu} = 3310$, 3215, 2970, 2930, 1690, 1630, 1525, 1435, 1285, 1225, 1170, 1150, 740 cm^{-1}; MS (ESI^+): *m/z* (%): 340 (100) $[M+Na]^+$; HRMS (ESI^+): *m/z* calcd for $C_{17}H_{23}N_3NaO_3$ $[M+Na]^+$: 340.1632; found: 340.1631; *m/z* calcd for $C_{17}H_{24}N_3O_3$ $[M+H]^+$: 318.1812; found: 318.1811.

***tert*-Butyl (2-(3-hydroxyureido)-2-(1*H*-indol-3-yl)ethyl)carbamate (8)**: A flask was charged with the amino compound **6** (100 mg, 0.36 mmol), phenyl *N*-hydroxycarbamate (56 mg, 0.36 mmol), and DMSO (1 mL). The mixture was stirred for 2 h at 50 °C. The crude solution was diluted in EtOAc (25 mL) and washed with brine and water. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. After flash chromatography (EtOAc/pentane, 7:3 then neat EtOAc), compound **8** was obtained as a white solid (60 mg, 50%): mp 188 °C; ^1H NMR (300 MHz, [D_6]DMSO): $\delta = 1.37$ (s, 9H), 3.32–3.48 (m, 2H), 5.12–5.20 (m, 1H), 6.68 (d, $J = 8.7$ Hz, 1H), 6.80 (t, $J = 5.4$ Hz, 1H), 6.97 (t, $J = 7.4$ Hz, 1H), 7.07 (t, $J = 7.4$ Hz, 1H), 7.23 (d, $J = 2.1$ Hz, 1H), 7.34 (d, $J = 7.4$ Hz, 1H), 7.61 (d, $J = 7.4$ Hz, 1H), 8.33 (br s, 1H), 8.52 (br s, 1H), 10.89 ppm (br s, 1H); ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta = 29.2$, 45.5, 46.9, 78.6, 112.3, 116.0, 119.3, 119.8, 121.9, 123.3, 127.1, 137.2, 156.7, 161.8 ppm; IR (neat): $\tilde{\nu} = 3415$, 3390, 3355, 3195, 2975, 1685, 1620, 1560, 1520, 1170 cm^{-1}; MS (ESI^+): *m/z* (%): 357 (100) $[M+Na]^+$; HRMS (ESI^+): *m/z* calcd for $C_{16}H_{23}N_4O_4$ $[M+H]^+$: 335.1714; found: 335.1713.

***tert*-Butyl (2-(benzylamino)-2-(5-bromo-1*H*-indol-3-yl)ethyl)carbamate (9)**: A 15% aqueous solution of titanium trichloride (9.1 mL, 8.6 mmol) was added to a stirred solution of indolic *N*-hydroxylamine **3cd** (1.83 g, 3.96 mmol) in MeOH (40 mL). The result-

ing mixture was stirred at room temperature for 30 min. A large excess of an aqueous solution of NaOH 20% saturated with NaCl was added. MeOH was then removed in vacuo and the crude mixture was extracted with EtOAc (3×20 mL). The combined organic layers were washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. After flash chromatography (EtOAc/pentane, 4:6 then 7:3), pure compound **9** was obtained as a white solid (1.20 g, 58%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=1.34 (s, 9H), 2.41 (br s, 1H), 3.23 (t, J=6.0 Hz, 2H), 3.59 (dd, AB system, J=13.5, $\delta_A-\delta_B$=42.0 Hz, 2H), 3.99 (t, J=6.0 Hz, 1H), 6.81 (br s, 1H), 7.16 (dd, J=8.7, J=1.8 Hz, 1H), 7.20–7.33 (m, 7H), 7.83 (d, J=1.8 Hz, 1H), 11.10 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=28.1, 46.0, 50.4, 54.4, 77.4, 110.8, 113.3, 115.1, 121.4, 123.2, 124.5, 126.3, 127.7, 127.9, 128.3, 135.2, 141.0, 155.6 ppm; IR (neat): $\tilde{\nu}$=3421, 3292, 3027, 1683, 1506, 1453, 1391, 1365, 1248, 1163, 1100, 884 cm^{-1}; MS (DCI; ammonia/isobutane, 63:37): m/z (%): 444 (100) and 446 (97) $[M+H]^+$; HRMS (ESI$^+$): m/z calcd for $C_{22}H_{27}BrN_3O_2$ $[M+H]^+$: 444.1281 and 446.1263; found: 444.1281 and 446.1264; elemental analysis: calcd for $C_{22}H_{26}BrN_3O_2$: C 59.46, H 5.86, N 9.46; found: C 59.25, H 5.92, N 9.33.

