From Phenothiazine to 3-Phenyl-1,4-benzothiazine Derivatives as Inhibitors of the *Staphylococcus aureus* NorA Multidrug Efflux Pump

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Overexpression of efflux pumps is an important mechanism by which bacteria evade effects of substrate antimicrobial agents and inhibition of such pumps is a promising strategy to circumvent this resistance mechanism. NorA is a *Staphylococcus aureus* multidrug efflux pump, the activity of which confers decreased susceptibility to many structurally unrelated agents, including fluoroquinolones, resulting in a multidrug resistant (MDR) phenotype. In this work, a series of 1,4-benzothiazine derivatives were designed and synthesized as a minimized structural template of phenothiazine MDR efflux pump inhibitors (EPIs) in an effort to identify more potent *S. aureus* NorA EPIs. Almost all derivatives evaluated showed good activity in combination with ciprofloxacin against *S. aureus* ATCC 25923; some were capable of completely restoring ciprofloxacin activity in a *norA*-overexpressing strain (SA-K2378). Compounds **6k** and **7j** displayed good activity against SA-1199B, a strain that also overexpresses *norA*, in an ethidium bromide (EtBr) efflux inhibition assay.

Introduction

The treatment of many infectious diseases may be compromised by resistance to antimicrobial agents. The emergence of resistance among bacteria to a wide variety of structurally unrelated antibacterial agents such as β-lactams, macrolides, tetracyclines, and fluoroquinolones as well as selected dyes and disinfectants has become a serious public health concern.[1]

Bacterial drug resistance is achieved by three general mechanisms which include enzymatic inactivation,[2] modification of the drug target,[3,4] and decreased intracellular drug concentration either by changes in membrane permeability[5] or overexpression of efflux pumps.[6] Efflux is a process by which drug molecules are removed from the cell by membrane-based proteins, resulting in sublethal drug concentrations at the active site.[7] The natural role of efflux pumps is to remove toxins that are encountered in the environment or produced during metabolism. This protective function enables bacteria to survive in hostile environments such as in the presence of antibiotics.[8] Bacteria have an array of antiporter-type efflux proteins, energized by the proton- or sodium-motive force or ATP hydrolysis, that extrude antibacterial drugs.[9] These pumps are capable of removing specific substrates such as tetracycline (SDR, specific drug resistance, i.e., TetK) or several structurally dissimilar substrates such as hydrophilic fluoroquinolones, various biocides, and dyes producing a multidrug resistance (MDR)[a] phenotype (i.e., NorA).[6,10]

One strategy used to circumvent efflux-mediated resistance is the search for new antibiotics that are poor pump substrates and thus bypass efflux systems.[11] A second approach is the development of efflux pump inhibitors (EPIs) capable of potentiating the activity of substrate antibiotics. Many inhibitors may bind to the same site as substrates and inhibit transport by a competitive or noncompetitive mechanism.[12] EPIs used in combination with antibiotics may not only increase antibacterial potency[13] but also expand the antibacterial spectrum and reduce the frequency of the emergence of target-based resistance.[14] Therefore, efflux pumps are viable antibacterial targets and identification and development of potent EPIs is a promising and valid strategy.[12]

Increasing drug resistance among Gram-positive bacteria is a significant health problem because these organisms are responsible for nearly one-third of all community-acquired infections.[15] In particular, methicillin-resistant *S. aureus* (MRSA) is one of most frequent nosocomial pathogens in developed countries.[16] For example, in England and Wales, the number of deaths due to MRSA increased from 51 in 1993 to 1629 in 2005. In 2005, infections caused by MRSA in the U.S. resulted in 19000 deaths.[17] Vancomycin-resistant (VRSA) and -insensitive (VISA) strains of *S. aureus* have also been identified, further complicating therapy.[17]

The most studied chromosomal efflux pump of *S. aureus* is NorA, a transporter belonging to the major facilitator superfamily (MFS). As mentioned previously, some MFS pumps, such as NorA, are capable of extruding multiple structurally dissimilar substrates.[18] Although the NorA binding pocket has never been studied, sequence homology and the sharing of a wide range of substrates and/or inhibitors with the *Bacillus subtilis* Bmr MDR pump and the plasmid-encoded *S. aureus* QacA MDR pump has led to the hypothesis that NorA may have a large hydrophobic binding site. Such a binding region would permit substrates to associate with the protein through a combination of hydrophobic effects and electrostatic attraction rather than by establishing a precise network of hydrogen bonds.

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[a] Abbreviations: EPI, efflux pump inhibitor, MDR, multidrug resistance, CPX, ciprofloxacin, EtBr, ethidium bromide.

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This structural peculiarity could explain the broad substrate specificity of MDR pumps.[19]

Recently, a structural characterization of NorA was attempted in silico. The three-dimensional model of NorA consists of 12 transmembrane helices connected by hydrophilic loops of varying lengths. Two hypothetical binding sites, including a central hydrophobic pocket and a periplasmic pocket, have been identified. The maximum docking scores of these sites with known inhibitors such as reserpine and verapamil provide supportive evidence for their potential importance.[20]

In recent years, many EPIs capable of potentiating the activity of antimicrobial substrates have been identified, although none has reached the clinic. Early EPIs included the plant alkaloid reserpine and the synthetic antihypertensive verapamil, but concentrations required for pump-inhibitory activity are too high to be clinically relevant.[21–23] Several nonantibiotic compounds such as omeprazole (proton pump inhibitor; antiulcer), paroxetine (antidepressant), chlorpromazine (neuroleptic), and respective derivatives have been shown to increase the antibacterial potency of pump substrates by inhibiting NorA of *S. aureus* and other MDR pumps of Gram-positive organisms.[23–28] Natural compounds such as epicatechin-gallate and epigallocatechin-gallate, and the synthetic acridone derivative GG918 (a P-glycoprotein inhibitor), have been shown to enhance the activity of selected antimicrobial agents, including fluoroquinolones, against efflux-mediated resistant Gram-positive bacteria.[29,30] Other natural products such as the porphyrin pheophorbide A and the flavonolignan 5′-methoxyhydnocarpin (5′-MHC), isolated from *Berberis* plants, also have been identified as NorA inhibitors.[31] Flavones and synthetic flavolignan analogues of 5′-MHC were evaluated and SAR studies carried out.[32] Inhibition of NorA by synthetic compounds such as phenyl nitroindoles and arylthiophenes significantly reduce the frequency at which ciprofloxacin-resistant *S. aureus* derivatives emerge.[33,34] To date, there are only a few examples of rationally designed inhibitors and very little work has been done with respect to the SAR of NorA inhibitors.[32,35]

The structural heterogeneity of compounds active in reversing MDR has made it difficult to establish SARs and complicates the design of new candidates. Indeed, the broad substrate affinity of MDR pumps suggests that there might be more than a single binding mechanism within the large binding site.[36] The role of lipophilicity, a characteristic that is generally considered important for MDR-reversing properties, remains unclear and no detailed SARs have been done showing any relationship between partition coefficients and efflux inhibition.[37] Successful correlations have been found only for highly homogeneous sets of molecules.[38] Thus, the rational design of new EPI candidates is difficult and new entities frequently are discovered serendipitously through testing large libraries of known molecules or by modifying known EPIs.

