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Synthesis and evaluation of new arylbenzo[*b*]thiophene and diarylthiophene derivatives as inhibitors of the NorA multidrug transporter of *Staphylococcus aureus*

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Abstract—The synthesis based on palladium catalytic coupling of 38 new-arylated benzo[*b*]thiophenes or thiophenes is described in a few steps. We also report the direct arylation of the position 3 of the benzo[*b*]thiophenic structure, a 'one pot' 2,5-heterodiarylation of thiophenes as well as the synthesis of precursors of amino-acids with a 2-arylated benzo[*b*]thiophene core. These compounds were evaluated on bacteria strains: most of them did not exhibit any antibiotic activity but were found to selectively inhibit the NorA multidrug transporter of *Staphylococcus aureus*. As such, they restored the activity of the NorA substrates ciprofloxacin against a resistant *S. aureus* strain in which this efflux pump is over-expressed.
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1. Introduction

As a consequence of the intense fight against infections, bacteria have evolved through numerous defences against antimicrobial agents. Amongst them, active efflux of drugs is being recognised as a major cause of resistance.[1,2] Efflux pumps have also been documented in mammalian cells (for example P-glycoprotein P-gp).[3] In bacteria, diverse antibacterial agents including β-lactams, macrolides, tetracyclines and fluoroquinolones are subjected to this efflux phenomenon. The resistance mechanism involves transporters that are capable of extruding a large variety of compounds out of the cells. These efflux pumps can induce specific or multidrug resistance (MDR). For example, the MsrA protein is specific and exports only certain macrolides and streptogramins,[4,5] whereas the NorA protein confers resistance to a wide range of structurally unrelated antibiotics and antiseptics such as acridines, ethidium bromide, pentamidine or more importantly fluoroquinolones (e.g., Ciprofloxacin Fig. 1).[6–8]

Although numerous small-molecule inhibitors of P-gp have been reported, only a few inhibitors of bacterial MDR pumps are known.[9,10] Compounds such as verapamil or reserpine can circumvent efflux-mediated MDR albeit none of them reached the clinical trials for this use. Thus, identification and the development of potent and non-toxic Efflux Pump Inhibitors (EPIs) are crucial aims of medical and pharmacological research. Influx, Inc. screened a library of compounds and selected the five most potents for further analyses. The structures feature highly conjugated aromatic rings such as phenyls, naphthalenes or indoles. The 2-arylindole INF55 (Fig. 1), although being a very simple structure, was active at concentrations below 5 μg/mL. In addition, it significantly decreased the IC50 of ciprofloxacin against *Staphylococcus aureus* SA-1199 at concentrations eight-fold lower than reserpine.[11]

From the assumption that the activity of INF55 was less dependent on the nitro functionality than the 2-arylindole, we tackled the synthesis of sulfur analogues of INF55. Indeed, benzo[*b*]thiophene derivatives were

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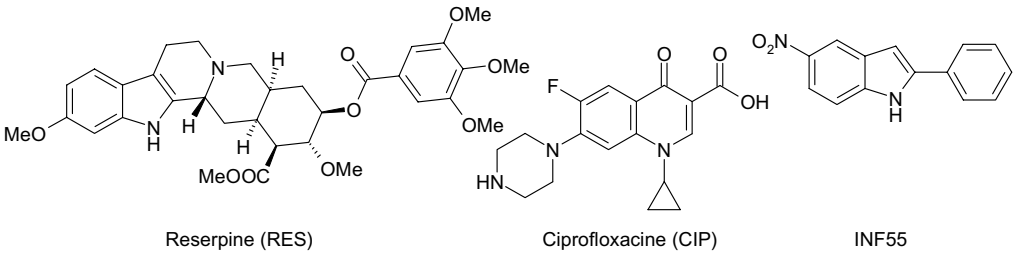


Figure 1.

found over the past 20 years to exhibit several biological properties as antiinflammatory,[12] antidepressive,[13] antipsychotic,[14] antithrombotic[15] and antiviral[16] agents. Some of these molecules also act as prostaglandin[17] or dopamine[18] receptor antagonists. In particular, many efforts have focused on the synthesis of 2-arylbenzo[*b*]thiophenes as some derivatives were found to be potent selective estrogen receptor modulators (SERMs)[19] and were also used as inhibitors of tubulin polymerisation.[20,21]

Additionally, we focused on the thiophene ring arylation. Although generally synthesised by intramolecular or intermolecular cyclisation,[22] recent advances described the direct arylation of the thiophene ring by palladium catalysis.[23,24] Only a few recent methods allowed a regioselective 2,5-heterodiarylation of the thiophene ring and were usually carried out in two steps.[25] We supposed that the presence of a functional group at position 3 would influence the regioselectivity of the arylation and therefore allow a regioselective diarylation of the thiophene ring.

Within the past 5 years, we have described the direct arylation of 3-substituted benzo[*b*]thiophenes by a phosphine-free catalytic system,[26] using either an ammonium salt (*n*-Bu_4NBr) or a crown-ether (DCH-18-C-6) as an additive. This system differs from those proposed by Miura[27] (use of 2 equiv of CuI) or Ohta[24] (base = NaOAc, solvent = DMA, cat. = $Pd(PPh_3)_4$) for direct arylation of the 2 position of the benzo[*b*]thiophene ring. However, we have shown in a recent paper that the addition of phosphine facilitates the arylation of unsubstituted benzo[*b*]thiophenes.[28]

In this paper, we describe the regioselective arylation of a series of benzo[*b*]thiophenes and thiophenes. This was controlled by the use of several different additives to the catalytic palladium species. We decided to synthesise a library for use in our continued effort to develop bacterial efflux inhibitors and we screened this library against strains of *S. aureus* susceptible and resistant to quinolone or macrolide antibacterial agents by active efflux processes.

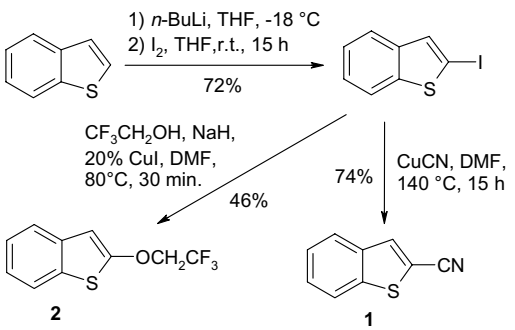
2. Synthesis

The synthesis of 2-functionalised benzo[*b*]thiophene precursors was undertaken in two steps from benzo[*b*]thiophene. Regioselective iodination followed by an aromatic nucleophilic substitution afforded 2-cyanobenzo[*b*]thiophene **1** and 2-(2,2,2-trifluoroethoxy)-benzo[*b*]thiophene **2** with 53% and 33% overall yields, respectively (Scheme 1).

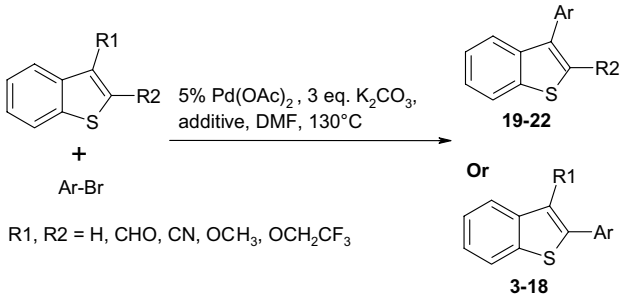
We subsequently studied the reactivity of benzo[*b*]thiophenes **1** and **2** in addition to that of 3-functionalised benzo[*b*]thiophenes (R = CN, CHO, OCH_3, OCH_2CF_3) with several arylbromides towards the Heck-type coupling in the presence of different additives: 1 equiv *n*-Bu_4NBr (method A), 1 equiv DCH-18-C-6 (method B) or 0.1 equiv PPh_3 (method C) (Scheme 2 and Table 1).

As benzo[*b*]thiophene-3-carboxaldehyde was rapidly degraded in the presence of crown ethers, the use of the quaternary ammonium salt was favoured (entries 1–7). It appeared that, due to the low reactivity of 2- and 3-bromopyridines towards the Heck-type coupling, yields of arylated compounds **10**, **15**–**17**, **19** and **21** were generally lower and often required the use of triphenylphosphine (method C) to reduce reaction times and improve the yields. This lack of reactivity was particularly pronounced for the arylation at position 3 as compounds **19** and **21** were isolated in only 17% and 34% yields, respectively (entries 17 and 19). In general, arylation at position 3 afforded the corresponding products in lower yields and higher reaction times than arylation at position 2 (entries 17–20).

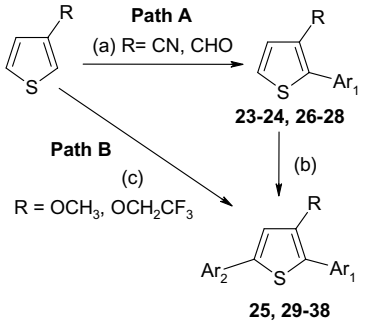
The arylation of 3-substituted thiophenes was undertaken according to the 3 methods described above (Scheme 3, path A, Table 2). It was found that in the presence of 1 equiv *n*-Bu_4NBr (method A) or 1 equiv DCH-18-C-6 (method B), thiophene-3-carboxaldehyde and 3-cyanothiophene were selectively arylated at position 2, affording compounds **27** and **28** in moderate yields (entries 5 and 6). The resulting compounds were



Scheme 1.



Scheme 2.



Scheme 3. Reagents: (a) method A or B; (b) method C; (c) method B.

then coupled at position 5 with triphenylphosphine as an additive (method C) in 30–44% yields (entries 7–10). The electron-rich thiophenes (R = OCH_3, OCH_2CF_3) were found to be more reactive towards coupling and a one-pot regioselective 2,5-diarylation was envisioned (Scheme 3, path B).

The first aryl halide was introduced stoichiometrically until a satisfactory conversion was obtained. Then an excess of the second aryl halide was added, affording 2,5-heterodiarylated thiophenes **33**–**34**, **36**–**37** which were isolated in good yields (30–39%) considering the number of possible by-products. It is noteworthy to outline the importance of the order of introduction of the two aryl halides. Indeed, when less reactive 3-bromopyridine was introduced first, no significant amount of by-products was isolated. On the contrary, when the most reactive aryl halide was introduced first, the resulting 2-arylthiophene was reactive enough to perform a second arylation, yielding significant amounts of 2,5-homodiarylated products **35** and **38** (entries 11 and 13).

As only a few syntheses of amino-acids bearing a benzo[*b*]thiophene core have been described so far,[29,30] it seemed interesting to combine the structures of both 2-arylbenzo[*b*]thiophenes and amino-acids. Starting from benzo[*b*]thiophene-3-carboxaldehyde and the 2-arylated compounds **4**–**6**, several precursors of amino-acids were synthesised. Amongst the classical methods such as Wittig–Horner reaction,[31] the Henry reaction[32] and the halogenation of the aldehyde function followed by a stereospecific substitution,[33] we preferred the Erlenmeyer condensation[34] with hippuric acid. This method was described once on 2-ethylbenzo[*b*]thiophene-3-carboxaldehyde by Neidlein and Salz,[35] affording the desired azalactone in 74% yield. According to a similar strategy, Ojima[36,37] described the synthesis of fluorinated analogues of tryptophan with excellent yields. Other described condensations[38] gave lower yields and a mixture of *E*/*Z* isomers, the major isomer being *Z* in every case. The synthesis comprised three steps: condensation of hippuric acid to yield azalactones **39a**–**d**, electrophilic opening of azalactones with methanol (**40a**–**d**) followed by hydrogenation of the alkenes to yield the protected amino-acids **41b**–**d** (Scheme 4).