***tert*-Butyl (2-(1-benzyl-3-hydroxyureido)-2-(5-bromo-1*H*-indol-3-yl)ethyl)carbamate (10)**: Compound **10** was synthesized by the same procedure as compound **8** with reaction time and temperature of 24 h at 50 °C. From indolic amine **9** (100 mg, 0.22 mmol) and phenyl *N*-hydroxycarbamate (41 mg, 0.27 mmol), pure compound **10** was obtained as a white solid (22 mg, 20%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.44 (s, 9H), 3.45–3.68 (m, 2H), 4.21 (dd, AB system, J=16.5, $\delta_A-\delta_B$=128.3 Hz, 2H), 4.98 (br s, 1H), 5.71 (t, J=7.2 Hz, 1H), 6.81 (br s, 1H), 6.93 (d, J=5.7 Hz, 2H), 7.10–7.30 (m, 6H), 7.40 (br s, 1H), 7.66 (d, J=2.4 Hz, 1H), 8.66 ppm (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=28.5, 42.8, 46.4, 51.2, 80.4, 111.6, 113.0, 113.8, 121.8, 124.3, 126.1, 127.0, 127.8, 128.7, 129.0, 135.0, 137.3, 156.7, 162.5 ppm; MS (ESI$^+$): m/z (%): 525 (100) and 527 (97) $[M+Na]^+$.

***tert*-Butyl (2-amino-2-(5-bromo-1*H*-indol-3-yl)ethyl)carbamate (11)**: Compound **11** was synthesized in a three-step sequence from indolic *N*-hydroxyamine **3cd** according to the procedure previously described.[27] Pure compound **11** was obtained as a white solid (overall yield: 23%): mp 151 °C; ^{1}H NMR (300 MHz, CD_3OD): δ=1.41 (s, 9H), 3.24–3.43 (m, 2H), 4.27 (dd, J_1=7.2, J_2=6.0 Hz, 1H), 7.18 (dd, J_1=8.4, J_2=1.8 Hz, 1H), 7.25 (s, 1H), 7.26 (d, J=8.4 Hz, 1H), 7.81 ppm (d, J=1.8 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ=28.8, 49.1, 49.5, 80.2, 113.1, 114.0, 118.0, 122.4, 124.2, 125.3, 129.4, 136.8, 156.7 ppm; IR (neat): $\tilde{\nu}$=3380, 3355, 3290, 3120, 3015, 2980, 2895, 1680, 1510, 1450, 1365, 1275, 1245, 1160, 915, 790 cm^{-1}; MS (ESI$^+$): m/z (%): 354 and 356 (12) $[M+H]^+$, 281 (100) and 283 (97); HRMS (ESI$^+$): m/z calcd for $C_{15}H_{21}BrN_3O_2$ $[M+H]^+$: 354.0812 and 356.0793; found: 354.0812 and 356.0793.