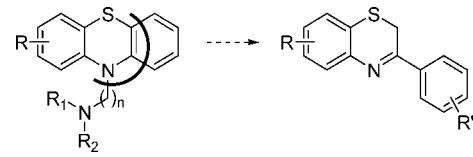


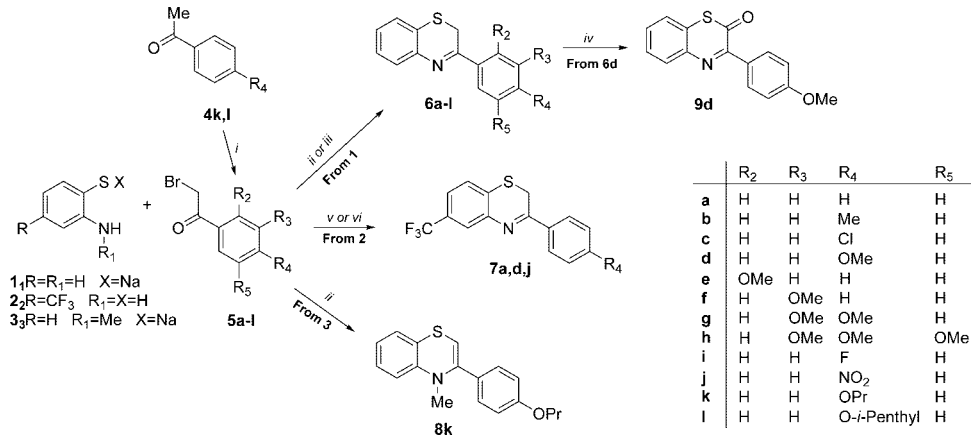
Figure 1. Structural modifications of the phenothiazine backbone leading to the 3-phenyl-2*H*-1,4-benzothiazines.

Some phenothiazine derivatives, which are known to inhibit the mammalian MDR pump P-glycoprotein,[39] are also inhibitors of NorA function.[27] In some cases, pump-inhibitory activity is synergized by intrinsic antibacterial activity and in others by a reduction in the proton motive force, which inhibits pump function by altering the membrane potential.[40,41] Therefore, the mechanism by which the phenothiazines inhibit efflux is multifactorial. Unfortunately, phenothiazine derivatives are recognized for their basic neurotropic activity. Indeed, many SAR studies have been carried out in an attempt to remove or decrease this effect.[42] Investigations have concentrated on the substituents in the aromatic ring, on the alkyl chain, the linker to protonable nitrogen of the same chain, as well as the substitution of nitrogen at N-10 with vinyl carbon (thioxanthene derivatives).[28]

Thinking that the intriguing phenothiazine moiety may be a valuable template for the synthesis of new and more potent MDR EPIs, we elected to pursue a structural modification of this backbone in an attempt to clarify its pharmacophoric moiety and identify derivatives having improved EPI activity compared to that of chlorpromazine. Our approach focused on the elimination of structural features responsible for neuroleptic activity through a structural minimization with the aim to identify the minimum pharmacophore entity responsible for EPI activity. We proceeded with drastic modifications such as the elimination of one ring of the tricyclic phenothiazine structure, resulting in a bicyclic benzothiazine scaffold, the elimination of the chain linked to the N-10 atom, carrying a protonable tertiary amine essential for the interaction with the dopaminergic receptor, and the insertion at the C-3 position of a substituted phenyl ring to ensure a better lipophilic character (Figure 1).

An entry set of benzothiazine derivatives was developed by synthesizing 3-phenyl-2*H*-1,4-benzothiazine (**6a**) as a prototype

Scheme 1[a]



	R_2	R_3	R_4	R_5
a	H	H	H	H
b	H	H	Me	H
c	H	H	Cl	H
d	H	H	OMe	H
e	OMe	H	H	H
f	H	OMe	H	H
g	H	OMe	OMe	H
h	H	OMe	OMe	OMe
i	H	H	F	H
j	H	H	NO_2	H
k	H	H	OPr	H
l	H	H	O-*i*-Penthyl	H

[a] (i) PHP, AcOH, 60°C; (ii) Et_2O, r.t.; (iii) Et_2O, r.t. and then HCl_g; (iv) PTSA, CH_2Cl_2, r.t.; (v) K_2CO_3, DMF, r.t.; (vi) Et_3N, EtOH/Et_2O, r.t.

of a new series (see Scheme 1). Analogues substituted at C-4′ with a lipophilic electron donor substituent as a methyl group (**6b**), an electron-withdrawing substituent such as a chlorine atom (**6c**), and a methoxy group to ensure a different level of hydrophobicity (**6d**) also were synthesized. Preliminary screening of compounds using a wild type *S. aureus* strain (ATCC 25923) in the presence and absence of ciprofloxacin (CPX) revealed variable intrinsic and synergistic activity (Table 1). Compounds **6a** and **6d** showed no intrinsic antibacterial activity (MIC > 512 μg/mL), but when the test strain was exposed to both CPX and 25 μg/mL of either compound, an 8-fold reduction in the CPX MIC was observed. In a similar manner, compounds **6b** and **6c** (MIC 64 μg/mL) reduced the CPX MIC 4-fold.

NorA inhibitory activity was then highlighted by testing this first set of compounds on modified strains in which the *norA* gene was deleted (SA-K1902) or overexpressed from a plasmid (SA-K2378). As expected, no compound exhibited a significant effect when coadministered with CPX against SA-K1902. When compounds **6c**, **6b**, and **6d** were coadministered with CPX against SA-K2378, MICs were decreased 4-, 8-, and 16-fold, respectively, with **6d** able to completely reverse the effect of NorA (MIC reduced from 2 to 0.12 μg/mL).

On the basis of these encouraging results, the series of benzothiazines was enlarged to include new derivatives with modifications on the phenyl linked to C-3 as a positional shift of the OMe or its increased presence (see **6e**−**h**), the introduction of F, NO_2, OPr, and O*i*-pentyl in the para position (**6i**−**l**, respectively). Other variations included the introduction of CF_3 at C-6 for derivatives **7a**, **7d**, and **7j** and introduction of a methyl group at the N-4 position, resulting in **8k**. Finally, sulfur atom oxidation to sulfone was performed resulting in compounds **12a** and **12c**, and hydrogenation of the double bond C-3/N-4 resulted in **15d** and **15m**.