The Erlenmeyer condensation conditions were first optimised and then performed in acetic anhydride with

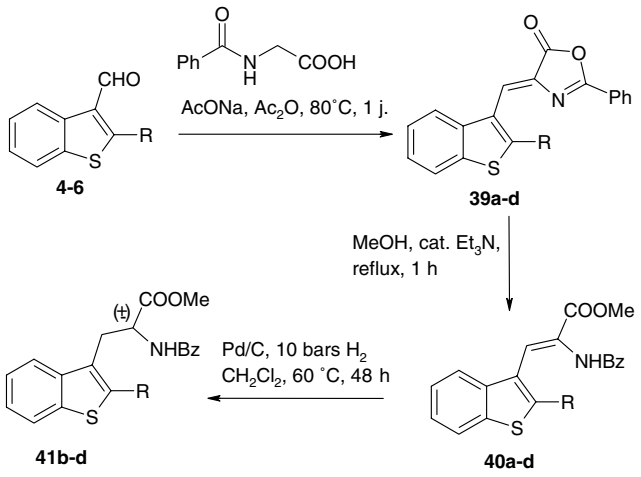
Table 1. Arylation of 2- or 3-functionalised benzo[*b*]thiophenes

Entry	Method	Reactant		Time (h)	Product		Isolated yield (%)
		R1	R2		Id.	Ar	
1	A	CHO	H	15	**3**	C_6H_5	47
2	A	CHO	H	4	**4**	4-OCH_3–C_6H_4	64
3	A	CHO	H	3	**5**	4-CF_3–C_6H_4	70
4	A	CHO	H	3	**6**	4-CN–C_6H_4	41
5	A	CHO	H	5	**7**	4-Cl–C_6H_4	58
6	A	CHO	H	30	**8**	3-Cl–C_6H_4	41
7	A	CHO	H	6	**9**	2-Cl–C_6H_4	59
8	C	CHO	H	48	**10**	3-Py	51
9	B	CN	H	1.5	**11**	4-Cl–C_6H_4	68
10	B	CN	H	2	**12**	3-Cl–C_6H_4	76
11	B	CN	H	4	**13**	3-OCH_3–C_6H_4	78
12	B	CN	H	15	**14**	3,4,5-OCH_3–C_6H_2	70
13	B	OCH_3	H	25	**15**	3-Py	66
14	C	OCH_3	H	48	**16**	2-Py	18
15	B	OCH_2CF_3	H	6	**17**	3-Py	59
16	B	OCH_2CF_3	H	0.75	**18**	2-CN–C_6H_4	76
17	C	H	CN	15	**19**	3-Py	17
18	C	H	CN	15	**20**	4-CF_3–C_6H_4	67
19	C	H	OCH_2CF_3	24	**21**	3-Py	34
20	C	H	OCH_2CF_3	3	**22**	2-CN–C_6H_4	69

Table 2. Arylation of 3-functionalised thiophenes

Entry	Method	R	Time (h)	Product			Isolated yield (%)
				Id. Nb.	Ar_1	Ar_2	
1	B[a]	OCH_3	4	**23**	2-CN–C_6H_4	H	51
2	C[a]	OCH_3	1.5	**24**	3-Py	H	68
				25	3-Py	3-Py	16[d]
3	C[a]	OCH_3	8	**26**	2-Py	H	40
4	A	CHO	10	**27**	C_6H_5	H	35
5	B	CN	18	**28**	3-Py	H	33
6	C	CHO[b]	18	**29**	C_6H_5	4-Cl–C_6H_4	43
7	C	CHO[b]	3	**30**	C_6H_5	3-Py	37
8	C	CN[c]	18	**31**	3-Py	4-CF_3–C_6H_4	42
9	C	CN[c]	18	**32**	3-Py	4-Cl–C_6H_4	44
10	B[a]	OCH_3	20	**33**	3-Py	3-CF_3–C_6H_4	39
11	B[a]	OCH_3	20	**34**	3-CF_3–C_6H_4	3-Py	37
				35	3-CF_3–C_6H_4	3-CF_3-C_6H_4	14[d]
12	B[a]	OCH_2CF_3	19	**36**	3-Py	4-Cl–C_6H_4	30
13	B[a]	OCH_2CF_3	17	**37**	4-Cl–C_6H_4	3-Py	39
				38	4-Cl–C_6H_4	4-Cl–C_6H_4	12[d]

[a] aryl halide (1 equiv) was added portionwise (0.2 equiv by 0.2 equiv).
[b] Thiophene = **27**.
[c] Thiophene = **28**.
[d] Obtained as a by-product alongside the compound of the same entry.



Scheme 4.

2.5–10 equivalents of hippuric acid. It was found that the reaction was extremely sensitive to steric hindrance as yields decreased dramatically when performed on 2-arylated compounds **4**–**6** (Table 3, compounds **39b**–**d**). In addition, the presence of an electron-donating group at the *para* position significantly lowered the yield (**39b**). Nevertheless, only one conformer, presumably the *Z* one according to the literature,[37,38] was isolated.

Table 3. Synthesis of amino-acid precursors

Compound type	R=	Isolated yields (%)		
		39	40	41
(a)	H	90	82	—
(b)	4-OCH_3–C_6H_4	44	94	100
(c)	4-CF_3–C_6H_4	57	87	100
(d)	4-CN–C_6H_4	62	92	100

The azalactone ring opening was straightforward and performed in excellent yields (compounds **40**: 82–94%). Eventually, hydrogenation under 10 bars H_2 in dichloromethane afforded the racemic protected amino-acids **41b–d** quantitatively. However, the non-arylated compound **40a** was degraded under these conditions, presumably, as described recently, by desulfurisation of the benzo[*b*]thiophene moiety.[28]

3. Biological evaluation

3.1. Microbiological screening

In these cellular assays, we were interested in compounds able to restore the activity against a resistant strain of an antibiotic used at a sub-minimal inhibitory concentration (MIC). We chose to work with *S. aureus*, since this Gram-positive species, devoid of an outer-membrane, has greater cell-wall permeability than the Gram-negative organisms.[39]

In a first screening assay, the compounds were used at a concentration of 100 mg/L against a susceptible strain of *S. aureus* (ATCC 25923). Ampicillin (MIC 8 mg/L) at 16 mg/L was used as a positive control. Growth of microorganisms was followed by measuring the absorbance, and only those compounds without effect on the growth at this concentration were submitted to the second screening. Two resistant bacteria harbouring efflux mechanisms were used in this case: (i) *S. aureus* SA-1199B (resistant to fluoroquinolones through overexpression of the NorA efflux pump and having a mutation in the A subunit of gyrase), which will be designated as *S. aureus* NorA, and its susceptible parental strain *S. aureus* SA-1199,[40] (ii) *S. aureus* MsrA (resistant to 14- and 15-membered macrolides, harbouring the multicopies plasmid pUL 5054).[4] Ciprofloxacin (CIP) has a MIC of 0.37 mg/L against susceptible *S. aureus* and of

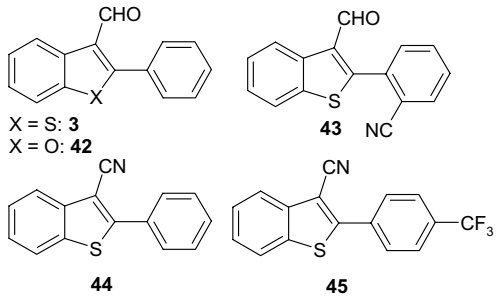


Figure 2. Additional compounds tested.

16 mg/L against *S. aureus* NorA. The gyrA mutation alone conferred a MIC of 2 mg/L. As we did not possess the resistant strain SA-1199-3[39] devoid of the gyrA mutation, we looked for a decrease of MIC up to the 2 mg/L level. Erythromycin (ERY) has MICs of 0.5 mg/L against the susceptible *S. aureus*, and of 128 mg/L against *S. aureus* MsrA.

The antibacterial activity of some of the assayed thiophene and benzothiophene compounds was evaluated on the susceptible strains. The benzofuran counterpart **42** of compound **3** in addition to some previously synthesised[26] 2-arylbenzo[*b*]thiophenes **43**–**45** was also tested (Fig. 2).

Biological assays against *S. aureus* NorA using 100 mg/L of the assayed compounds in the presence of a sub-MIC concentration (MIC/8, i.e., 2 mg/L) of ciprofloxacin, and against *S. aureus* MsrA in the presence of a sub-MIC concentration (MIC/4, i.e., 32 mg/L) of erythromycin are presented in Table 4. All other compounds described herein but not listed in the table were inactive in all assays. Compounds **15**, **17** and **25** displayed antibacterial activity against both susceptible and resistant strains (entries 9, 10, 15). As the MICs against the susceptible *S. aureus* were only moderate, these compounds were not studied further. The diarylated thiophene **29** exhibited a specific but poor activity on *S. aureus* MsrA strain and was also not studied further.

Only compounds which specifically restore the activity of CIP against *S. aureus* NorA were retained. The concentration of these compounds required for the recovery of the activity of ciprofloxacin at half of this concentration (2 mg/L) against the resistant strain was then measured, and the results are reported in parentheses in column 4 of Table 4.

The isobologram (Fig. 3) shows the effective concentration of six assayed compounds able to confer ciprofloxacin a given MIC. As a comparison, compounds **3** and **10** (highly active) were compared to less active compounds such as **5**, **7**, **11** and **12**. There is obviously a syn-

Table 4. Evaluation of antibacterial activity

Entry	Id. Nb.	Antibacterial activity[a] against S[b] and R[c] *S. aureus*			Comments
		S[d]	R NorA+CIP 2 mg/L[e]	R MsrA+ERY 32 mg/L	
1	**3**	–	++ (12.5)	–	NorA inhibitor
2	**4**	–	++ (12.5)	–	NorA inhibitor
3	**5**	–	++ (25)	–	NorA inhibitor
4	**7**	–	++	++	Non-specific inhibitor
5	**10**	–	++ (25)	+	Non-specific inhibitor
6	**11**	–	++ (100)	+	Non-specific inhibitor
7	**12**	–	++ (100)	–	NorA inhibitor
8	**13**	–	++ (100)	–	NorA inhibitor
9	**15**	++ (100)	++	++	Antibacterial
10	**17**	++ (100)	++	++	Antibacterial
11	**18**	–	++ (50)	–	NorA inhibitor
12	**19**	–	++ (50)	–	NorA inhibitor
13	**22**	–	++ (50)	++	Non-specific inhibitor
14	**24**	–	++ (100)	–	NorA inhibitor
15	**25**	++ (100)	++	++	Antibacterial
16	**27**	–	–	+	
17	**29**	–	–	++	MsrA inhibitor
18	**33**	–	++ (50)	–	NorA inhibitor
19	**35**	–	–	+	
20	**40a**	–	++ (100)	++	Non-specific inhibitor
21	**40d**	–	++ (100)	–	NorA inhibitor
22	**41d**	+	++ (100)	–	Poor antibacterial
23	**42**	–	++ (12.5)	–	NorA inhibitor
24	**43**	–	++ (100)	–	NorA inhibitor
25	**44**	–	–	+	
26	**45**	–	–	+	

[a] ++, no bacterial growth; +, <10% of growth of the susceptible strain; –, no effect.
[b] S, susceptible *S. aureus* ATCC 25923.
[c] R, resistant strains 1199B *S. aureus* NorA, or *S. aureus* MsrA.
[d] The number in parentheses is the MIC (mg/L) against the S strain.
[e] The number in parentheses is the concentration of the assayed compound able to restore the activity of ciprofloxacin at 4 mg/L.

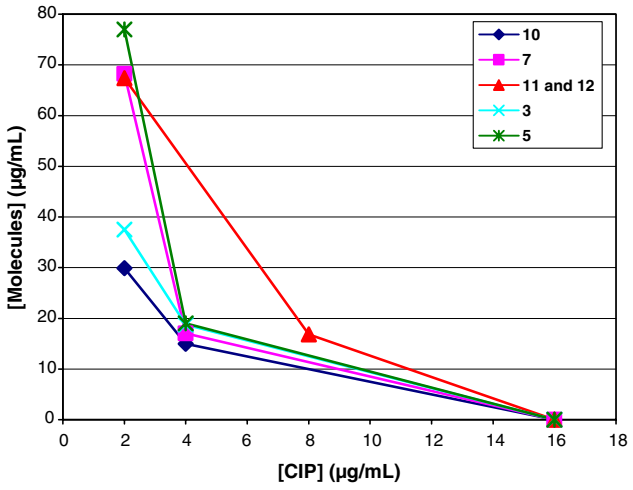


Figure 3. Isobologram of the association between CIP and six inhibitors for *S. aureus* NorA.

ergy between CIP and compounds **3** or **10**, whereas the effect is only poor for other compounds.

3.2. Effects on antibacterial accumulation in *S. aureus* 1199B NorA

As compound **3**, in association with ciprofloxacin, showed a strong effect against *S. aureus* NorA, we carried out its characterisation as a potential NorA inhibitor. We first measured fluorometrically the efflux of ethidium bromide (EtBr, antibiotic DNA-fluorescing agent substrate of the NorA pump) and then the uptake of CIP by *S. aureus* NorA. The effect of the assayed compounds on antibacterial accumulation was compared with those of known efflux pump inhibitors such as reserpine or carbonyl cyanide *m*-chlorophenylhydrazone (CCCP, a non-specific proton gradient dissipater, Fig. 4).

Figure 5 illustrates the effect of benzo[*b*]thiophenes **3** and **10** and of the furan analogue **42,** at a concentration of 20 mg/L, as compared to that of the specific inhibitor reserpine, on the efflux of EtBr by the resistant *S. aureus* NorA as a function of time. Without inhibitor, the accumulation level reached after 10 min was 45% of the initial concentration. Upon addition of reserpine, **3**, **10** or **42**, 72–78% of the initial EtBr concentration remained after 10 min.