***tert*-Butyl (2-formamido-2-(5-bromo-1*H*-indol-3-yl)ethyl)carbamate (12a)**: A solution of BtCHO (85 mg, 0.58 mmol) in THF (2 mL) was added to a solution of indolic amine **11** (205 mg, 0.58 mmol) in THF (8 mL) at 0 °C. The resulting mixture was stirred for 10 min at 0 °C and it was then allowed to warm to room temperature. After 30 min, the THF was removed in vacuo, then CH_2Cl_2 (50 mL) and an aqueous solution of NaOH (2 N, 20 mL) were added. After extraction, the organic solution was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography with EtOAc/pentane (10:90 then 99:1) yielded pure compound **12a** as a white solid (140 mg, 63%): ^{1}H NMR (300 MHz, CD_3OD): δ=1.41 (s, 9H), 3.41–3.60 (m, 2H), 5.43 (dd, J_1=8.0, J_2=6.1 Hz, 1H), 7.18–7.29 (m, 3H), 7.79 (d, J=1.8 Hz, 1H), 8.14 ppm (s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ=28.8, 45.5, 46.3, 80.3, 113.4, 114.1, 114.5, 122.4, 124.8, 125.6, 129.2, 136.8, 163.4, 168.5 ppm; IR (neat): $\tilde{\nu}$=3270, 3170, 3080, 2975, 2930, 1690, 1655, 1530, 1450, 1380, 1290, 1255, 1150, 790, 755 cm^{-1}; MS (ESI$^+$): m/z (%): 404 (100) and 406 (97) $[M+Na]^+$; HRMS (ESI$^+$): m/z calcd for $C_{16}H_{21}BrN_3O_3$ $[M+H]^+$: 382.0761 and 384.0742; found: 382.0760 and 384.0741.

***tert*-Butyl (2-acetamido-2-(5-bromo-1*H*-indol-3-yl)ethyl)carbamate (12b)**: Compound **12b** was obtained from indolic amine **11** (177 mg, 0.5 mmol) and acetyl chloride (23 μL, 0.6 mmol) by the same procedure as compound **7b**. The product was a white solid (150 mg, 76%): ^{1}H NMR (300 MHz, CD_3OD): δ=1.41 (s, 9H), 1.97 (s, 3H), 3.43–3.58 (m, 2H), 5.34 (dd, J_1=7.8, J_2=6.3 Hz, 1H), 7.19 (dd, J_1=8.6, J_2=1.7 Hz, 1H), 7.26 (s, 1H), 7.27 (d, J=8.6 Hz, 1H), 7.77 ppm (d, J=1.7 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ=22.8, 28.8, 45.4, 47.7, 80.2, 113.3, 114.1, 114.9, 122.4, 124.7, 125.5, 129.4, 136.8, 158.6, 172.9 ppm; IR (neat): $\tilde{\nu}$=3425, 3325, 2980, 2930, 1680, 1640, 1530, 1460, 1365, 1275, 1165, 885, 800 cm^{-1}; MS (ESI$^+$): m/z (%): 418 (100) and 420 (97) $[M+Na]^+$; HRMS (ESI$^+$): m/z calcd for $C_{17}H_{23}BrN_3O_3$ $[M+H]^+$: 396.0917 and 398.08999; found: 396.0916 and 398.0897.

***tert*-Butyl (2-benzamido-2-(5-bromo-1*H*-indol-3-yl)ethyl)carbamate (12c)**: Compound **12c** was obtained from indolic amine **11** (60 mg, 0.17 mmol) and benzoyl chloride (20 μL, 0.17 mmol) by the same procedure as compound **7b**. The product was a white solid (45 mg, 58%): mp 187 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=1.33 (s, 9H), 3.44–3.52 (m, 2H), 5.42–5.49 (m, 1H), 7.00 (t, J=5.8 Hz, 1H), 7.17 (d, J=6.4 Hz, 1H), 7.32 (d, J=8.8 Hz, 1H), 7.38 (s, 1H), 7.44 (t, J=7.2 Hz, 2H), 7.51 (t, J=7.2 Hz, 1H), 7.82–7.88 (m, 3H), 8.55 (d, J=8.8 Hz, 1H), 11.14 ppm (br s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=28.1, 44.1, 46.2, 77.6, 111.1, 113.3, 114.5, 121.0, 123.4, 124.2, 127.3, 128.0, 130.9, 134.61, 134.65, 134.71, 155.8, 165.8 ppm; IR (neat): $\tilde{\nu}$=3375, 3310, 1661, 1629, 1519, 1276, 1163 cm^{-1}; MS (ESI$^+$): m/z (%): 480 (100) and 482 (97) $[M+Na]^+$; HRMS (ESI$^+$): m/z calcd for $C_{22}H_{25}BrN_3O_3$ $[M+H]^+$: 458.1074 and 460.1056; found: 458.1069 and 460.1050.