Chemistry

The synthetic route giving rise to the 3-phenyl-1,4-benzothiazine derivatives **6a**−**l**, **7a**,**d**,**j**, and **8k** entailed the reaction of appropriate 2-aminothiophenol or its sodium salt **1**,[43] **2**, and **3**[44] with a substituted α-bromo acetophenone **5a**−**f**, **5g**,[45] and **5h**[46]−**l** (Scheme 1). α-Bromo acetophenones **5k**,**l** were obtained by bromuration with pyridinium perbromide hydrobromide (PHP) of correspondent acetophenones **4k**[47] and **4l**.[47]

Compounds **6a**,[48] **6d**,[44] **6j**[44]−**l**, **7a**, and **7j** were obtained in low yields (32−43%) because they were accompanied by dimerization compounds,[48] a byproduct of spontaneous self-oxidation at the C-2 position in polar solvents. A slight modification of the classical procedure resulted in compounds **6b**,[49] **6c**,[49] **6e**−**i**, and **7d**, which were obtained as hydrochlorides in better yields. The N-4 methyl derivative **8k** was prepared in the same manner by reacting 2-methylamino thiophenol sodium salt **3**[44] with α-bromo-4′-propoxyacetophenone **5k**.

The direct oxidation of the benzothiazine sulfur atom of **6d**[44] to the corresponding sulfoxide and/or sulfone derivative was prevented by the high reactivity of the C-2 position, which resulted in the corresponding 3-[4-(methyloxy)phenyl]-2*H*-1,4-benzothiazin-2-one **9d**.[44] 3-Phenyl-1,4-benzothiazine-1,1-dioxide derivatives **12a**[50]and **12c** were obtained, as illustrated in (Scheme 2) by reacting the sodium salt of 2-nitro-benzensulfinic acid **10**[51] with substituted α-bromo acetophenones **5a**,**c** to obtain the nitrosulphones **11a**[50] and **11c** that were then reduced with H_2/Raney-Ni to afford, after spontaneous cyclization, the expected sulfones **12a**[50] and **12c** in high yields.

Reacting the 2-nitrobenzenesulfenyl chloride **13** with substituted acetophenones **4d**,**m** resulted in the 2-[(2-nitrophenyl)thio]-1-phenylethanones **14d**[44] and **14m**, which then were reduced with $LiAlH_4$ to afford, after spontaneous cyclization, the expected 3-phenyl-3,4-dihydro-2*H*-1,4-benzothiazines **15d** and **15m** in good yields (Scheme 3).

Results and Discussion

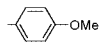
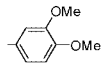
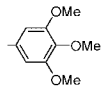
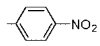
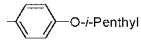
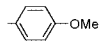
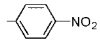
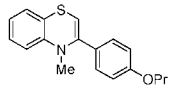
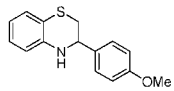
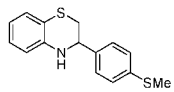
In this study, a series of 1,4-benzothiazine derivatives was designed and synthesized as possible minimizing structures of phenothiazine MDR EPIs and developed as a template to obtain more effective *S. aureus* NorA EPIs. A microbiological approach was used to assess EPI function by determining the intrinsic antimicrobial activity of synthesized compounds against wild-type *S. aureus* ATCC 25923 and the effect of combining these compounds with CPX on the same strain. Successive steps involved an evaluation of synergistic antimicrobial activity against SA-K1902 (*norA*−) and SA-K2378, which overexpresses *norA* from a multicopy plasmid. To confirm the inhibitory mechanism an ethidium bromide (EtBr), efflux inhibition assay was carried out using SA-1199B, a well-characterized strain that overexpresses *norA*.[52]

The modification of the 3-phenyl ring on the **6a** prototype involved the shifting of a methoxy group to the para, meta, and ortho positions, giving rise to compounds **6d**, **6f**, and **6e**, respectively. When combined with CPX at a concentration of 25 μg/mL, MIC reductions of 4- to 8-fold versus *S. aureus* ATCC 25923 and 8- to 16-fold versus SA-K2378 were observed (Table 1). Compounds **6g** (dimethoxy) and **6h** (trimethoxy) potentiated the activity of CPX by 4- to 16-fold versus ATCC 25923 and SA-K2378, respectively. Elongation of the ethereal alkyl chain at C-4′ of **6d** to *n*-propyloxy resulted in the same activity for **6k** (CPX MIC decreased ≥8-fold) against *S. aureus* ATCC 25923 but a lesser effect was observed against SA-K2378 (4-fold). Introduction of an *i*-pentyl chain **6l** had a detrimental effect with a reduction of activity against both *S. aureus* ATCC 25923 and SA-K2378 observed. Substitution at C-4′ with an electron withdrawing group give different results: NO_2 **6j** resulted in diminished synergistic activity when combined with CPX, while fluorine **6i** potentiated activity by 16-fold against SA-K2378. A hydrophobic electron-donor methyl group **6b** also was quite beneficial, resulting in a 4- to 8-fold potentiation of CPX activity versus the ATCC strain and SA-K2378, respectively.

Further modifications were carried out on the benzothiazine nucleus. The introduction at C-6 of CF_3, a hydrophobic electron-withdrawing group that is also present both in phenothiazine and thioxanthene EPI derivatives (i.e., fluphenazine and flupentixol, respectively) gave rise to compounds **7a**, **7d**, and **7j**. These compounds had variable intrinsic activities (**7a** = **7j** > **7d**), and when combined with CPX at 25 μg/mL, **7j** potentiated the activity of CPX by ≥8- and ≥32-fold against *S. aureus* ATCC 25923 and SA-K2378, respectively. The 4′-unsubstituted **7a** and 4′-methoxy **7d** derivatives exhibited a low activity on the wild-type strain; however, against SA-K2378 the activity for **7a** was very strong with a MIC reduction of 16-fold at both 25 and 12.5 μg/mL.