Figure 6 shows the effect of **3**, reserpine and CCCP (20 mg/L) on EtBr efflux by *B. subtilis*. The activity of

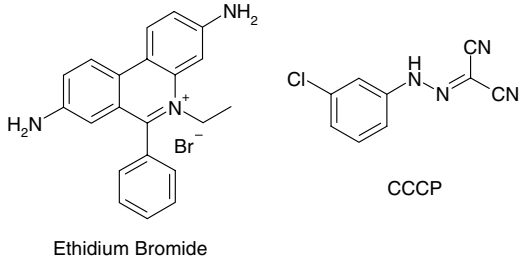


Figure 4.

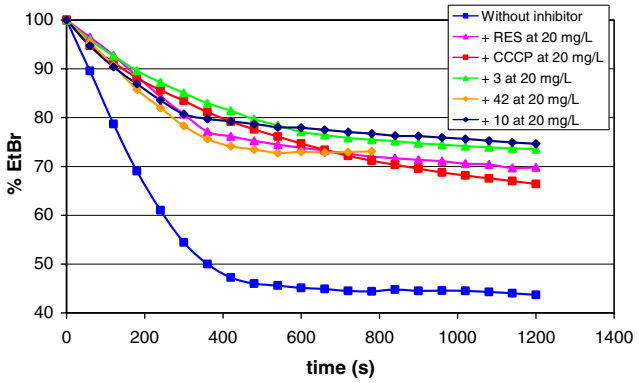


Figure 5. Efflux of ethidium bromide (EtBr) by *S. aureus* NorA.

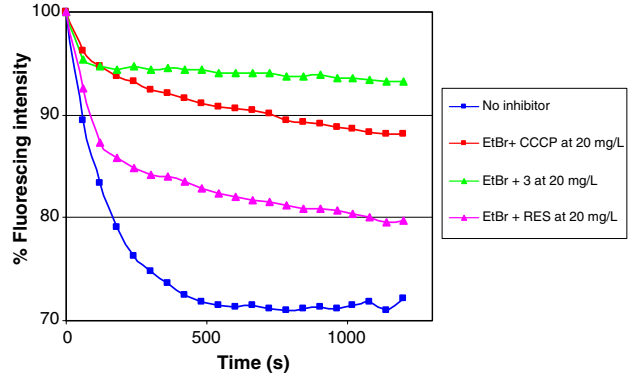


Figure 6. Efflux of ethidium bromide (EtBr) by *B. subtilis* ΔΔNA.

3 was notable, as only 5% of efflux took place after 10 min.

The effect of reserpine, CCCP and compound **3** on the uptake of EtBr by *S. aureus* NorA is illustrated in Figure 7. The effect of the non-specific gradient dissipater CCCP was maximum, with twice the amount of EtBr accumulated. However, when compared with reserpine, compound **3** was more efficient at 20 mg/L and retained a similar activity at 10 mg/L.

Finally, the effect of compound **3** on the uptake of CIP by susceptible *S. aureus* 1199 and its resistant parent *S. aureus* NorA 1199B was evaluated (Fig. 8). The intracellular concentration of ciprofloxacin reached a plateau after 5 minutes at 0.045 fluorescence arbitrary units (about 40 ng CIP/10^9 colony forming units, CFU) and, in the absence of any inhibitor, this level was stable. The addition of compound **3** (20 mg/L) at 10 min increased CIP uptake from 0.045 to 0.07 AU (about 60 ng CIP/10^9 CFU). This level is similar to that obtained for the susceptible *S. aureus* (0.08 AU), on which compound **3**, as expected, had no influence on the CIP uptake.

3.3. Cytotoxicity assays

To evaluate the potential of the active molecules as antibacterial agents, the cytotoxicity of some of the active compounds was measured at concentrations of 10 µM

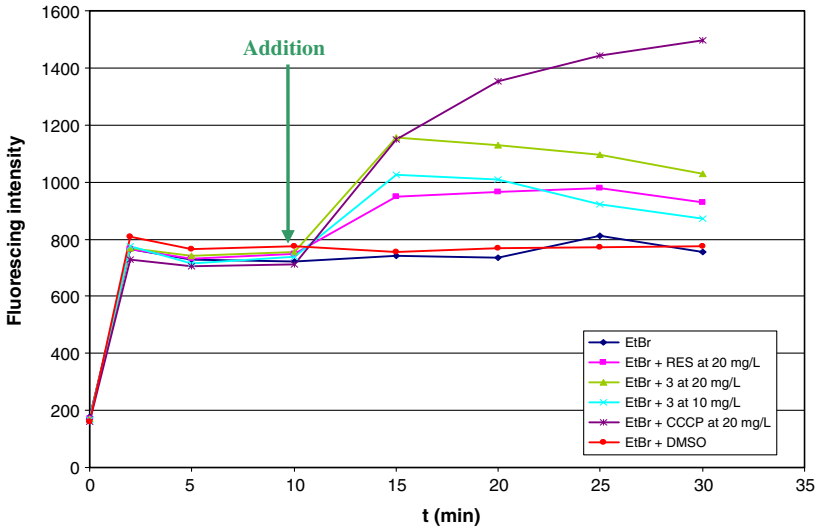


Figure 7. Uptake of ethidium bromide by *S. aureus* NorA.

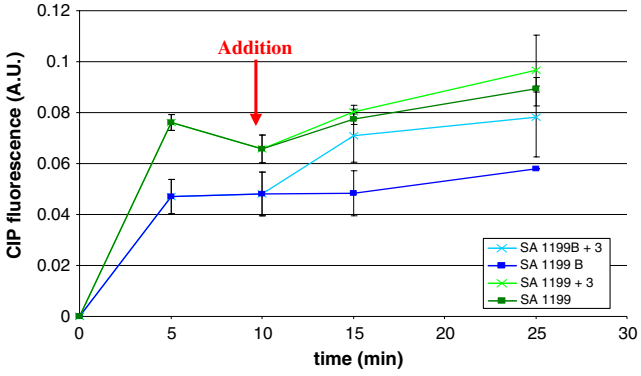


Figure 8. Uptake of ciprofloxacin (CIP) by resistant *S. aureus* NorA 1199B and susceptible *S. aureus* 1199.

on KB, MCF7 and MCF7R cells. Taxotere® was used as a reference at 0.25 nM (entry 1). The results are shown in Table 5 as % of cellular growth inhibition.

Most molecules present a moderate level of cytotoxicity. Compound **22** is the most cytotoxic at 10 μM (entry 11), whereas **10**, **23**, **33** are to be considered as non-toxic (entries 6, 12, 14). Amongst the most potent inhibitors, **3**, a molecule thoroughly evaluated, has a moderate level of toxicity (entry 2). Other active molecules are interesting such as **33** (entry 14) and**40d** (entry 15), which are not toxic, as well as **12** (entry 8) and **19** (entry 10), only slightly toxic.

It is difficult to establish structure–toxicity relationships at such an early stage of this research and with so few molecules tested. For instance the presence of an alde-

Table 5. Evaluation of the cytotoxicity

Entry	Name or Id. Nb.	Cytotoxicity as % of inhibition of cellular growth			Comments
		KB cells	MCF7	MCF7R	
1	Taxotere®	87	31	4	
2	**3**	42	51	37	NorA inhibitor at 12.5 mg/L
3	**4**	89	62	80	NorA inhibitor at 12.5 mg/L
4	**5**	88	73	59	NorA inhibitor at 25 mg/L
5	**7**	67	69	35	Non-specific inhibitor
6	**10**	18	3	0	Non-specific inhibitor
7	**11**	29	27	18	Non-specific inhibitor
8	**12**	23	36	20	NorA inhibitor at 50 mg/L
9	**18**	56	49	39	NorA inhibitor at 50 mg/L
10	**19**	37	18	22	NorA inhibitor at 75 mg/L
11	**22**	100	92	97	Non-specific inhibitor
12	**23**	11	17	4	
13	**27**	33	16	0	
14	**33**	7	0	0	NorA inhibitor at 50 mg/L
15	**40d**	24	0	2	NorA inhibitor at 100 mg/L
16	**41d**	35	12	32	Poor antibacterial
17	**42**	67	47	37	NorA inhibitor at 12.5 mg/L
18	**43**	51	38	33	NorA inhibitor at 100 mg/L
19	**44**	45	20	32	

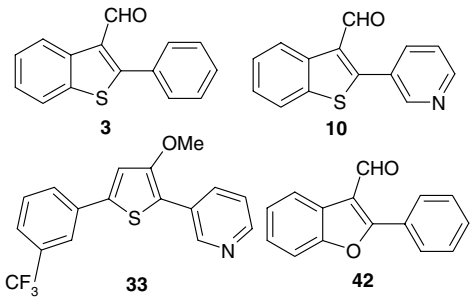


Figure 9. Benzothiophene, thiophene and benzofuran structures.

hyde function on one thiophene ring seemed to be unfavourable (**4** and **5** are toxic), but aldehydes **10** and **27** are not toxic, whereas **22**, with the highest toxicity, is devoid of an aldehyde function.

4. Conclusion

We have applied our previously developed methodology to the synthesis of a series of new-arylated benzo[*b*]thiophenes and thiophenes. Some 3-arylated benzo[*b*]thiophenes were synthesised after slight modifications of the Heck type coupling conditions. The change of the additive also allowed the synthesis of 2,5-heterodiarylated thiophenes in relatively good yield. Finally, some compounds bearing an aldehyde moiety were further functionalised into amino-acid precursors.

Molecules **3**, **10**, **33**, **42** (Fig. 9), which were assayed as synergistic with ciprofloxacin against resistant bacteria harbouring an efflux pump, and as inhibitors of the efflux or accumulation of ethidium bromide or ciprofloxacin, two substrates of the NorA pump, may be considered as potential bacterial efflux pump inhibitors (EPIs) according to the criteria of Lomovskaya.[41] They enhance the activity of ciprofloxacin, have no significant effect on a susceptible strain and increase intracellular level of antibiotics, which is an additional confirmation that their mode of action is that of an EPI. The investigation of a series of analogues of these molecules to accede to compounds with higher efficiency/toxicity ratio seems reasonable, since, as pointed out by others,[41–43] the combination of a broad-spectrum MDR pump inhibitor with antibiotics known as substrates of the pump could reduce the mortality that might result from serious infections caused by Gram-positive organisms.

5. Experimental

5.1. Chemistry

The experimental details for the synthesis and the physical chemical data for compounds **43**–**45** have already been described previously.[26] Compound **42** was synthesised by arylation of benzo[*b*]furan according to reference 22 followed by a regioselective carbonylation at position 3 with $TiCl_4$ and Cl_2CHOCH_3.[44]

Reactants and solvents have been supplied by Acros, Aldrich, Alfa Aesar, Fluka and Lancaster. Flash chromatographies were performed with Merck Si 60 (40–63 μm) silica. Melting points were measured on a Köfler bench and are uncorrected. Mass spectra were recorded with a GC/MS FISONS INSTRUMENT MD 800 coupled with a gas chromatogram. NMR spectra were recorded on either a Bruker AMX 300 (^{1}H: 300 MHz; ^{13}C: 75 MHz) or a Bruker DPX 500, 500 MHz. Elemental analyses were performed by the 'Service Central d'Analyse du CNRS' (Solaize, France).

5.1.1. 2-(2,2,2-Trifluoroethoxy)benzo[*b*]thiophene (2). To 2,2,2-trifluoroethanol (3 g; 30 mmol) in DMF (3 mL) was added portionwise sodium hydride (0.72 g; 30 mmol) at 0 °C under argon. The reaction mixture was stirred at 80 °C for 20 min before adding 2-iodobenzo[*b*]thiophene (1.35 g; 5 mmol) and CuI (0.162 g, 1 mmol). After 30 min, the reaction mixture was cooled and filtered on a short pad of silica, eluting with dichloromethane (20 mL). The filtrate was then successively washed with 1 M HCl (2× 20 mL) and water (20 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The yellow oil was purified by column chromatography (silica, cyclohexane) to afford 533 mg of a colourless liquid of **6** (yield = 46%); mp 70–71 °C; Anal. found: C 51.99, H 3.25%, calcd for $C_{10}H_7F_3OS$: C 51.72, H 3.04%; δ_H (300 MHz, $CDCl_3$) 4.47 (q, 2H, J = 7.9 Hz), 6.49 (s, 1H), 7.26 (ddd, 1H, J = 1.2; 7.4; 7.7 Hz), 7.33 (ddd, 1H, J = 1.3; 7.4; 7.7 Hz), 7.58 (d, 1H, J = 7.7 Hz), 7.65 (dd, 1H, J = 1.2; 7.7 Hz); δ_C (75 MHz, $CDCl_3$) 69.7 (q, CH_2, J = 36 Hz), 101.7 (CH), 122.2 (CH), 122.6 (CH), 123.2 (q, CF_3, J = 266 Hz), 123.7 (CH), 125.0 (CH), 132.6 (C), 137.8 (C), 162.7 (C) ppm; *m*/*z* 232 (M^+, 80), 149 (70), 121 (100).