***tert*-Butyl (2-(5-bromo-1*H*-indol-3-yl)-2-(2,2,2-trifluoroacetamido)ethyl)carbamate (12d)**: Compound **12d** was obtained from indolic amine **11** (80 mg, 0.23 mmol) and trifluoroacetic anhydride (35 μL, 0.25 mmol) by the same procedure as compound **7b** as a white solid (70 mg, 69%): mp 176 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=1.36 (s, 9H), 3.43–3.48 (m, 2H), 5.25–5.32 (m, 1H), 7.03 (t, J=5.7 Hz, 1H), 7.20 (dd, J_1=8.7, J_2=2.0 Hz, 1H), 7.34 (d, J=8.7 Hz, 1H), 7.38 (d, J=2.0 Hz, 1H), 7.72 (s, 1H), 9.63 (d, J=8.7 Hz, 1H), 11.24 ppm (br s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=28.0, 43.4, 46.6, 77.8, 111.4, 112.3, 113.5, 115.9 (J_{C-F}=288.8 Hz), 120.6, 123.7, 127.7, 155.9 (J_{C-F}=288.8 Hz), 127.7, 134.7, 155.6 (J_{C-F}=15.8 Hz), 156.0 ppm; IR (neat): $\tilde{\nu}$=3440, 3325, 1675, 1535, 1455, 1275, 1160 cm^{-1}; MS (ESI$^+$): m/z: 472 and 474 $[M+Na]^+$; HRMS (ESI$^+$): m/z calcd for $C_{17}H_{20}BrFN_3O_3$ $[M+H]^+$: 450.0635 and 452.0616; found: 450.0629 and 452.0610.

***tert*-Butyl (2-(5-bromo-1*H*-indol-3-yl)-2-(nicotinamido)ethyl)carbamate (12e)**: Compound **12e** was obtained from indolic amine **11** (80 mg, 0.23 mmol) and nicotinoyl chloride (48 mg, 0.27 mmol) by the same procedure as compound **7b** as a white solid (60 mg, 58%): mp 195 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=1.32 (s, 9H), 3.46–3.50 (m, 2H), 5.43–5.49 (m, 1H), 7.03 (t, J=6.0 Hz, 1H), 7.18 (d, J=8.4 Hz, 1H), 7.32 (d, J=8.4 Hz, 1H), 7.40 (s, 1H), 7.49 (dd, J_1=8.0, J_2=4.8 Hz, 1H), 7.83 (s, 1H), 8.19 (d, J=8.0 Hz, 1H), 8.68 (d, J=3.6 Hz, 1H), 8.77 (d, J=8.8 Hz, 1H), 9.02 (s, 1H), 11.16 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=29.1, 45.1, 47.3, 78.6, 112.2, 114.4, 115.1, 121.9, 124.2, 124.4, 125.3, 129.0, 131.1, 135.7,

136.0, 149.5, 152.6, 156.8, 165.4 ppm; IR (neat): $\tilde{\nu}$ = 3325, 3210, 2970, 1680, 1635, 1545, 1270, 1165 cm^{-1}; MS (ESI^+): *m/z*: 459 and 461 $[M+H]^+$; HRMS (ESI^+): *m/z* calcd for $C_{21}H_{24}BrN_4O_3$ $[M+H]^+$: 459.1026 and 461.1008; found: 459.1024 and 461.1006.

***tert*-Butyl (2-(4-bromophenyl)acetamido-2-(5-bromo-1*H*-indol-3-yl)ethyl)carbamate (12 f)**: Compound **12 f** was obtained from indolic amine **11** (60 mg, 0.17 mmol) and 2-(4-bromophenyl)acetyl chloride (204 μL, 0.20 mmol) by the same procedure as compound **7 b** as a white solid (50 mg, 54%): mp 131 °C; ^1H NMR (300 MHz, CD_3OD): δ = 1.34 (s, 9H), 3.29–3.43 (m, 2H), 3.41 (s, 2H), 5.17–5.23 (m, 1H), 6.82 (t, *J* = 6.0 Hz, 1H), 7.17 (dd, J_1 = 8.4, J_2 = 1.6 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.29–7.31 (m, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.66 (s, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 11.13 ppm (br s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ = 29.1, 42.6, 45.1, 46.3, 78.6, 112.1, 114.3, 115.2, 120.4, 122.0, 124.5, 124.8, 128.9, 131.9, 132.1, 135.8, 136.8, 156.6, 170.0 ppm; IR (neat): $\tilde{\nu}$ = 3425, 3340, 1680, 1635, 1535, 1490, 1460, 1275, 1170 cm^{-1}; MS (ESI^+): *m/z*: 572, 573, 574, 575, 576, and 577 $[M+Na]^+$; HRMS (ESI^+): *m/z* calcd for $C_{23}H_{25}Br_2N_3O_3$ $[M+H]^+$: 550.0335, 552.0316, 554.0300; found: 550.0328, 552.0310, 554.0292.