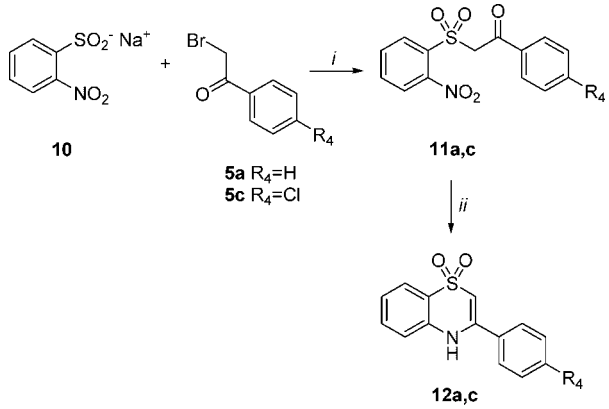
To obtain further information on the SAR of this new class of EPIs, other modifications were carried out. Methylation at N-4, with consequent stabilization of the endocyclic double bond in the C-2/C-3 positions, gave derivative **8k** that caused a slight reduction of CPX MIC (4-fold) only for SA-K2378. The 3-phenyl benzothiazine-1,1-dioxide **12a** and its 4′-chloro derivative **12c**, obtained by S-1 oxidation, had minimal potentiation activity. Reduction of the endocyclic double bond at the C-3/N-4 positions resulted in the 3,4-dihydro-2*H*-1,4-benzothiazine **15d**, which had half the potentiation activity observed with **6d**

Table 1. Evaluation of Antibacterial Activity

Compd.	R	Ar	n	Intrinsic activity MICs (μg/mL) *S. aureus* ATCC 25923	CPX MICs (μg/mL) Conc. of inhibitor (μg/mL)	*S. aureus* ATCC 25923	Modified *S. aureus* K1902 (*norA*-)	Modified *S. aureus* K2378 (*norA*++)
6a	H		0	>512	25	0.06 (8)[a]	0.12	2.00
6b	H		0	64	25	0.12 (4)	0.12	0.25 (8)
6c	H		0	64	25	0.12 (4)	0.12	0.50 (4)
6d	H		0	>512	25 12.5 6.25	≤0.06 (≥8)	0.12 0.12 0.12	0.12 (16) 0.50 (4) 1.00 (2)
6e	H		0	64	25	0.12 (4)	0.12	0.25 (8)
6f	H		0	64	25	0.12 (4)	0.12	0.25 (8)
6g	H		0	128	25 12.5 6.25	0.12 (4)	0.06 (2) 0.12 0.12	0.12 (16) 0.50 (4) 1.00 (2)
6h	H		0	32	25 12.5 6.25	0.12 (4)	0.06 (2) 0.12 0.12	0.12 (16) 0.50 (4) 1.00 (2)
6i	H		0	64	25 12.5 6.25	0.12 (4)	0.06 (2) 0.12 0.12	0.12 (16) 1.00 (2) 1.00 (2)
6j	H		0	>128	25	0.25 (2)	0.12	1.00 (2)
6k	H		0	128	25	≤0.06 (≥8)	0.12	0.50 (4)
6l	H		0	>128	25	0.12 (4)	0.12	1.00 (2)
7a	CF_3		0	32	25 12.5 6.25	0.25 (2)	0.12 0.12 0.12	0.12 (16) 0.12 (16) 0.50 (4)
7d	CF_3		0	64	25	0.25 (2)	0.12	0.25 (8)
7j	CF_3		0	32	25 12.5 6.25	≤0.06 (≥8)	0.06 (2) 0.06 (2) 0.12	≤0.06 (≥32) 0.12 (16) 0.25 (8)
12a	H		2	128	25	0.25 (2)	0.12	1.00 (2)
12c	H		2	128	25	0.12 (4)	0.12	1.00 (2)
8k				>512	25	0.50	0.12	0.50 (4)
15d				>128	25 12.5 6.25	0.25 (2)	0.12 0.12 0.12	0.25 (8) 0.50 (4) 0.50 (4)
15m				>128	25 12.5 6.25	≤0.06 (≥8)	0.12 0.12 0.12	0.12 (16) 0.25 (8) 0.25 (8)
chlorpromazine				128	25	0.50	≤ 0.03 (≥ 4)	0.25 (8)
reserpine				128	25	0.25 (2)	0.06 (2)	0.50 (4)
ciprofloxacin						0.5	0.12	2.00

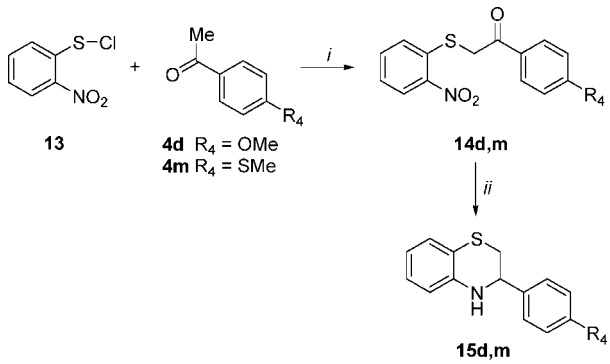
[a] *n*-Fold reduction of CPX MIC.

Scheme 2[a]



[a] (i) DMF, r.t.; (ii) H_2, Raney-Ni, r.t.

Scheme 3[a]



[a] (i) CH_3CN dry, reflux; (ii) $LiAlH_4$, THF dry, r.t.

against SA-K2378, and its 4′-thio analogue **15m**, which was equipotent to compound **6d** against both strains.

Many of the tested compounds, such as **6b**, **6d**−**i**, **6k**, **7a**, **7d**, **7j**, **15d**, and **15m**, displayed the same or better activity than the reference compounds reserpine and chlorpromazine against *S. aureus* ATCC 25923 and SA-K2378 when coadministered with CPX. In some cases (see **6b**, **6e**, **6f**, **6h**, **6i**, **7a**, **7d**, and **7j** in Table 1), the strong activity results, at least for the wild-type strain, from NorA pump inhibition and from their weak intrinsic antibacterial activity that can give a synergistic interaction with CPX. Compounds **6d**, **6g**, and **15m**, which are devoid of any antibacterial activity, are able to restore CPX susceptibility in SA-K2378 to the same level as that observed for the SA-K1902 (*norA*-) by complete inhibition of NorA.

The 2-fold reductions in CPX MICs observed for compounds **6 g**−**i** and **7j** against SA-K1902 (*norA*-deleted strain) are within the accepted variance of microdilution susceptibility testing and are thus not significant. Interestingly, some compounds demonstrated greater activity against ATCC 25923 than against the *norA*-overexpressing strain SA-K2378 (see **6a** and **6k** data in Table 1), and some had the opposite effect (see **6 g**−**i**). In the latter case, the explanation is most likely related to inhibition of NorA. In cases where the activity of a compound was greater against the ATCC strain than against the *norA*-overexpressing strain, the explanation may be as simple as the fact that these are two very different genetic backgrounds. The *S. aureus* genome encodes many putative pumps and most have not been characterized.[53] It is feasible that baseline expression of a known or undefined pump(s) capable of CPX transport susceptible to the activity of **6a** or **6k** in ATCC 25923 is greater than that of either SA-K1902 or its *norA*-overexpressing derivative SA-K2378. In addition to NorA, characterized *S. aureus* MDR efflux pumps that do transport CPX include NorB, NorC, and MepA.[53] Gene expression analyses would be required to determine if any of these are at play in ATCC 25923, but is beyond the scope of this study.