5.1.2. Typical arylation procedure. A suspension of potassium carbonate (3.75 mmol), *n*-Bu_4NBr (1.25 mmol), DCH-18-C-6 (dicyclohexyl-18-crown-6) (1.25 mmol) or PPh_3 (0.125 mmol), substituted benzothiophene or thiophene (1.25 mmol) and aryl halide (0.75 mmol) in *N*,*N*-dimethylformamide (1 mL) was stirred under argon at 140 °C for 5 min. Palladium diacetate (0.0625 mmol) was then added and the resulting mixture was allowed to stir for the time indicated, adding aryl halide (0.375 by 0.375 mmol) stepwise until no further change of the chemical yield (determined by GC) was detected. After cooling to room temperature, the mixture was filtered over Celite®, rinsed with dichloromethane (10 mL) and then successively washed with brine (10 mL), a saturated sodium thiosulfate solution (10 mL) and water (10 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated to give a brown residue. This was then purified by flash column chromatography (silica, cyclohexane/ethyl acetate 95:5) to afford the pure desired compound.

5.1.2.1. 2-Phenylbenzo[*b*]thiophene-3-carboxaldehyde (3). This was prepared in 47% yield according to method A. Pale yellow viscous solid; mp <50 °C; Anal. found: C 75.11, H 4.56, O 7.06, S 13.23%, calcd for $C_{15}H_{10}OS$: C 75.60, H 4.23, O 6.71, S 13.46%; δ_H (500 MHz, $CDCl_3$) 7.44 (ddd, 1H, J = 1.1; 7.1; 7.9 Hz), 7.50–7.62 (m, 6H), 7.82 (d, 1H, J = 7.9 Hz), 8.80 (d, 1H, J = 8.1 Hz), 10.07 (s, 1H, CHO); δ_C (75 MHz, $CDCl_3$) 122.0 (CH),

125.6 (CH), 126.2 (CH), 126.7 (CH), 129.3 (2CH), 130.4 (CH), 130.5 (C), 131.0 (2CH), 132.0 (C), 137.5 (C), 138.4 (C), 161.1 (C), 187.1 (CHO) ppm; *m/z* 238 (M^+, 360), 237 (100), 221 (60).

5.1.2.2. 2-(3′-Chlorophenyl)-benzo[*b*]thiophene-3-carboxaldehyde (8). This was prepared in 41% yield according to method A. Yellow powder; mp 134–136 °C; Anal. found: C 66.11, H 3.47, Cl 13.12%, calcd for $C_{15}H_9ClOS$: C 66.05, H 3.33, Cl 13.00%; δ_H (500 MHz, $CDCl_3$) 7.43 (ddd, 1H, J = 0.6; 6.1; 7.1 Hz), 7.45 (m, 1H), 7.46 (ddd, 1H, J = 1.2; 7.3; 8.1 Hz), 7.49 (ddd, 1H, J = 2.0; 7.1; 7.3 Hz), 7.52 (ddd, 1H, J = 1.3; 7.3; 8.0 Hz), 7.58 (ddd, 1H, J = 0.6; 1.6; 2.0 Hz), 7.83 (ddd, 1H, J = 0.7; 1.2; 8.0 Hz), 8.77 (ddd, 1H, J = 0.7; 1.3; 8.1 Hz), 10.04 (s, 1H); δ_C (75 MHz, $CDCl_3$) 122.1 (CH), 125.7 (CH), 126.5 (CH), 126.9 (CH), 129.2 (CH), 130.5 (CH), 130.5 (CH), 130.7 (CH), 131.0 (C), 133.7 (C), 135.4 (C), 137.3 (C), 138.4 (C), 158.8 (C), 186.6 (CHO) ppm; *m/z* 273 (M+1, 50), 272 (M^+, 98), 271 (M−1, 80), 237 (90), 208 (100), 163 (90).

5.1.2.3. 2-(2′-Chlorophenyl)-benzo[*b*]thiophene-3-carboxaldehyde (9). This was prepared in 59% yield according to method A. Yellow powder; mp 129–130 °C; Anal. found: C 66.19, H 3.38, Cl 12.86%, calcd for $C_{15}H_9ClOS$: C 66.05, H 3.33, Cl 13.00%; δ_H (500 MHz, $CDCl_3$) 7.41 (ddd, 1H, J = 1.0; 7.3; 7.5 Hz), 7.45–7.50 (m, 2H), 7.51–7.59 (m, 3H), 7.87 (d, 1H, J = 8.2 Hz), 8.78 (d, 1H, J = 7.9 Hz), 9.82 (s, 1H); δ_C (75 MHz, $CDCl_3$) 122.1 (CH), 125.6 (CH), 126.4 (CH), 126.7 (CH), 127.2 (CH), 130.6 (CH), 130.8 (C), 131.6 (CH), 132.0 (C), 133.1 (CH), 134.8 (C), 136.5 (C), 139.1 (C), 156.6 (C), 186.5 (CHO) ppm; *m/z* 273 (M+1, 3), 272 (M^+, 5), 271 (M−1, 3), 237 (100), 208 (70), 163 (70).

5.1.2.4. 2-(3′-Pyridyl)-benzo[*b*]thiophene-3-carboxaldehyde (10). This was prepared in 51% yield according to method C. Pale orange powder; mp 96–98 °C; Anal. found: C 70.27, H 3.70, N 5.83%, calcd for $C_{14}H_9NOS$: C 70.27, H 3.79, N 5.85%; δ_H (300 MHz, $CDCl_3$) 7.45–7.52 (m, 2H), 7.55 (ddd, 1H, J = 1.3; 7.3; 8.3 Hz), 7.88 (d, 1H, J = 7.6 Hz), 7.92 (ddd, 1H, J = 1.7; 1.9; 7.9 Hz), 8.76 (dd, 1H, J = 1.8; 5.0 Hz), 8.79 (dd, 1H, J = 1.7; 8.3 Hz), 8.86 (d, 1H, J = 1.9 Hz), 10.04 (s, 1H, CHO); δ_C (75 MHz, $CDCl_3$) 122.1 (CH), 124.0 (CH), 125.7 (CH), 126.7 (CH), 127.0 (CH), 128.4 (C), 131.5 (C), 137.3 (C), 138.0 (CH), 138.6 (C), 150.7 (CH), 151.4 (CH), 156.2 (C), 186.1 (CHO) ppm; *m/z* 239 (M^+, 70), 238 (M−1, 100), 210 (50).

5.1.2.5. 2-(3′-Methoxyphenyl)-benzo[*b*]thiophene-3-carbonitrile (13). This was prepared in 78% yield according to method B. White powder; mp 92–94 °C; Anal. found C 72.79, H 4.21, N 5.17, S 12.16%, calcd for $C_{16}H_{11}NOS$: C 72.43, H 4.18, N 5.28, S 12.16%; δ_H (300 MHz, $CDCl_3$) 3.90 (s, 3H), 7.04 (m, 1H), 7.44–7.51 (m, 5H), 7.86 (d, 1H, J = 7.7 Hz), 7.98 (d, 1H, J = 8.1 Hz); δ_C (75 MHz, $CDCl_3$) 55.6 (CH_3), 102.3 (C), 113.5 (CH), 115.3 (C), 116.6 (CH), 120.8 (CH), 122.5 (CH), 122.8 (CH), 126.3 (CH), 126.3 (CH), 130.5 (CH), 132.8 (C), 137.5 (C), 139.3 (C), 155.0 (C), 160.2 (C) ppm; *m/z* 265 (M^+, 100), 236 (20), 222 (20).

5.1.2.6. 2-(3′,4′,5′-Trimethoxyphenyl)-benzo[*b*]thiophene-3-carbonitrile (14). This was prepared in 70% yield according to method B. Beige crystals; mp 148–150 °C; Anal. found C 66.64, H 4.63, N 4.31%, calcd for $C_{18}H_{17}NO_3S$: C 66.44, H 4.65, N 4.30%; δ_H (300 MHz, $CDCl_3$) 3.93 (s, 3H), 3.96 (s, 6H), 7.13 (s, 2H), 7.46–7.51 (m, 2H), 7.83 (d, 1H, J = 7.9 Hz), 7.96 (d, 1H, J = 7.9 Hz); δ_C (75 MHz, $CDCl_3$) 56.5 ($2CH_3$), 61.2 (CH_3), 101.8 (C), 105.7 (2CH), 115.5 (C), 122.4 (CH), 122.6 (CH), 126.2 (CH), 126.3 (CH), 126.9 (C), 137.2 (C), 139.3 (C), 140.2 (C), 153.8 (2C), 155.2 (C) ppm; *m/z* 325 (M^+, 100).

5.1.2.7. 2-(2′-Pyridyl)-3-methoxybenzo[*b*]thiophene (16). This was prepared in 18% yield according to method C. Pink oil; Anal. found: C 68.86, H 4.59, N 5.69%, calcd for $C_{14}H_{11}NOS$: C 69.68, H 4.59, N 5.80%; δ_H (300 MHz, $CDCl_3$) 4.01 (s, 3H, CH_3), 7.18 (ddd, 1H, J = 1.1; 4.9; 7.6 Hz), 7.34–7.41 (m, 2H), 7.74 (dd, 1H, J = 1.9; 8.1 Hz), 7.77–7.82 (m, 2H), 8.25 (ddd, 1H, J = 1.7; 7.6; 8.1 Hz), 8.63 (ddd, 1H, J = 0.9; 1.7; 4.9 Hz); δ_C (75 MHz, $CDCl_3$) 61.0 (CH_3), 121.4 (CH), 121.6 (CH), 122.3 (CH), 123.3 (CH), 124.3 (CH), 125.8 (CH), 128.9 (C), 134.6 (C), 136.8 (CH), 137.5 (C), 149.5 (CH), 151.8 (C), 162.8 (C) ppm; *m/z* 240 (M−1, 70), 226 (20), 212 (100), 197 (40).

5.1.2.8. 3-(3′-Pyridyl)-benzo[*b*]thiophene-2-carbonitrile (19). This was prepared in 17% yield according to method C. White powder; mp 132–133 °C; Anal. found: C 70.41, H 3.42, N 11.50%, calcd for $C_{14}H_8N_2S$: C 71.16, H 3.41, N 11.86%; δ_H (300 MHz, $CDCl_3$) 7.51 (ddd, 1H, J = 1.0; 7.5; 8.1 Hz), 7.55 (ddd, 1H, J = 1.2; 3.8; 7.9 Hz), 7.61 (ddd, 1H, J = 1.1; 7.5; 8.1 Hz), 7.81 (d, 1H, J = 8.1 Hz), 7.93 (d, 1H, J = 8.1 Hz), 7.98 (ddd, 1H, J = 1.2; 1.3; 7.9 Hz), 8.78 (d, 1H, J = 3.8 Hz), 8.88 (s, 1H); δ_C (75 MHz, $CDCl_3$) 107.8 (C), 114.6 (C), 123.3 (CH), 124.4 (CH), 124.9 (CH), 126.6 (CH), 128.8 (CH), 129.0 (C), 136.7 (C), 137.3 (CH), 141.5 (C), 144.9 (C), 150.1 (CH), 150.8 (CH) ppm; *m/z* 236 (M^+, 100).

5.1.2.9. 3-(4′-Trifluorotoluyl(α,α,α))-benzo[*b*]thiophene-2-carbonitrile (20). This was prepared in 67% yield according to method C. Crystalline orange solid; mp 135–136 °C; Anal. found C 63.11, H 2.56, N 4.72, F 18.05%, calcd for $C_{16}H_8NF_3S$: C 63.32, H 2.61, N 4.61, F 18.80%; δ_H (300 MHz, $CDCl_3$) 7.51 (ddd, 1H, J = 0.9; 7.3; 8.1 Hz), 7.61 (ddd, 1H, J = 1.1; 7.3; 8.1 Hz), 7.75 (d, 2H, J = 8.4 Hz), 7.81 (dd, 1H, J = 0.9; 8.1 Hz), 7.85 (d, 2H, J = 8.4 Hz), 7.94 (d, 1H, J = 8.1 Hz); δ_C (75 MHz, $CDCl_3$) 107.1 (C), 114.4 (C), 122.9 (CH), 124.6 (CH), 126.2 (q, 2CH, J = 4 Hz), 126.2 (CH), 127.6 (q, CF_3, J = 272 Hz), 128.0 (CH), 129.9 (2CH), 131.5 (q, C, J = 32 Hz), 135.1 (C), 135.9 (C), 141.2 (C), 146.7 (C) ppm; *m/z* 304 (20), 303 (M^+, 100), 234 (20), 233 (30).