***tert*-Butyl (3-oxopropyl)carbamate (13)**: In a dry flask, 3-aminopropanol (3.0 g, 40 mmol) was dissolved in dry CH_2Cl_2 (40 mL) at 0 °C under argon. Boc_2O (10.5 g, 48 mmol) was then added portionwise. The solution was stirred at room temperature for 24 h. After addition of CH_2Cl_2, the organic layer was washed twice with a saturated Na_2CO_3 aqueous solution and water, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The crude material was purified by filtration through a short pad of silica (EtOAc/pentane, 4:6) to afford the desired carbamate as a colorless oil (6.16 g, 88%). A flask was charged with the carbamate (875 mg, 5.0 mmol), EtOAc (25 mL), and IBX (3.5 g, 12.5 mmol). The heterogeneous solution was heated at reflux with stirring for 3 h. The mixture was then filtered to remove the excess of IBX and the solvent was evaporated. The desired aldehyde **13** was obtained without any further purification as a colorless liquid (820 mg, 95%): ^1H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9H), 2.70 (t, *J* = 5.7 Hz, 2H), 3.42 (q, *J* = 5.7 Hz, 2H), 4.97 (br s, 1H), 9.81 ppm (t, *J* = 0.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.4, 34.2, 44.4, 79.6, 155.9, 201.4 ppm.

(*Z*)-*N*-(3-((*tert*-butoxycarbonyl)amino)propylidene)-1-phenylmethanamine oxide (14): *N*-Benzylhydroxyamine (585 mg, 4.76 mmol) and aldehyde **13** (800 mg, 4.62 mmol) were dissolved in CH_2Cl_2 (15 mL) at room temperature. Anhydrous $MgSO_4$ (2.5 g) was added to this solution. The mixture was stirred for 3 h at room temperature. The solution was then filtered and evaporated in vacuo. The resulting oil was purified by flash chromatography (neat EtOAc, then EtOAc with 3–6% of MeOH) to afford the desired product **14** as a colorless oil (1.20 g, 91%): ^1H NMR (300 MHz, $CDCl_3$): δ = 1.42 (s, 9H), 2.65 (q, *J* = 6.3 Hz, 2H), 3.31 (q, *J* = 6.3 Hz, 2H), 4.86 (s, 2H), 5.16 (br s, 1H), 6.80 (t, *J* = 6.3 Hz, 1H), 7.34–7.41 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.9, 28.5, 37.6, 69.4, 128.3, 129.0, 129.3, 132.8, 137.2 ppm; HRMS (ESI^+): *m/z* calcd for $C_{15}H_{23}N_2O_3$ $[M+H]^+$: 279.1703; found: 279.1705.