Table 2. Inhibition of EtBr Efflux by SA-1199B[a]

compd	%[b]	compd	%[b]
6a	0.0	**6l**	16.9
6d	19.5	**7a**	8.4
6e	0.0	**7j**	69.7
6g	22.8	**8k**	0.0
6h	23.6	**15d**	10.4
6i	0.0	**15m**	28.2
6j	19.6	reserpine	81.6
6k	84.7	chlorpromazine	81.0

[a] All compounds were tested at a concentration of 50 μM. [b] Percent efflux inhibition.

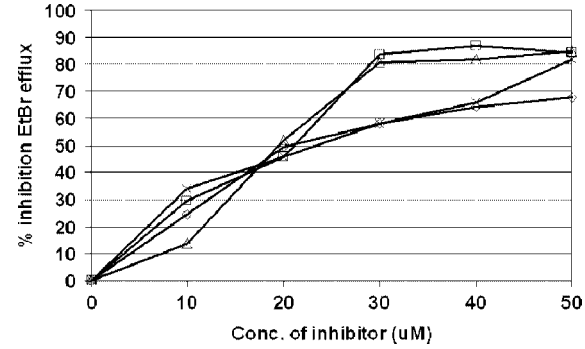


Figure 2. Effect of compounds **6k** (□), **7j** (◊), reserpine (Δ), and chlorpromazine (×) on EtBr efflux of SA-1199B.

Several derivatives (**6a**,**d**,**e**,**g**−**l**, **7a**,**j**, **8k**, and **15d**,**m**) were evaluated for their abilities to interfere with EtBr efflux from SA-1199B, employing a 50 μM concentration. None of these compounds had any intrinsic antibacterial activity against this strain (data not shown). **6k** and **7j** showed good efflux inhibitory activity; **6k** was equipotent to reserpine and chlorpromazine (84.7, 81.6, and 81.0% inhibition of efflux, respectively) (Table 2). A dose−response efflux inhibition curve was generated for **6k** and **7j**, the most active compounds. Reserpine and chlorpromazine were used as comparators, and the data are presented in Figure 2. Compound **6k** reduced NorA-mediated efflux as well as reserpine at all tested concentrations.

Isobolograms demonstrating the effect of combining **6k**, **7j**, reserpine, and chlorpromazine on CPX MICs against SA-K1902 and SA-K2378 are shown in Figure 3. It can be seen that the CPX MICs for SA-K1902 (*norA*-) were constant at all the concentrations of **6k**, indicating no significant activity against non-NorA pumps that this strain may express. With respect to **7j** and chlorpromazine, CPX MICs decreased 4-fold at concentrations greater than 6.25 and 12.5 μg/mL, respectively (Figure 3a). Compound **7j** and chlorpromazine have weak antibacterial activity against this strain (MIC 25 μg/mL), and it is possible that as this concentration is approached, the antimicrobial effect produces the MIC changes observed. The small MIC reduction produced by 3.13 μg/mL of reserpine, which is devoid of any antimicrobial activity against this strain, is probably the result of inhibition of one or more non-NorA efflux pumps that have CPX as a substrate.

For SA-K2378, which overexpresses *norA* from a multicopy plasmid, there is a gradual reduction of CPX MICs across all tested concentrations of both **6k** and **7j** (Figure 3b). The CPX MIC is reduced to a value identical to that of SA-K1902 at a

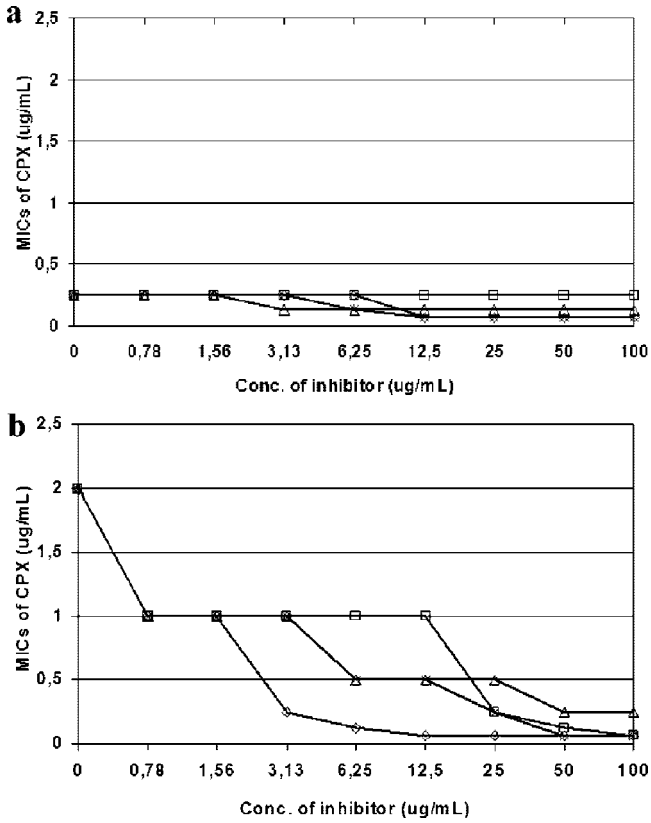


Figure 3. Effect of combining **6k** (□), **7j** (◊), reserpine (Δ), and chlorpromazine (×) on ciprofloxacin MICs against SA-K1902 (**a**) and SA-K2378 (**b**).

concentration of 25 μg/mL for compound **6k** and 12.5 μg/mL for **7j**. **6k** is devoid of any antimicrobial activity against this strain (MIC >100 μg/mL), and **7j** has weak activity (MIC 50 μg/mL). The fact that a concentration of no more than one-fourth the MIC of compound **7j** reduces the ciprofloxacin MIC to that of the *norA*-null parent strain strongly suggests that the mechanism of the effect is the complete inhibition of NorA activity.

Data collected in this study highlight that 3-phenyl-1,4-benzothiazine derivatives obtained by structural minimization of phenothiazine EPIs can be effective inhibitors of NorA. These results indicate that the benzothiazine moiety is the key feature for activity of the two different series of derivatives. The SAR for this new class of benzothiazine EPIs has shown that substituents on the C-3 phenyl ring play an important role and in particular 4′-methoxy and 4′-thiomethyl groups give potent compounds (**6d** and **15m**) against wild-type and *norA*-overexpressing strain SA-K2378. Propyloxy compound **6k** displays good activity against both a wild-type strain and in the EtBr efflux inhibition assay against the *norA*-overexpressing strain SA-1199B. A nitro group in the C-4′ position, when coupled with the presence of a trifluoromethyl group in the C-6 position of the benzothiazine nucleus, gives the potent compound **7j** characterized by good efflux inhibitory activity and in combination susceptibility testing with CPX. Oxidation of the S-1 position or N-4 methylation gave less active compounds, which were probably the result of an increased polarity and/or planarity of the benzothiazine nucleus.