5.1.2.10. 2-(2,2,2-Trifluoroethoxy)-3-(3′-pyridyl)-benzo-[*b*]thiophene (21). This was prepared in 34% yield according to method C. Orange oil; Anal. found: C 59.29, H 3.39, N 4.49%, calcd for $C_{15}H_{10}F_3NOS$: C 58.25, H 3.26, N 4.53%; δ_H (300 MHz, $CDCl_3$) 4.40 (q, 2H, J = 8.0 Hz), 7.32–7.38 (m, 2H), 7.42 (dd, 1H, J = 4.9; 7.9 Hz), 7.60 (ddd, 1H, J = 1.0; 2.1; 8.1 Hz), 7.73 (ddd, 1H, J = 0.9; 1.9; 8.1 Hz),

7.86 (ddd, 1H, J = 1.5; 1.5; 7.9 Hz), 8.63 (dd, 1H, J = 1.5; 4.9 Hz), 7.94 (d, 1H, J = 1.5 Hz); δ_C (75 MHz, $CDCl_3$) 71.4 (q, CH_2, J = 36 Hz), 116.9 (C), 121.9 (CH), 122.6 (q, CF_3, J = 279 Hz), 122.7 (CH), 123.6 (CH), 125.5 (CH), 124.6 (CH), 125.5 (CH), 128.7 (C), 131.9 (C), 136.9 (C), 137.1 (CH), 148.7 (CH), 150.4 (CH), 157.9 (C) ppm; *m/z* 309 (M^+, 70), 226 (50), 198 (100).

5.1.2.11. 2-(2,2,2-Trifluoroethoxy)-3-(2′-cyanophenyl)-benzo[*b*]thiophene (22). This was prepared in 69% yield according to method C. Pale pink solid; mp 130–131 °C; δ_H (300 MHz, $CDCl_3$) 4.48 (dq, 1H, J = 7.9; 11.9 Hz), 4.69 (dq, 1H, J = 8.1; 11.9 Hz), 7.33–7.43 (m, 3H), 7.48–7.59 (m, 2H), 7.68–7.78 (m, 2H), 7.83 (d, 1H, J = 7.7 Hz); δ_C (75 MHz, $CDCl_3$) 71.0 (q, CH_2, J = 36 Hz), 113.9 (C), 116.4 (C), 118.5 (C), 121.8 (CH), 122.7 (CH), 124.6 (CH), 125.5 (CH), 125.9 (q, CF_3, J = 264 Hz), 128.4 (CH), 131.3 (CH), 131.7 (C), 132.9 (CH), 133.6 (CH), 136.5 (C), 136.9 (C), 158.6 (C) ppm; *m/z* 333 (M^+, 40), 250 (30), 222 (100).

5.1.2.12. 2-(2′-Cyanophenyl)-3-methoxythiophene (23). This was prepared in 51% yield according to method B. Yellow oil; Anal. found: C 67.13, H 4.46, N 6.45%, calcd for $C_{12}H_9NOS$: C 66.95, H 4.21, N 6.51%; δ_H (300 MHz, $CDCl_3$) 3.93 (s, 3H, OCH_3), 6.97 (d, 1H, J = 5.5 Hz), 7.34 (d, 1H, J = 5.5 Hz), 7.37 (ddd, 1H, J = 1.3; 6.8; 7.7 Hz), 7.55 (ddd, 1H, J = 0.7; 1.3; 7.5 Hz), 7.59 (ddd, 1H, J = 1.3; 6.8; 7.5 Hz), 7.72 (ddd, 1H, J = 0.7; 1.3; 7.5 Hz); δ_C (75 MHz, $CDCl_3$) 58.6 (OCH_3), 112.9 (C), 116.5 (CH), 118.8 (C), 125.3 (CH), 127.4 (CH), 131.4 (CH), 132.6 (CH), 133.8 (CH), 136.5 (CH), 142.8 (C), 155.0 (C) ppm; *m/z* 215 (M^+, 100), 200 (65), 186 (20).

5.1.2.13. 2-(3′-Pyridyl)-3-methoxythiophene (24). This was prepared in 68% yield according to method C. Colourless oil; Anal. found: C 63.03, H 4.97, N 7.45%, calcd for $C_{10}H_9NOS$: C 62.80, H 4.74, N 7.32%; δ_H (300 MHz, $CDCl_3$) 3.94 (s, 3H, OCH_3), 6.95 (d, 1H, J = 5.6 Hz), 7.23 (d, 1H, J = 5.6 Hz), 7.28 (dd, 1H, J = 4.0; 8.0 Hz), 8.00 (ddd, 1H, J = 0.6; 2.3; 8.0 Hz), 8.43 (d, 1H, J = 4.0 Hz), 8.97 (s, 1H); δ_C (75 MHz, $CDCl_3$) 58.4 (OCH_3), 115.6 (C), 117.0 (CH), 123.1 (CH), 123.3 (CH), 129.6 (C), 133.3 (CH), 146.7 (CH), 147.2 (CH), 154.8 (C) ppm; *m/z* 191 (M^+, 100), 176 (40), 148 (40).

5.1.2.14. 2,5-Di(3′-pyridyl)-3-methoxythiophene (25). Isolated as a by-product of the previous synthesis (53 mg) as yellow viscous solid (yield = 16%); δ_H (300 MHz, $CDCl_3$) 3.97 (s, 3H, OCH_3), 7.20 (s, 1H), 7.27 (dd, 1H, J = 4.9; 8.1 Hz), 7.29 (dd, 1H, J = 4.9; 7.9 Hz), 7.83 (ddd, 1H, J = 1.4; 2.1; 8.1 Hz), 8.03 (ddd, 1H, J = 1.3; 1.9; 7.9 Hz), 8.44 (dd, 1H, J = 1.4; 4.9 Hz), 8.53 (dd, 1H, J = 1.3; 4.9 Hz), 8.85 (d, 1H, J = 2.1 Hz), 8.94 (d, 1H, J = 1.9 Hz); δ_C (75 MHz, $CDCl_3$) 58.6 (OCH_3), 113.8 (CH), 116.6 (C), 123.5 (CH), 123.6 (CH), 129.1 (C), 129.5 (C), 133.1 (CH), 134.1 (CH), 136.9 (C), 146.0 (CH), 147.2 (CH), 147.9 (CH), 149.1 (CH), 155.1 (C) ppm; *m/z* 268 (M^+, 100), 253 (80), 225 (30), 122 (80).

5.1.2.15. 2-(2′-Pyridyl)-3-methoxythiophene (26). This was prepared in 40% yield according to method C. Yellow oil; Anal. found: C 62.70, H 4.72, N 7.10%, calcd for $C_{10}H_9NOS$: C 62.80, H 4.74, N 7.32%; δ_H (300 MHz, $CDCl_3$) 3.99 (s, 3H, OCH_3), 6.93 (d, 1H, J = 5.6 Hz), 7.05 (ddd, 1H, J = 1.1; 4.9; 7.5 Hz), 7.28 (d, 1H, J = 5.6 Hz), 7.65 (ddd, 1H, J = 1.9; 7.5; 8.0 Hz), 8.08 (ddd, 1H, J = 0.9; 1.1; 8.0 Hz), 8.53 (ddd, 1H, J = 0.9; 1.9; 4.9 Hz); δ_C (75 MHz, $CDCl_3$) 58.9 (OCH_3), 117.2 (CH), 120.7 (CH), 121.0 (CH), 121.6 (C), 126.1 (CH), 136.7 (CH), 149.4 (CH), 152.7 (C), 155.9 (C) ppm; *m/z* 191 (M+1, 50), 190 (M^+, 50), 162 (100).

5.1.2.16. 2-(3′-Pyridyl)-3-cyanothiophene (28). This was prepared in 33% yield according to method B. Yellow powder; mp 119–120 °C; Anal. found: C 64.19, H 3.23, N 14.43%, calcd for $C_{10}H_6N_2S$: C 64.49, H 3.25, N 15.04%; δ_H (300 MHz, $CDCl_3$) 7.34 (d, 1H, J = 5.3 Hz), 7.42 (d, 1H, J = 5.3 Hz), 7.43 (ddd, 1H, J = 0.8; 4.9; 8.0 Hz), 8.12 (ddd, 1H, J = 1.8; 2.2; 8.0 Hz), 8.67 (dd, 1H, J = 1.8; 4.9 Hz), 8.93 (d, 1H, J = 2.2 Hz); δ_C (75 MHz, $CDCl_3$) 107.7 (C), 115.3 (C), 123.9 (CH), 126.8 (CH), 127.7 (C), 130.7 (CH), 134.9 (CH), 148.5 (CH), 149.6 (C), 150.7 (CH) ppm; *m/z* 186 (M^+, 100), 159 (20).

5.1.2.17. 2-(Phenyl)-5-(4″-chlorophenyl)-thiophene-3-carboxaldehyde (29). This was prepared in 43% yield according to method B. Beige oil; δ_H (300 MHz, $CDCl_3$) 7.37 (d, 2H, J = 8.5 Hz), 7.47–7.53 (m, 6H), 7.56 (d, 2H, J = 8.5 Hz), 9.86 (s, 1H, CHO); δ_C (75 MHz, $CDCl_3$) 122.2 (CH), 126.0 (C), 127.2 (2CH), 129.1 (C), 129.2 (2CH), 129.4 (2CH), 129.8 (CH), 130.1 (2CH), 131.3 (C), 131.7 (C), 138.1 (C), 155.7 (C), 185.9 (CHO) ppm; *m/z* 300 (40, M+2), 299 (40, M+1), 298 (100, M^+), 297 (70), 270 (40), 234 (30); SMHR CI found: 299.0296, calcd for $C_{17}H_{11}ClOS,H^+$: 299.0297.

5.1.2.18. 2-(Phenyl)-5-(3″-pyridyl)-thiophene-3-carboxaldehyde (30). This was prepared in 37% yield from compound **27** according to method C. Yellow powder; mp 129–130 °C; δ_H (300 MHz, $CDCl_3$) 7.35–7.65 (m, 6H), 7.81 (s, 1H), 7.91 (ddd, 1H, J = 1.7; 1.9; 7.9 Hz), 8.57 (d, 1H, J = 3.6 Hz), 8.89 (br s, 1H), 9.87 (s, 1H, CHO); δ_C (75 MHz, $CDCl_3$) 123.2 (CH), 123.9 (CH), 129.2 (2CH), 129.9 (CH), 130.2 (2CH), 131.1 (C), 133.2 (CH), 137.4 (C), 138.1 (C), 139.8 (C), 147.0 (CH), 149.4 (CH), 156.1 (C), 185.7 (CHO) ppm; *m/z* 265 (M^+,100), 264 (80), 237 (20); SMHR CI found: 266.0641, calcd for $C_{16}H_{11}NOS,H^+$: 266.0640.

5.1.2.19. 2-(3′-pyridyl)-5-(3″-(α,α, α)-trifluorotoluyl)-3-cyanothiophene (31). This was prepared in 42% yield from compound **28** according to method C. Yellow powder; mp 145–146 °C; Anal. found: C 61.62, H 3.07, N 8.29%, calcd for $C_{17}H_9F_3N_2S$: C 61.81, H 2.75, N 8.48%; δ_H (300 MHz, $CDCl_3$) 7.46 (ddd, 1H, J = 0.6; 4.9; 7.9 Hz), 7.56 (s, 1H), 7.60 (m, 1H), 7.65 (d, 1H, J = 7.9 Hz), 7.78 (d, 1H, J = 7.7 Hz), 7.83 (br s, 1H), 8.18 (ddd, 1H, J = 1.5; 2.3; 7.9 Hz), 8.71 (dd, 1H, J = 1.5; 4.9 Hz), 8.98 (d, 1H, J = 2.3 Hz); δ_C (75 MHz, $CDCl_3$) 108.4 (C), 115.0 (C), 122.9 (q, CH, J = 4 Hz), 123.9 (q, CF_3, J = 273 Hz), 124.0 (CH), 125.8 (q, CH, J = 4 Hz), 126.9 (CH), 129.3 (C), 130.1 (CH), 132.0 (q, C, J = 33 Hz), 132.9 (CH), 134.7 (CH), 141.0 (C), 143.7 (C), 148.4 (CH), 149.1 (C), 151.0 (CH) ppm; *m/z* 330 (M^+, 100).