***tert*-Butyl (3-(benzyl(hydroxy)amino)-3-(5-bromo-1*H*-indol-3-yl)propyl)carbamate (15)**: In a dry flask, under an argon atmosphere, freshly distilled acetyl chloride (513 μL, 7.19 mmol) was added to cold dry MeOH (15 mL). The mixture was stirred for 10 min at 0 °C, then a mixture of 5-bromoindole (705 mg, 3.60 mmol) and nitrone **14** (1.0 g, 3.60 mmol) in dry MeOH (15 mL) was added. After the addition, the mixture was stirred at 0 °C for 6 h. The solution was then quenched with saturated $NaHCO_3$ aqueous solution. The aqueous layer was extracted three times with CH_2Cl_2. The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The crude material was purified by flash chromatography (EtOAc/pentane, 4:6). The solid was washed with pentane to afford the desired hydroxyamine **15** as a white solid (685 mg, 40%): ^1H NMR (300 MHz, $[D_6]DMSO$): δ = 1.36 (s, 9H), 1.81–1.92 (m, 1H), 2.22–2.33 (m, 1H), 2.88–2.97 (m, 2H), 3.51 (ABq system, *J* = 13.8, $\delta_A-\delta_B$ = 17.4 Hz, 2H), 3.99 (dd, *J* = 7.8, *J* = 6.0 Hz, 1H), 6.74 (br s, 1H), 7.15–7.28 (m, 6H), 7.33 (d, *J* = 9.0 Hz, 1H), 7.34 (s, 1H), 7.71 (s, 1H), 7.90 (d, *J* = 1.8 Hz, 1H), 11.16 ppm (s, 1H); ^{13}C NMR (75 MHz, $[D_6]DMSO$): δ = 28.2, 33.0, 38.0, 60.4, 61.6, 77.2, 110.9, 113.1, 113.2, 122.5, 123.1, 125.8, 126.3, 127.6, 128.7, 128.8, 135.0, 139.5, 155.4 ppm; IR (neat): $\tilde{\nu}$ = 3410, 3290, 2970, 1735, 1685, 1510, 1455 cm^{-1}; MS (ESI^+): *m/z* (%): 474 (100) and 476 (97) $[M+H]^+$; HRMS (ESI^+): *m/z* calcd for $C_{23}H_{29}BrN_3O_3$ $[M+H]^+$: 474.1387 and 476.1369; found: 474.1380 and 476.1367.

Biology

Bacterial strains: Four *S. aureus* strains were used.[8c,d,16b,33] The wild-type clinical isolate *S. aureus* SA-1199, the NorA-overproducing mutant SA-1199B, and the fluoroquinolone-resistant strain SA-K2378, which overexpresses the NorA efflux pump and was produced by cloning *norA* and its promoter into plasmid pCU1 and introducing the construct into SA-K1902, were generously provided by G. W. Kaatz (John D. Dingell Department of Veterans Affairs Medical Center, Detroit, IL, USA). *S. aureus* ATCC 25923 was purchased from the Institut Pasteur (CRBIP, Paris, France). All strains were grown at 37 °C in Mueller–Hinton (MH) broth (Bio-Rad, Mitry Mory, France) or spread on MH agar plates for counting. Colony forming unit (CFU) monitoring was carried out by counting the colonies present in 2×10 μL of serial log dilutions of bacteria inoculum spotted on MH agar plates. Plates were examined for growth after one night at 37 °C.

Media, antibiotics, and culture conditions: MH broth (Bio Rad) was used for all bacteria overnight cultures, susceptibility testing, and ethidium bromide accumulation experiments. Ciprofloxacin was obtained from Sigma–Aldrich (Saint Quentin Fallavier, France). Reserpine was purchased from Alfa Aesar (Schiltigheim, France). Stock solutions (ciprofloxacin and EtBr) were prepared in sterile water, except for reserpine, which was dissolved in DMSO.

Susceptibility testing/MIC determination: For each tested strain, a bacterial inoculum of 5×10^5 CFU mL^{-1} was prepared by diluting an overnight culture in sterile MH broth. The inoculum was checked by CFU counting. Indolic compounds (100 μL) were dispensed in a 96-well microplate after twofold serial dilutions in MH broth by using a Biomek 2000 Workstation (Beckman Coulter, Villepinte, France). The bacterial inoculum (100 μL; prepared as described above) was then added into each well. The growth of bacterial strains was measured by using a DTX 880 microplate reader (Beckman Coulter, Villepinte, France) by monitoring absorption at 620 nm after 0, 2, 4, 6, and 24 h of incubation at 37 °C. All of the tested compounds were dissolved in DMSO at a concentration of 10 mg mL^{-1} before dilution into MH broth for use in MIC determination. The MIC value was defined as the minimal concentration of the indolic derivatives that completely inhibited cell growth during 24 h incubation at 37 °C. Indolic molecules were first tested at final concentrations ranging from 0.125–128 mg L^{-1}. For compounds with solubilities lower than 128 mg L^{-1} in MH broth, the concentration range was adjusted with the maximal concentration decreased to 8 μg mL^{-1}. The highest final DMSO concentration used (2.56% v/v) induced no significant bacterial growth inhibition. The MIC determination with ciprofloxacin (8 mg L^{-1} for SA-1199B, 2 mg L^{-1} for

SA-K2378, and 0.25 mg L^{-1} for SA-1199) was performed as a control on each microplate. The accepted variance in the MIC values can be estimated to a twofold difference due to the microdilution method used. All experiments were at least duplicated in two independent runs.