To investigate if there is a correlation between NorA inhibitory activity and the physicochemical parameters calculated in silico,[54] seven compounds were selected based on their EtBr efflux inhibitory activity and were compared with the reference compounds reserpine and chlorpromazine. The first group, consisting of **6a**, **6e**, and **6i**, did not show any EtBr efflux inhibition. The second group, including **6d** and **15m**, displayed moderate EtBr efflux inhibitory activity (20−30% inhibition), and the third group, including **6k**, **7j**, reserpine, and chlorpromazine, exhibited high activity (more than 50% efflux inhibition). Several in silico parameters were calculated for these groups, and some of these molecular descriptors seem to correlate with efflux inhibition (Table 3).

The physicochemical data reported in Table 3 suggest that the more active compounds share with reserpine and chlorpromazine some properties that may contribute to confer inhibitory activity of EtBr efflux by NorA pump. Relatively to EPIs compounds, the NorA inhibitory activity seems to be related both to hydrophobic properties (Log $P > 3.9$, W1 > 1050, SOLY < –4, and %FU4 < 57.6) and molecular dimension (MW > 280, $V > 600$, and $G > 1.35$). To better understand the relative weight of each physicochemical property on NorA pump inhibition, a PLS model was built taking into account the X descriptor matrix (Table 3) and the percent of EtBr efflux on SA-1199B. The model confirms that Log *P* is directly correlated with efflux inhibition. Furthermore, water solubility (SOLY) as well as percentage of uncharged molecules at pH 4 (%FU4) were strongly but inversely correlated to NorA inhibition. Polarity (PSA), molecular dimension (MW, *V*, and *G*), and hydrophilic accessible volume (W1) are less correlated with inhibition of EtBr efflux. The model therefore could suggest that H-bond pattern is more relevant than molecular size and shape in respect to efflux.

In conclusion, the 3-phenyl-1,4-benzothiazine derivatives, designed by structural minimization of phenothiazine NorA pump inhibitors, show modest or no intrinsic antistaphylococcal activity and restore, in a concentration-dependent manner, the antibacterial activity of CPX against *norA*-overexpressing *S. aureus* strains. The EPI activity of some of these benzothiazine derivatives was superior to that shown for the progenitor compound chlorpromazine and the reference compound reserpine both against *S. aureus* ATCC 25923 and SA-K2378.

Experimental Section

Bacterial Strains. The strains of *S. aureus* employed were ATCC 25923 (wild-type), SA-1902 (*norA*-deleted), and SA-1199B (overexpressing *norA* and also possesses an A116E *GrlA* substitution).[52,55] In addition SA-K2378, which overexpresses *norA* from a multicopy plasmid, also was used. This strain was produced by cloning *norA* and its promoter into plasmid pCU1 and then introducing the construct into SA-K1902.[56]

Microbiologic Procedures. All microbiologic procedures employed *S. aureus* ATCC 29523, SA-K1902, and SA-K2378. MICs were determined in duplicate by microdilution techniques according to CLSI guidelines.[57] The effect of combining reserpine and chlorpromazine or scalar dilutions of freshly prepared solutions of each synthesized compound on the MICs of CPX also was determined. Checkerboard combination studies using CPX and **6k** or **7j** were performed as described previously.[58]

EtBr Efflux. The loss of EtBr from *S. aureus* SA-1199B was determined fluorometrically as previously described.[59] Experiments were performed in duplicate, and the results were expressed as the mean total efflux over a 5 min time course. The effect of increasing concentrations of reserpine, **6k**, and **7j** on the EtBr efflux of SA-1199B also was determined. EtBr efflux of SA-1199B in the presence of test compounds was compared to that determined in their absence and percent reduction in efflux was calculated.

Synthesis. All reactions were routinely checked by thin-layer chromatography (TLC) on silica gel 60_{F254} (Merck) and visualized using UV illumination. Flash column chromatography was performed on Merck silica gel 60 (mesh 230−400) using the indicated

Table 3. Physicochemical Properties of Selected Compounds

compd	activity[a]	MW	Log *P*	*V*[b]	*G*[c]	W1[d]	SOLY[e]	PSA[f]	%FU4[g]
6a	0.0	225.31	3.0	531.4	1.26	890.9	−3.66	39.4	92.0
6e	0.0	255.34	2.9	584.4	1.30	956.1	−3.60	50.8	78.3
6i	0.0	243.30	3.2	534.5	1.27	1031.1	−3.45	39.4	87.0
6d	19.5	255.34	3.1	590.2	1.32	1017.8	−3.68	50.8	23.5
15m	28.2	273.42	4.5	623.5	1.33	1034.6	−4.46	65.6	18.2
6k	84.7	283.34	4.0	669.6	1.39	1086.5	−4.43	50.8	23.5
7j	69.7	338.30	3.9	644.8	1.40	1303.0	−4.19	76.6	57.6
reserpine	81.6	608.68	4.0	1275.0	1.79	1784.2	−6.05	126.1	0.2
chlorpromazine	81.0	318.86	5.2	719.5	1.37	1067.0	−4.25	33.0	<0.1

[a] Percent inhibition of EtBr efflux from SA-1199B. [b] *V*: molecular volume. [c] *G*: globularity. [d] W1: hydrophilic accessible volume. [e] SOLY: intrinsic solubility in water. [f] PSA: polar surface area. [g] %FU4: % fraction of uncharged molecules at pH 4.

solvents. Yields were of purified product and were not optimized. Melting points were determined in capillary tubes (Electrothermal model 9100) and are uncorrected. Elemental analyses were performed on a Carlo Erba elemental analyzer (model 1106), and the data for C, H, and N are within 0.4% of the theoretical values. ^{1}H NMR spectra were recorded at 200 MHz (Bruker Avance AC 200) or 400 MHz (Bruker Advance-DRX 400), with $CDCl_3$ as solvent, unless otherwise indicated, and with Me_4Si as the internal standard. The chemical shift (δ) values are reported in ppm, and the coupling constants (*J*) are given in Hz. The abbreviations used are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. The spectral data are consistent with the assigned structures. Reagents and solvents were purchased from common commercial suppliers and were used as received. For routine aqueous workup, the reaction mixture was extracted with CH_2Cl_2 or EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated with a Büchi rotary evaporator at low pressure. All starting materials were commercially available, unless otherwise indicated.