5.1.2.20. 2-(3′-Pyridyl)-5-(4″-chlorophenyl)-3-cyanothiophene (32). This was prepared in 54% yield from compound **28** according to method C. Yellow powder; mp 183-184 °C; Anal. found: C 64.17, H 3.08, N 8.76, Cl 12.09%, calcd for $C_{16}H_9ClN_2S$: C 64.75, H 3.06, N 9.44, Cl 11.95%; δ_H (300 MHz, $CDCl_3$): 7.38–7.43 (m, 1H), 7.40 (d, 2H, J = 8.6 Hz), 7.44 (s, 1H), 7.50 (d, 2H, J = 8.6 Hz), 8.14 (ddd, 1H, J = 1.6; 2.0; 8.0 Hz), 8.68 (dd, 1H, J = 1.6; 4.8 Hz), 8.95 (d, 1H, J 2.0 Hz); δ_C (75 MHz, $CDCl_3$) 108.2 (C), 115.1 (C), 124.0 (CH), 126.1 (CH), 127.2 (2CH), 127.6 (C), 129.6 (2CH), 130.5 (C), 134.6 (CH), 135.2 (C), 144.2 (C), 148.3 (CH), 148.3 (C), 150.8 (CH) ppm; *m*/*z* 298 (M+2, 30), 296 (M^+, 100), 261 (10).

5.1.3. General procedure for the 'one pot' Heck type arylation. A suspension of potassium carbonate (3 equiv), DCH-18-C-6 (1 equiv mmol), thiophene (1 equiv mmol) and aryl halide (0.5 equiv) in *N*,*N*-dimethylformamide (1 M) was stirred under argon at 100 °C for 5 min. Palladium diacetate (5% mol) was then added and the resulting mixture was allowed to stir at 100 °C, adding portion wise the 0.5 equiv of the aryl halide until no further change of the chemical yield (determined by GC) was detected. An excess of the second aryl halide was then added and the mixture was allowed to stir until no further change of the chemical yield. After cooling to room temperature, the mixture was filtered over Celite®, rinsed with dichloromethane (30 mL) and then successively washed with a saturated KCl solution (2× 10 mL) and water (10 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated to give a brown residue. The latter was then purified by flash column chromatography (silica, cyclohexane/ethyl acetate 95:5) to afford the pure desired compound.

5.1.3.1. 2-(3′-Pyridyl)-5-(3″-(α,α,α)-trifluorotoluyl)-3-methoxythiophene (33). This product was synthesised from 3-methoxythiophene (154 mg, 1.35 mmol) and 3-bromopyridine (213 mg, 1.35 mmol). After 7 h of stirring, 3-bromo-(α,α,α)-trifluorotoluene (303 mg, 2 mmol) was added and the reaction was left for an additional 13 h at 100 °C. After purification, 187 mg of compound **33** was isolated as a beige powder (yield = 39%); mp 106–107 °C; Anal. found: C 60.48, H 3.64, N 4.17%, calcd for $C_{17}H_{12}F_3NOS$: C 60.89, H 3.61, N 4.18%; δ_H (300 MHz, $CDCl_3$) 3.97 (s, 3H, OCH_3), 7.23 (s, 1H), 7.30 (m, 1H), 7.48–7.58 (m, 2H), 7.77 (d, 1H, J = 7.2 Hz), 7.84 (s, 1H), 8.07 (d, 1H, J = 8.1 Hz), 8.47 (br s, 1H), 9.02 (bs 1H); δ_C (75 MHz, $CDCl_3$) 59.0 (OCH_3), 114.0 (CH), 116.8 (C), 121.9 (q, CH, J = 4 Hz), 124.0 (q, CF_3, J = 273 Hz), 124.5 (q, CH, J = 4 Hz), 128.4 (CH), 128.4 (C), 129.7 (CH), 131.5 (q, C, J = 32 Hz), 133.6 (CH), 133.6 (CH), 134.7 (C), 139.3 (C), 147.3 (CH), 147.6 (CH), 155.4 (C) ppm; *m*/*z* 335 (M^+, 100), 320 (95), 292 (85), 223 (25).

5.1.3.2. 2-(3′-(α,α,α)-Trifluorotoluyl)-5-(3″-pyridyl)-3-methoxythiophene (34). This product was synthesised from 3-methoxythiophene (194 mg, 1.7 mmol) and 3-bromo-(α,α,α)-trifluorotoluene (382 mg, 1.7 mmol). After 10 h of stirring, 3-bromopyridine (537 mg, 3.4 mmol) was added and the reaction was left for an additional 10 h at 100 °C. After purification, 212 mg of compound **34** was isolated as a yellow powder (yield = 38%); mp 114–115 °C; Anal. found: C 61.12, H 3.39, F 16.90, N 4.47%, calcd for $C_{17}H_{12}F_3NOS$: C 60.89, H 3.61, F 17.00, N 4.18%; δ_H (300 MHz, $CDCl_3$) 4.00 (s, 3H, OCH_3), 7.20 (s, 1H), 7.31 (dd, 1H, J = 4.9; 7.9 Hz), 7.46–7.49 (m, 2H), 7.84 (ddd, 1H, J = 1.7; 2.2; 7.9 Hz), 7.91 (m, 1H), 8.06 (br s, 1H), 8.54 (dd, 1H, J = 1.7; 4.9 Hz), 9.05 (d, 1H, J = 2.2 Hz); δ_C (75 MHz, $CDCl_3$) 59.0 (OCH_3), 114.1 (CH), 119.2 (C), 123.1 (q, CH, J = 4 Hz), 123.3 (q, CH, J = 4 Hz), 124.2 (q, CF_3, J = 272 Hz), 129.1 (CH), 129.7 (CH), 130.0 (C), 131.0 (q, C, J = 32 Hz), 132.3 (CH), 133.9 (C), 136.9 (C), 146.3 (CH), 149.0 (CH), 155.1 (C) ppm; *m*/*z* 335 (100, M^+), 320 (70), 292 (25), 223 (20), 189 (70).

5.1.3.3. 2,5-Di(3′-(α,α,α)-trifluorotoluyl)-3-methoxythiophene (35). Isolated as a by-product of the previous synthesis (40 mg) as a beige solid (yield = 14%); mp 73–74 °C; δ_H (300 MHz, $CDCl_3$) 4.01 (s, 3H, OCH_3), 7.22 (s, 1H), 7.45–7.55 (m, 4H), 7.77 (bd, 1H, J = 7.5 Hz), 7.87 (br s, 1H), 7.90 (m, 1H), 8.06 (s, 1H); δ_C (75 MHz, $CDCl_3$) 59.0 (OCH_3), 114.2 (CH), 119.2 (C), 121.9 (q, CH, J = 4 Hz), 123.1 (q, CH, J = 4 Hz), 123.4 (q, CH, J = 4 Hz), 124.1 (q, CF_3, J = 272 Hz), 124.3 (q, CF_3, J = 273 Hz), 124.6 (q, CH, J = 4 Hz), 128.4 (CH), 129.2 (CH), 129.7 (CH), 129.8 (CH), 131.1 (q, C, J = 33 Hz), 131.6 (q, C, J = 32 Hz), 134.0 (C), 134.8 (C), 139.0 (C), 155.1 (C) ppm; *m*/*z* 402 (15, M^+), 290 (10), 189 (100).

5.1.3.4. 2-(3′-Pyridyl)-5-(4″-chlorophenyl)-3-(2,2,2-trifluoroethoxy)thiophene (36). This product was synthesised from 3-trifluoroethoxythiophene (182 mg, 1 mmol) and 3-bromopyridine (159 mg, 1 mmol). After 5 h of stirring, 4-chlorobromobenzene (290 mg, 1.5 mmol) was added and the reaction was left for an additional 14 h at 100 °C. After purification, 111 mg of compound **36** was isolated as an orange powder (yield = 30%); mp 97–98 °C; δ_H (300 MHz, $CDCl_3$) 4.50 (q, 2H, CH_2, J = 8.1 Hz), 7.07 (s, 1H), 7.34–7.42 (m, 1H), 7.37 (d, 2H, J = 7.9 Hz), 7.50 (d, 2H, J = 7.9 Hz), 8.04 (d, 1H, J = 7.7 Hz), 8.57 (br s, 1H), 8.98 (br s, 1H); δ_C (75 MHz, $CDCl_3$) 68.8 (q, CH_2, J = 36 Hz), 114.0 (CH), 119.4 (C), 123.1 (q, CF_3, J = 279 Hz), 126.5 (2CH), 129.3 (CH), 129.3 (C), 129.4 (2CH), 131.9 (C), 134.0 (CH), 134.4 (C), 140.8 (C), 147.5 (CH), 147.8 (CH), 152.0 (C) ppm; *m*/*z* 371 (M+2, 15), 369 (M^+, 50), 288 (15), 286 (40), 258 (20), 251 (20), 122 (100).

5.1.3.5. 2-(4′-Chlorophenyl)-5-(3″-pyridyl)-3-(2,2,2-trifluoroethoxy)thiophene (37). This product was synthesised from 3-trifluoroethoxythiophene (182 mg, 1 mmol) and 4-chlorobromobenzene (192 mg, 1 mmol). After 3 h of stirring, 3-bromopyridine (237 mg, 1.5 mmol) was added and the reaction was left for an additional 15 h at 100 °C. After purification, 144 mg of compound **37** was isolated as a red powder (yield = 39%); mp 162–163 °C; δ_H (300 MHz, $CDCl_3$) 4.43 (q, 2H, CH_2, J = 8.1 Hz), 7.13 (s, 1H), 7.35 (ddd, 1H, J = 0.7; 4.8; 8.1 Hz), 7.39 (d, 2H, J = 8.5 Hz), 7.65 (d, 2H, J = 8.5 Hz), 7.83 (ddd, 1H, J = 1.7; 2.5; 8.1 Hz), 8.56 (dd, 1H, J = 1.7; 4.8 Hz), 8.86 (d, 1H, J = 2.5 Hz); δ_C

(75 MHz, $CDCl_3$) 68.9 (q, CH_2, J = 36 Hz), 115.4 (CH), 123.6 (C), 123.9 (q, CF_3, J = 278 Hz), 124.0 (CH), 128.4 (2CH), 129.1 (2CH), 129.8 (C), 130.5 (C), 132.6 (CH), 133.4 (C), 136.8 (C), 146.2 (CH), 149.0 (CH), 151.2 (C) ppm; *m/z* 371 (M+2, 20), 369 (M^+, 60), 288 (15), 286 (50), 251 (100), 157 (30), 155 (90).

5.1.3.6. 2,5-Di(4′-chlorophenyl)-3-(2,2,2-trifluoroethoxy)thiophene (38). Isolated as a by-product of the previous synthesis (50 mg) as a yellow solid (yield = 12%); mp 99–101 °C; δ_H (300 MHz, $CDCl_3$) 4.39 (q, 2H, CH_2, J = 8.1 Hz), 6.87 (s, 1H), 7.35 (d, 2H, J = 8.8 Hz), 7.36 (d, 2H, J = 8.6 Hz), 7.50 (d, 2H, J = 8.8 Hz), 7.63 (d, 2H, J = 8.6 Hz); δ_C (75 MHz, $CDCl_3$) 68.9 (q, CH_2, J = 35 Hz), 114.6 (CH), 124.0 (q, CF_3, J = 277 Hz), 126.5 (2CH), 128.3 (2CH), 128.7 (C), 129.1 (2CH), 129.4 (2CH), 130.8 (C), 132.2 (C), 133.1 (C), 134.2 (C), 139.7 (C), 151.1 (C) ppm; *m/z* 406 (M+4, 20), 404 (M+2, 60), 402 (M^+, 80), 323 (5), 321 (15), 319 (20), 286 (30), 284 (98), 157 (40), 155 (100); SMHR CI found: 402.9936, calcd for $C_{18}H_{11}Cl_2F_3OS,H^+$: 402.9938.