Ethidium bromide accumulation assays: The accumulation of ethidium bromide by SA cells with or without inhibitors was determined by fluorimetry. SA strains were grown overnight in MH broth, diluted 20-fold in fresh, pre-warmed MH broth, and grown in a water bath at 37 °C under agitation (130 rpm) for 3.5 h, which corresponds to the mid-log phase of growth. Samples (10 mL) were aseptically withdrawn from the culture, the bacteria were harvested by centrifugation (4000 *g*, 10 min), the supernatant was discarded, and the bacterial cells were resuspended in fresh sterile pre-warmed MH broth (20 mL) containing EtBr (final concentration: 10 mg $L^{<M->1}$) and incubated again in a water bath at 37 °C under agitation (130 rpm) for 30 min. Aliquots (1 mL) were taken at fixed intervals (0, 5, 10, 15, 20, 25, and 30 min) for fluorescence measurement (λ_{ex} = 530 nm, λ_{em} = 600 nm). After 10 min, the test compounds were added (final concentration: 5 or 20 mg L^{-1}) and the fluorescence was measured for an additional 20 min. The fluorescence intensity difference between the compound-containing assay and the fluorescence baseline before addition was indicative of the ability of the compound to inhibit the efflux of EtBr. The result was expressed as a percentage of the fluorescence difference after and before the addition of the potential efflux pump inhibitor. The fluorescence intensity (in arbitrary units) before addition was calculated as the mean of the fluorescence at 0, 5, and 10 min. Fluorescence intensity after inhibitor addition was calculated as the mean of the fluorescence at 15, 20, 25, and 30 min. It was checked that the fluorescence remained constant during the experiment in the absence of tested compound. Reserpine was used as a positive control. Experiments were repeated two or three times and were expressed as mean values ± SD.

Cellular toxicity tests: In vitro cytotoxicity was assayed on three cell lines: KB (human mouth carcinoma), MCR5 (human lung fibroblast), and HCT116 (human colon tumor). Cells were plated in 96-well tissue-culture microplates in complete medium (200 μL) and treated 24 h later with stock solution of compounds (2 μL) dissolved in DMSO by using a Biomek 3000 apparatus (Beckman–Coulter). Controls received the same volume of DMSO (1% final volume). After 72 h of exposure, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) reagent (Promega) was added and incubated for 3 h at 37 °C. The absorbance was monitored at 490 nm and the results were expressed as the inhibition of cell proliferation calculated as the ratio [1−(OD_{490} treated/OD_{490} control)]×100 in triplicate experiments. Results are presented as the percentage of cellular growth inhibition in the presence of 10^{-5} M and 10^{-6} M concentrations of the tested indolic derivatives.

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Keywords: antibiotics · efflux pumps · indoles · inhibitors · *Staphylococcus aureus* · structure–activity relationships

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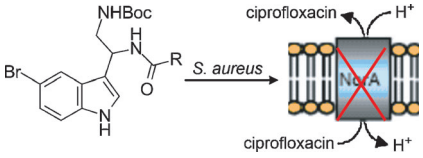
A pump turn off: 1-(1*H*-Indol-3-yl)-ethanamine derivatives such as the one shown here, were synthesized through simple chemical modifications and were shown to be efficient NorA efflux pump inhibitors. They are able to restore ciprofloxacin activity against fluoroquinolone-resistant *Staphylococcus aureus* strains.



A. Hequet, O. N. Burchak, M. Jeanty, X. Guinchard, E. Le Pihive, L. Maigre, P. Bouhours, D. Schneider, M. Maurin, J.-M. Paris, J.-N. Denis, C. Jolivalt**

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1-(1*H*-Indol-3-yl)ethanamine Derivatives as Potent *Staphylococcus aureus* NorA Efflux Pump Inhibitors