2-Bromo-1-(4-propoxyphenyl)ethanone (5k). Pyridinium perbromide hydrobromide (3.50 g, 11.0 mmol) was added to a solution of 1-(4-propoxyphenyl)ethanone **4k**[47] (1.30 g, 7.3 mmol) in acetic acid (15 mL) and warmed to 55−60 °C for 30 min under magnetic stirring. After cooling, the mixture was poured in water, neutralized with $NaHCO_3$, and extracted with EtOAc (3 × 50 mL). The organic extracts were dried with Na_2SO_4, and the solvent was removed under reduced pressure to obtain a residue that was purified by flash column chromatography with a mixture of EP:Et_2O 95:5 to obtain 0.90 g (yield 48%) of pure 2-bromo-1-(4-propoxyphenyl)ethanone **5k** as colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.05 (3H, t, *J* = 7.45 Hz, $OCH_2CH_2CH_3$), 1.75−1.95 (2H, m, $OCH_2CH_2CH_3$), 4.00 (2H, t, *J* = 6.55 Hz, $OCH_2CH_2CH_3$), 4.40 (2H, s, CH_2Br), 6.95 (2H, d, *J* = 9.02 Hz, H-2, H-6), 7.95 (2H, d, *J* = 9.02 Hz H-3, H-5).

2-Bromo-1-[4-(isopentyloxy)phenyl]ethanone (5l). Obtained by the previous procedure, from 4-(isopentyloxyphenyl)ethanone **4l**,[47] as a yellow oil in 44% yield, after purification by column chromatography (EP/Et_2O 95:5). ^{1}H NMR ($CDCl_3$): δ 1.00 (6H, d, *J* = 6.40 Hz, $OCH_2CH_2CH(CH_3)_2$), 1.55−1.95 (3H, m, $OCH_2CH_2CH(CH_3)_2$), 4.05 (2H, t, *J* = 6.55 Hz, $OCH_2CH_2CH(CH_3)_2$), 4.40 (2H, s, CH_2Br), 6.95 (2H, d, *J* = 9.00 Hz, H-2, H-6), 7.95 (2H, d, *J* = 9.00 Hz, H-3, H-5).

Procedure for the Synthesis of Substituted 3-Phenyl-2*H*-1,4-benzothiazines 6a−l and 8k. To a suspension of sodium 2-aminothiophenate **1**[43] (1.5 equiv) in dry Et_2O (30 mL) was added dropwise to a solution of appropriate 2-bromo-1-phenylethanone (1.0 equiv) in the same solvent. The reaction mixture was maintained at room temperature under nitrogen atmosphere and magnetic stirring until 2-bromo-1-phenylethanone disappeared (TLC). Then, for compounds **6a**,[48] **6d**,[44] **6j**[44]**−l**, and **8k**, the mixture was filtered to eliminate NaBr and the organic solvent was removed under reduced pressure to obtain a residue that was purified by flash column chromatography to give the target compounds. For compounds **6b**,[49] **6c**,[49] and **6e−i**, after filtration, the reaction mixture was bubbled with HCl_g until the target compound was completely precipitated as hydrochloride.

3-(2-Methoxyphenyl)-2*H*-1,4-benzothiazine Hydrochloride (6e). Obtained from 2-bromo-1-[2-(methyloxy)phenyl]ethanone **5e** as brown solid in 62% yield after crystallization with cycloesane/Et_2O; mp 137.5−138 °C. ^{1}H NMR ($CDCl_3$): δ 3.95 (5H, s, OCH_3 and CH_2), 7.04 (1H, d, *J* = 8.43 Hz, H-3′), 7.20 (1H, t, *J* = 7.19 Hz, H-5′), 7.30−7.45 (3H, m, H-7, H-4′ and H-6′), 7.66 (1H, t, *J* = 7.35 Hz, H-6), 8.10 (1H, d, *J* = 7.59 Hz, H-5), 8.45−8.55 (1H, m, H-8). Anal. ($C_{15}H_{14}ClNOS$) C, H, N.

3-(3-Methoxyphenyl)-2*H*-1,4-benzothiazine Hydrochloride (6f). Obtained from 2-bromo-1-[3-(methyloxy)phenyl]ethanone **5f** as brown solid in 55% yield after crystallization with cycloesane/Et_2O; mp 159−160 °C. ^{1}H NMR ($CDCl_3$): δ 3.75 (2H, s, CH_2), 3.95 (3H, s, OCH_3), 7.10−7.25 (2H, m, H-2′ and H-5′), 7.30−7.45 (3H, m, H-5, H-7 and H-4′), 7.55−7.65 (1H, m, H-6′), 7.80−7.95 (2H, m, H-6 and H-8). Anal. ($C_{15}H_{14}ClNOS$) C, H, N.

3-(3,4-Dimethoxyphenyl)-2*H*-1,4-benzothiazine Hydrochloride (6g). Obtained from 1-[3,4-bis(methyloxy)phenyl]-2-bromoethanone **5g**[45] as pale-brown solid in 47% yield after crystallization with cycloesane/Et_2O; mp 198−198.5 °C. ^{1}H NMR ($CDCl_3$): δ 4.10 (5H, s, OCH_3 and CH_2), 4.30 (3H, s, OCH_3), 7.10 (1H, d, *J* = 8.85 Hz, H-5′), 7.35−7.55 (3H, m, H-6, H-7 and H-6′), 7.83 (1H, d, *J* = 6.64 Hz, H-5), 8.64 (1H, s, H-2′), 8.82 (1H, d, *J* = 7.7 Hz, H-8). Anal. ($C_{16}H_{16}ClNO_2S$) C, H, N.

3-(3,4,5-Trimethoxyphenyl)-2*H*-1,4-benzothiazine Hydrochloride (6h). Obtained from 2-bromo-1-[3,4,5-tris(methyloxy)phenyl]ethanone **5h**[46] as brown solid in 41% yield after crystallization with cycloesane/Et_2O; mp 157−158 °C. ^{1}H NMR ($CDCl_3$): δ 3.93 (2H, s, CH_2), 4.03 (3H, s, OCH_3), 4.08 (6H, s, OCH_3), 7.35−7.45 (3H, m, H-5, H-6 and H-7), 7.65 (2H, s, H-2′ and H-6′), 8.55 (1H, d, *J* = 7.7 Hz, H-8). Anal. ($C_{17}H_{18}ClNO_3S$) C, H, N.