5.1.3.7. 4-(Benzo[*b*]thiophene-3-ylmethylene)-2-phenyl-4H-oxazol-5-one (39a). A mixture of benzo[*b*]thiophene-3-carboxaldehyde (300 mg, 1.85 mmol), sodium acetate (228 mg, 2.78 mmol), hippuric acid (829 mg, 4.63 mmol) and acetic anhydride (9 mL) was heated at 80 °C for 5 h. The reaction mixture was concentrated several times with cyclohexane and methanol (20 mL) was added. The resulting precipitate was collected on a glass filter and washed with small portions of methanol to afford **39a** (506 mg, 89% yield) as yellow crystals; mp 224–226 °C; Anal. found: C, 70.80; H, 3.59; N, 4.50; O, 10.65%; calcd for $C_{18}H_{11}NO_2S$: C, 70.80; H, 3.63; N, 4.59; O, 10.48%; δ_H (500 MHz, $CDCl_3$) 7.45 (ddd, 1H, J = 0.8; 7.6; 8.0 Hz), 7.52 (ddd, 1H, J = 1.1; 7.6; 8.0 Hz), 7.55 (dd, 2H, J = 7.7; 7.9 Hz), 7.61 (s, 1H), 7.63 (m, 1H), 7.93 (d, 1H, J = 7.6 Hz), 8.05 (d, 1H, J = 7.6 Hz), 8.21 (dd, 2H, J = 1.6; 7.7 Hz), 9.14 (s, 1H); δ_C (125 MHz, $CDCl_3$) 121.3 (CH), 121.6 (CH), 121.7 (CH), 123.1 (CH), 125.4 (CH), 125.7 (C), 128.5 (2CH), 129.2 (2CH), 129.7 (C), 133.4 (C), 133.6 (CH), 135.8 (CH), 138.3 (C), 139.6 (C), 163.8 (NCO), 167.6 (CO_2) ppm; *m/z* 306 (M+1, 5), 305 (M^+, 15), 172 (15), 105 (100), 77 (90).

5.1.3.8. 4-(2-(4′-Methoxyphenyl)-benzo[*b*]thiophene-3-ylmethylene)-2-phenyl-4H-oxazol-5-one (39b). A mixture of **4** (1500 mg, 5.59 mmol), sodium acetate (689 mg, 8.40 mmol), hippuric acid (5017 mg, 28 mmol) and acetic anhydride (15 mL) was heated at 80 °C for 18 h. The reaction mixture was concentrated several times with cyclohexane and methanol (50 mL) was added. The resulting precipitate was collected on a glass filter and washed with small portions of methanol to give a crude product. Recrystallisation from $MeOH/CH_2Cl_2$ (10:1) gave **39b** (1020 mg, 44% yield) as yellow crystals; mp 206–208 °C; Anal. found: C, 72.69; H, 4.15; N, 3.42; O, 11.67%; calcd for $C_{25}H_{17}NO_3S$: C, 72.97; H, 4.16; N, 3.40; O, 11.66% δ_H (300 MHz, $CDCl_3$) 3.88 (s, 3H), 7.02 (d, 2H, J = 8.5 Hz), 7.39–7.54 (m, 7H), 7.60 (m, 1H), 7.85 (d, 1H, J = 7.5 Hz), 8.09 (d, 2H, J = 7.2 Hz), 8.69 (d, 1H, J = 8.1 Hz) ppm; *m/z* 412.1 (MH^+).

5.1.3.9. 4-(2-(4′-Benzotrifluoride)-benzo[*b*]thiophene-3-ylmethylene)-2-phenyl-4H-oxazol-5-one (39c). A mixture of **5** (500 mg, 1.63 mmol), sodium acetate (201 mg, 2.45 mmol), hippuric acid (730 mg, 4.07 mmol) and acetic anhydride (5 mL) was heated at 80 °C for 18 h. The reaction mixture was concentrated several times with cyclohexane and methanol (10 mL) was added. The resulting precipitate was collected on a glass filter and washed with small portions of methanol to afford **39c** (420 mg, 57% yield) as yellow crystals; mp 184–186 °C; Anal. found: C, 66.43; H, 3.22; N, 3.09%; calcd for $C_{25}H_{14}F_3NO_2S$: C, 66.81; H, 3.14; N, 3.12%; δ_H (300 MHz, $CDCl_3$) 7.44 (s, 1H), 7.45–7.77 (m, 9H), 7.90 (d, 1H, J = 7.2 Hz), 7.98 (d, 2H, J = 7.7 Hz), 8.52 (d, 1H, J = 7.6 Hz) ppm; *m/z* 450.1 (MH^+).

5.1.3.10. 4-(2-(4′-Benzonitrile-)benzo[*b*]thiophene-3-ylmethylene)-2-phenyl-4H-oxazol-5-one (39d). A mixture of **6** (250 mg, 0.95 mmol), sodium acetate (117 mg, 1.43 mmol), hippuric acid (851 mg, 4.75 mmol) and acetic anhydride (10 mL) was heated at 80 °C for 18 h. The reaction mixture was concentrated several times with cyclohexane and methanol (10 mL) was added. The resulting precipitate was collected on a glass filter and washed with small portions of methanol to afford **39d** (188 mg, 48% yield) as yellow crystals; mp 224–225 °C; Anal. found: C 73.42, H 3.41, N 6.89, O 7.94%, calcd for $C_{25}H_{14}N_2O_2S$: C 73.88, H 3.47, N 6.89, O 7.87%; δ_H (300 MHz, $CDCl_3$) 7.43 (s, 1H), 7.45–7.7.57 (m, 4H), 7.61 (tt, 1H, J = 1.5; 7.4 Hz), 7.67 (d, 2H, J = 8.5 Hz), 7.75 (d, 2H, J = 8.5 Hz), 7.90 (d, 1H, J = 7.1 Hz), 7.96 (d, 2H, J = 7.7 Hz), 8.45 (d, 1H, J = 7.4 Hz) ppm; *m/z* 407.0 (MH^+).

5.1.3.11. Methyl-(*Z*)-2-benzylamino-3-benzo[*b*]thiophene-propenoate (40a). A solution of **39a** (300 mg, 0.98 mmol) and triethylamine (0.3 mL) in methanol (50 mL) was refluxed for 2 h. Recrystallisation of the crude mixture from CH_2Cl_2/cyclohexane (20:80) gave **40a** (270 mg, 82% yield) as white crystals; mp 185–186 °C; Anal. found: C, 67.46; H, 4.25; N, 4.06; O, 14.02%; calcd for $C_{19}H_{15}NO_3S$: C, 67.64; H, 4.48; N, 4.15; O, 14.23%; δ_H (500 MHz, $CDCl_3$) 3.89 (s, 3H), 7.39 (ddd, 1H, J = 1.1; 7.3; 7.9 Hz), 7.44 (ddd, 1H, J = 1.2; 7.3; 7.9 Hz), 7.46–7.50 (m, 2H), 7.56 (dd, 1H, J = 1.5; 7.4 Hz), 7.72 (s, 1H), 7.78 (s, 1H), 7.79 (s, 1H), 7.85 (dd, 1H, J = 1.2; 7.3 Hz), 7.87 (m, 2H), 7.92 (dd, 1H, J = 1.1; 7.3 Hz); δ_C (75 MHz, $CDCl_3$) 53.0 (OCH_3), 121.8 (CH), 122.9 (CH), 123.3 (CH), 124.9 (CH), 125.2 (CH), 127.7 (2 CH), 128.9 (2 CH), 129.0 (CH), 129.1 (C), 132.5 (CH), 133.6 (C), 138.4 (C), 139.4 (C), 165.8 (CO), 165.9 (CO_2Me) ppm; *m/z* 338.0 (MH^+).

5.1.3.12. Methyl-(*Z*)-2-benzylamino-3-(2-(4′-methoxyphenyl)-benzo[*b*]thiophene)-propenoate (40b). A solution of **39b** (500 mg, 1.22 mmol) and triethylamine (0.34 mL) in methanol (40 mL) was refluxed for 2 h. Recrystallisation of the crude mixture from CH_2Cl_2/cyclohexane (20:80) gave **40b** (480 mg, 89% yield) as white crystals; mp 156–158 °C; Anal. found: C, 70.25; H, 4.81; N, 3.05; O, 14.72%; calcd for $C_{26}H_{21}NO_4S$: C, 70.41; H, 4.77; N, 3.16; O, 14.43%; δ_H (300 MHz, $CDCl_3$) 3.85 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.90

(d, 2H, $J = 8.9$ Hz), 7.27–7.48 (m, 9H), 7.53 (d, 2H, $J = 8.9$ Hz), 7.73 (d, 1H, $J = 7.4$ Hz), 7.78 (d, 1H, $J = 7.4$ Hz) ppm; δ_C (75 MHz, $CDCl_3$) 53.2 (CH_3), 55.8 (CH_3), 114.9 (2CH), 122.4 (CH), 122.6 (CH), 123.1 (CH), 124.9 (C), 125.0 (CH), 125.1 (CH), 126.8 (C), 127.6 (C), 127.8 (2CH), 128.7 (2CH), 131.8 (2CH), 132.3 (CH), 133.6 (C), 139.0 (C), 139.3 (C), 143.6 (C), 160.6 (C), 165.1 (CO), 165.4 (CO_2Me) ppm; *m*/*z* 444.0 (MH^+).

5.1.3.13. Methyl-(*Z*)-2-benzylamino-3-(2-(4′-benzotrifluoride)-benzo[*b*]thiophene)-propenoate (40c). A solution of **39c** (300 mg, 0.67 mmol) and triethylamine (0.1 mL) in methanol (50 mL) was refluxed for 2 h. Recrystallisation of the crude mixture from CH_2Cl_2/cyclohexane gave **40c** (281 mg, 88% yield) as white crystals; mp 175–176 °C; Anal. found: C, 64.99; H, 3.81; N, 2.94%; calcd for $C_{26}H_{18}F_3NO_3S$: C, 64.86; H, 3.77; N, 2.91%; δ_H (300 MHz, $CDCl_3$) 3.91 (s, 3H, OCH_3), 7.26–7.46 (m, 7H), 7.50 (s, 1H), 7.57–7.64 (m, 3H), 7.73 (d, 2H, $J = 8.2$ Hz), 7.79 (dd, 1H, $J = 1.5$; 7.2 Hz), 7.82 (dd, 1H, $J = 1.5$; 7.2 Hz); δ_C (75 MHz, $CDCl_3$) 53.1 (CH_3), 121.4 (CH), 122.5 (CH), 123.2 (CH), 124.1 (q, CF_3, $J = 270$ Hz), 125.0 (CH), 125.4 (CH), 125.9 (q, 2CH, $J = 4$ Hz), 127.3 (C), 127.4 (2CH), 127.5 (C), 128.5 (2CH), 129.6 (2CH), 130.5 (q, C, $J = 32$ Hz), 132.2 (CH), 133.2 (C), 137.9 (C), 138.5 (C), 139.1 (C), 141.0 (C), 164.7 (CO), 165.3 (CO_2Me) ppm; *m*/*z* 482.0 (MH^+).

5.1.3.14. Methyl-(*Z*)-2-benzylamino-3-(2-(4′-benzonitrile)-benzo[*b*]thiophene)-propenoate (40d). A solution of **39d** (180 mg, 0.44 mmol) and triethylamine (0.3 mL) in methanol (50 mL) was refluxed for 2 h. The reaction mixture was concentrated to give a crude product, which was then purified by flash chromatography (CH_2Cl_2) to afford **40d** (170 mg, 88% yield) as white crystals; mp >110 °C; Anal. found: C, 71.15; H, 4.17; N, 4.06; O, 10.89%; calcd for $C_{26}H_{18}N_2O_3S$: C, 71.22; H, 4.14; N, 6.39; O, 10.95%; δ_H (300 MHz, $CDCl_3$) 3.91 (s, 3H), 7.31–7.42 (m, 4H), 7.43–7.50 (m, 3H), 7.46 (s, 1H), 7.59–7.66 (m, 3H), 7.72–7.77 (m, 3H), 7.82 (dd, 1H, $J = 1.7$; 7.1 Hz); δ_C (75 MHz, $CDCl_3$) 53.5 (CH_3), 112.4 (C), 118.9 (C), 121.8 (CH), 122.9 (CH), 123.7 (CH), 125.4 (CH), 125.9 (CH), 127.7 (2 CH), 127.9 (C), 128.4 (C), 128.9 (2 CH), 130.3 (2 CH), 132.6 (CH), 132.9 (2 CH), 133.5 (C), 138.5 (C), 139.2 (C), 139.5 (C), 140.5 (C), 165.0 (CO), 165.5 (CO_2Me) ppm; *m*/*z* 439.0 (MH^+).

5.1.3.15. (±)-*N*-Benzoyl-3-(2-(4′-methoxyphenyl)-benzo[*b*]thiophene)-alanine methyl ester (41b). A solution of **40b** (300 mg, 0.676 mmol) in dichloromethane (10 mL) was charged in a 40 mL autoclave containing 10% palladium/carbon (300 mg). The atmosphere was replaced by argon and pressurised to 20 bars with hydrogen at room temperature. The autoclave was heated at 60 °C and left stirring for 48 h. The catalyst was then separated from the reaction mixture by filtration over silica and the solvent was removed in vacuo to afford **41b** (301 mg, 100% yield) as white crystals; mp 112–114 °C; Anal. found: C, 70.44; H, 5.62; N, 3.13; O, 14.24%; calcd for $C_{26}H_{23}NO_4S$: C, 70.09; H, 5.20; N, 3.14; O, 14.36%; δ_H (300 MHz, $CDCl_3$) 3.54 (s, 3H, OCH_3), 3.63 (m, 2H), 3.85 (s, 3H, OCH_3), 5.04 (m, 1H), 6.47 (m, 1H, NH), 6.95 (d, 2H, $J = 8.7$ Hz), 7.32–7.57 (m, 9H), 7.84 (d, 1H, $J = 7.4$ Hz), 7.95 (d, 1H, $J = 7.9$ Hz); δ_C (75 MHz, $CDCl_3$) 28.9 (CH_2), 52.5 (CH_3), 52.9 (CH), 55.4 (CH_3), 114.4 (2CH), 122.1 (CH), 122.2 (CH), 124.4 (CH), 124.6 (CH), 125.7 (C), 126.3 (C), 127.1 (2CH), 128.4 (2CH), 131.0 (2CH), 131.7 (CH), 133.5 (C), 139.0 (C), 140.3 (C), 141.3 (C), 159.7 (C), 166.8 (CO), 172.1 (CO_2Me) ppm; *m*/*z* 446.1 (MH^+).

5.1.3.16. (±)-*N*-Benzoyl-3-(2-(4′-benzotrifluoride)-benzo[*b*]thiophene)-alanine methyl ester (41c). A solution of **40c** (100 mg, 0.21 mmol) in dichloromethane (10 mL) was charged in a 40 mL autoclave containing 10% palladium/carbon (100 mg). The atmosphere was replaced by argon and pressurised to 20 bars with hydrogen at room temperature. The autoclave was heated at 60 °C with stirring for 48 h. After the catalyst was separated from the reaction mixture by filtration over silica, the solvent was removed in vacuo to afford **41c** (100 mg, 100% yield) as white crystals; mp 138–140 °C; Anal. found: C, 65.03; H, 4.54; N, 3.02; F, 11.32%; calcd for $C_{26}H_{20}F_3NO_3S$: C, 64.59; H, 4.17; N, 2.90; F, 11.79%; δ_H (300 MHz, $CDCl_3$) 3.48 (s, 3H, OCH_3), 3.64 (m, 2H), 5.06 (m, 1H), 6.42 (m, 1H, NH), 7.31–7.52 (m, 7H), 7.58 (d, 2H, $J = 8.5$ Hz), 7.63 (d, 2H, $J = 8.5$ Hz), 7.85 (dd, 1H, $J = 1.2$; 6.8 Hz), 7.97 (dd, 1H, $J = 1.2$; 6.9 Hz) ppm; δ_C (75 MHz, $CDCl_3$) 29.1 (CH_2), 52.6 (CH_3), 52.7 (CH), 122.4 (CH), 122.6 (CH), 124.4 (q, CF_3, $J = 271$ Hz), 124.9 (CH), 125.2 (CH), 125.9 (q, 2CH, $J = 4$ Hz), 127.0 (2CH), 127.4 (C), 128.6 (2CH), 130.2 (2CH), 130.4 (q, C, $J = 32$ Hz), 131.9 (CH), 133.4 (C), 138.1 (C), 139.3 (C), 139.6 (C), 140.2 (C), 166.7 (CO), 172.0 (CO_2Me) ppm; *m*/*z* 484.0 (MH^+).

5.1.3.17. (±)-*N*-Benzoyl-3-(2-(4′-benzonitrile)-benzo[*b*]-thiophene)-alanine methyl ester (41d). A solution of **40d** (100 mg, 0.23 mmol) in dichloromethane (10 mL) was charged in a 40 mL autoclave containing 10% palladium/carbon (100 mg). The atmosphere was replaced by argon and pressurised to 20 bars with hydrogen at room temperature. The autoclave was heated at 60 °C with stirring for 48 h. After the catalyst was separated from the reaction mixture by filtration over silica, the solvent was removed in vacuo to afford **41d** (100 mg, 100% yield) as white crystals; mp 126–129 °C; Anal. found: C, 70.90; H, 5.13; N, 6.01; O, 11.03%; calcd for $C_{26}H_{20}N_2O_3S$: C, 70.89; H, 4.58; N, 6.36; O, 10.90%; δ_H (300 MHz, $CDCl_3$) 3.48 (s, 3H, OCH_3), 3.64 (m, 2H), 5.03 (m, 1H), 6.48 (d, 1H, $J = 7.7$ Hz), 7.34–7.54 (m, 7H), 7.56 (d, 2H, $J = 7.5$ Hz), 7.60 (d, 2H, $J = 7.5$ Hz), 7.84 (dd, 1H, $J = 1.3$; 7.3 Hz), 7.95 (dd, 1H, $J = 1.2$; 7.4 Hz); δ_C (75 MHz, $CDCl_3$) 29.2 (CH_2), 52.6 (CH_3), 52.7 (CH), 111.9 (C), 118.5 (C), 122.5 (CH), 122.7 (CH), 125.0 (CH), 125.4 (CH), 127.1 (2 CH), 127.8 (C), 128.6 (2 CH), 130.4 (2 CH), 132.1 (CH), 132.6 (2 CH), 133.2 (C), 139.1 (C), 139.2 (C), 139.4 (C), 140.2 (C), 166.5 (CO), 171.9 (CO_2Me) ppm; *m*/*z* 441.1 (MH^+).

5.1.3.18. 2-Phenylbenzo[*b*]furan-3-carboxaldehyde (42). This was prepared in 47% yield according to reference 44. Pale yellow powder; mp: 68–70 °C (unrecrystal-

lised); δ_H (300 MHz, $CDCl_3$) 7.26–7.37 (m, 2H), 7.43–7.57 (m, 4H), 7.73–7.82 (m, 2H), 8.18–8.21 (m, 1H), 10,26 (s, 1H, CHO); δ_C (75 MHz, $CDCl_3$) 111.5 (CH), 117.8 (C), 123.0 (CH), 125.2 (CH), 125.7 (C), 126.4 (C), 128.9 (C), 129.5 (4CH), 131.4 (CH), 154.3 (C), 165.7 (C), 187.0 (CHO) ppm.

5.2. Biological evaluation

5.2.1. Bacterial strains. The following bacterial strains were used: *S. aureus* (ATCC 25923, Gram-positive susceptible strain), *S. aureus* SA-1199B[40] (harbouring resistance to fluoroquinolones through over-expression of the NorA efflux pump and called *S. aureus* NorA) and its susceptible parental strain *S. aureus* SA-1199, *S. aureus* MsrA[4] (resistant to 14- and 15-membered macrolides, harbouring the multicopies plasmid pUL 5054 and called *S. aureus*) MsrA. *Bacillus subtilis* ΔΔNA was obtained thanks to Professor A. A. Neyfakh.[8] Plasmid pBEV, carrying the NorA gene under the control of a strong *B. subtilis* promoter, was introduced into *B. Subtilis* ΔΔ (*B. subtilis* BD170/*bmr::cat blt::erm*), a strain with the two genes encoding the efflux pumps Bmr and Blt.[45] Strains were grown in Mueller–Hinton broth (MH, Bio-Rad) or Luria–Bertani broth (LB, Difco) at 37 °C.

5.2.2. Determination of antibacterial susceptibilities. The antibacterial activity of the assayed compounds was carried out in liquid medium by using a Biomek 2000 robot (Beckman) and microtitration plates. The final volume in each well was 200 μl. Five microliters of a DMSO solution of the compound was added to each well containing MH medium and 10^6 CFU/mL (colony forming units) of the bacteria. The final molecular concentration was therefore 100 mg/L. Ampicillin was used as a positive control and 2 μl of DMSO as a negative control. The plates were incubated at 37 °C, and bacterial growth was monitored at 650 nm after 1, 2, 8 and 24 h of growth. The compound was considered as very active (indicated as ++ in the tables) if there was no bacterial growth, as active (+ in the tables) if bacterial growth was less than 10% of the negative control and as not active (− in the tables) if there was no effect or less than 90% of inhibition of growth. The minimal inhibitory concentrations (MICs) were determined by using a twofold dilution technique in Mueller–Hinton broth, to which 10^6CFU of bacteria per milliliter was added. The MIC is defined as the lowest concentration at which there was no visible growth after 18 h at 37 °C. Experiments were carried out in triplicate.

5.2.3. Inhibition of resistance mechanisms. Experiments were carried out as above, but using the resistant strains, and looking for the effect of combining the assayed compound and the antibiotic to which the strains were resistant. For that purpose, ciprofloxacin (*S. aureus* NorA) or erythromycin (*S. aureus* MsrA) was added to each well at a concentration of MIC/8, that is, 2 mg/L and 32 mg/L, respectively. The molecule, added at a concentration of 100 mg/L, was considered as a possible good (++) or fair (+) inhibitor of the pump if bacterial growth was totally, or at least 90%, inhibited, respectively. Reserpine, a plant alkaloïd inhibiting several MDR efflux pumps,[8] was used at 20 mg/L as a positive control for SA-1199B.

For the construction of an isobologram, that is, the respective concentrations of antibacterial agent and inhibitor able to restore the activity of the former, the twofold dilution technique in Mueller–Hinton broth was applied to the inhibitor, starting from a concentration of 100 mg/L in the lines of the microplate, and to the antibiotic, starting from its MIC against the resistant strain, in the columns. The isobologram was drawn with the active concentrations of the molecule as a function of the concentration of ciprofloxacin.

5.2.4. Ethidium bromide efflux. Efflux of ethidium bromide (EtBr) from cells was performed essentially as described previously.[46] LB medium was inoculated with an overnight culture of NorA *S. aureus* 1199B and cultured at 37 °C with agitation to an A_{660} of 0.7–0.8. The cells were first loaded by incubation with EtBr (10 mg/L) in the presence of the protonophore carbonyl cyanide *m*-chlorophenylhydrazone, CCCP (20 mg/L), during 10 min at 37 °C. Cells were then centrifuged and resuspended in 1 mL of LB medium at 37 °C without EtBr, the inhibitors CCCP or reserpine (20 mg/L) or compound **3** being eventually added at this moment, and the fluorescence was continuously measured for 10 minutes. In the case of *B. subtilis* ΔΔNA, the culture was transferred by centrifugation into the buffer phosphate-buffered saline (NaCl 137 mM, KCl 2.7 mM, Na_2HPO_4 8 mM and KH_2PO_4 1.5 mM, pH 7.2) plus 0.5% w/w glucose and prewarmed at 37 °C. The cells were then loaded with EtBr and processed as for *S. aureus*. All experiments were performed at least three times. Results are expressed as percentage of the initial fluorescence.

5.2.5. Ethidium bromide accumulation. A culture of R NorA strain (10 mL) at an A_{660} of 0.7–0.8 was prepared as above and kept at 37 °C with agitation. One milliliter was kept and its fluorescence at λ_{ex} = 530 nm and λ_{em} = 600 nm was taken as zero time. Ethidium bromide was added at a final concentration of 10 mg/L to the culture. Samples were taken after 2 min, 5 min and then every 5 min over 30 min, and the fluorescence was measured. To assay the influence of inhibitors, the same protocol was used, but after 10 min the bacterial suspension was divided in two, and the inhibitor was added to one of them. The results are expressed as fluorescence arbitrary units (AU).

5.2.6. Ciprofloxacin accumulation. Ciprofloxacin uptake was performed as previously described[47] using the silicone oil method. Briefly, cells were grown in LB medium to an OD_{660} of 0.7–0.8, then washed twice and concentrated 20-fold in 100 mM phosphate buffer, pH 7.2, containing 1 mM $MgSO_4$ and 0.4% glucose. Following 5 min pre-incubation at 37 °C, ciprofloxacin was added at 10 mg/L to the cell suspension. At various time intervals, 0.5 mL samples were removed, placed on 0.5 mL aliquots of silicone oil (density 1.03, Fluka), centrifuged and immediately frozen. Efflux pump inhibitors were

added 6 min after addition of the fluoroquinolone. The tubes were cut in the middle of the silicone layer and inverted to eliminate the residual oil. The cell pellets were resuspended in 1 mL of lysis buffer (0.1 M glycine–HCl pH 3.0) and lysed by incubation at 100 °C for 10 min. Fluorescence of supernatants was measured and compared with a ciprofloxacin standard curve (λ_{ex} = 242 nm and λ_{em} = 487 nm) established into bacterial lysate. A blank was done using bacterial lysate without ciprofloxacin. CIP accumulation was then expressed in nanograms of product per 10^9 CFU.

5.2.7. Cytotoxicity assays. Cytotoxicity assays were performed by Thierry Cresteil at the Institut de Chimie des Substances Naturelles, UPR2301 CNRS, at Gif-sur-Yvette, France, using concentrations of 10^{-5} M of the assayed molecules and KB, MCF7 and MCF7R cells. Taxotere at $2.5\,10^{-10}$ M was used as a reference. The results are shown as % of cellular growth inhibition.

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