3-(4-Fluorophenyl)-2*H*-1,4-benzothiazine Hydrochloride (6i). Obtained from 2-bromo-1-(4-fluorophenyl)ethanone **5i** as yellow solid in 53% yield after crystallization with cycloesane/Et_2O; mp 187−188 °C. ^{1}H NMR ($CDCl_3$): δ 4.05 (2H, s, CH_2), 7.35−7.55 (5H, m, H-6, H-2′, H-3′, H-5′ and H-6′), 8.53−8.78 (3H, m, H-5, H-6 and H-8). Anal. ($C_{14}H_{11}ClFNS$) C, H, N.

3-(4-Propoxyphenyl)-2*H*-1,4-benzothiazine (6k). Obtained from 2-bromo-1-(4-propoxyphenyl)ethanone **5k** as yellow solid in 32% yield after purification by flash column chromatography (CH_2Cl_2/EP 60:40 → 40:60); mp 115−117 °C. ^{1}H NMR ($CDCl_3$): δ 1.05 (3H, t, *J* = 7.35 Hz, $OCH_2CH_2CH_3$), 1,75−1,95 (2H, m, $OCH_2CH_2CH_3$), 3.90 (2H, s, CH_2), 4.10 (2H, t, *J* = 6.50 Hz, $OCH_2CH_2CH_3$), 6.98 (2H, d, *J* = 8.64 Hz, H-3′, H5′), 7.10 (1H, dt, *J* = 7.53 and 1.49 Hz, H-7), 7.23 (1H, dt, *J* = 7.77 and 1.67 Hz, H-6), 7.33 (1H, dd, *J* = 7.56 and 1.63 Hz, H-5), 7.47 (1H, dd, *J* = 7.81 and 1.56 Hz, H-7), 7.98 (2H, d, *J* = 8.64, H-2′,H-6′). Anal. ($C_{17}H_{17}NOS$) C, H, N.

3-[4-(Isopentyloxy)phenyl]-2*H*-1,4-benzothiazine (6l). Obtained from 2-bromo-1-[4-(isopentyloxy)phenyl]ethanone **5l** as yellow semisolid in 39% yield after purification by flash column chromatography (CH_2Cl_2/EP 60:40 → 40:60). ^{1}H NMR ($CDCl_3$): δ 1.00 (6H, d, *J* = 6.42 Hz, $OCH_2CH_2CH(CH_3)_2$), 1.60−1.95 (3H, m, $OCH_2CH_2CH(CH_3)_2$), 2,45 (1H, bs, NH), 4.10 (2H, t, *J* = 6.50 Hz, $OCH_2CH_2CH(CH_3)_2$), 5.95 (1H, s, H-2), 7.00 (2H, d, *J* = 8.98, H-3′, H5′), 7.20−7.50 (3H, m, H-5, H-6, H-7), 7.60 (1H, dd, *J* =

8.95 and $J = 2.05$, H-8), 8.05 (2H, d, $J = 8.98$, H-2′,H-6′). Anal. ($C_{19}H_{21}NOS$) C, H, N.

4-Methyl-3-(4-propoxyphenyl)-4*H*-1,4-benzothiazine (8k). Obtained from 2-(methylamino)benzenethiol sodium salt **3**[44] and 2-bromo-1-(4-propoxyphenyl)ethanone **5k** as yellow solid in 35% yield after purification by flash column chromatography (CH_2Cl_2/EP 60:40 → 40:60). mp 91−93 °C. ^{1}H NMR ($CDCl_3$): δ 1.05 (3H, t, $J = 7.45$ Hz, $OCH_2CH_2CH_3$), 1.80−1.95 (2H, m, $OCH_2CH_2CH_3$), 3.65 (3H, s, NCH_3), 4.00 (2H, t, $J = 6.55$ Hz, $OCH_2CH_2CH_3$), 6.30 (1H, s, H-2), 7.00 (2H, d, $J = 8.70$ Hz, H-3′, H-5′), 7.32 (2H, d, $J = 8.70$ Hz, H-2′, H-6′), 7.40 (1H, t, $J = 7.12$ Hz, H-6), 7.55 (1H, d, $J = 8.55$ Hz, H-8), 7.65 (1H, t, $J = 7.01$ Hz, H-7), 8.50 (1H, d, $J = 8.04$ Hz, H-5). Anal. ($C_{18}H_{19}NOS$) C, H, N.

3-(4-Methoxyphenyl)-2*H*-1,4-benzothiazin-2-one (9d[44]). Solid *m*-chloroperbenzoic acid (0.20 g, 1.17 mmol) was added to a solution of 3-[4-(methyloxy)phenyl]-2*H*-1,4-benzothiazine **6d**[44] (0.10 g, 0.39 mmol) in CH_2Cl_2 (10 mL) and maintained under magnetic stirring for 30 min at room temperature. The mixture then was poured in water, neutralized with $NaHCO_3$, and extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were dried with Na_2SO_4, and the solvent was removed under reduced pressure to obtain a residue that was purified by flash column chromatography with $CHCl_3$ to obtain 0.078 g (yield 74%) of pure 3-(4-methoxyphenyl)-2*H*-1,4-benzothiazin-2-one **9d**[44] as yellow solid; mp 111−113 °C. ^{1}H NMR ($CDCl_3$): δ 3.80 (3H, s, OCH_3), 6.95 (2H, d, $J = 8.95$ Hz, H-3′, H-5′), 7.30−7.55 (3H, m, H-5, H-6, H-7), 7.90 (1H, d, $J = 9.35$ Hz, H-8), 8.20 (2H, d, $J = 8.95$ Hz, H-2′, H-6′). Anal. ($C_{15}H_{11}NO_2S$) C, H, N.

Procedure for the Synthesis of Substituted 3-Phenyl-6-(trifluoromethyl)-2*H*-1,4-benzothiazines 7a,j. A suspension of 2-amino-4-(trifluoromethyl)benzenethiol hydrochloride **2** (1.2 equiv) and K_2CO_3 (3.0 equiv) in dry DMF (20 mL) was maintained at room temperature under nitrogen atmosphere and magnetic stirring for 45 min, then a solution of appropriate 2-bromo-1-phenylethanone (1.0 equiv) in the same solvent was added dropwise. When 2-bromo-1-phenylethanone disappeared (TLC), the mixture was poured in water and extracted with EtOAc (3 × 50 mL). The organic extracts were dried with Na_2SO_4, and the solvent was removed under reduced pressure to obtain a residue that was purified by flash column chromatography (EP:Et_2O 95:5 → 50:50) to give the target compounds.

3-Phenyl-6-(trifluoromethyl)-2*H*-1,4-benzothiazine (7a). Obtained from 2-bromo-1-phenylethanone **5a** as yellow solid in 43% yield; mp 83.5−84 °C. ^{1}H NMR ($CDCl_3$): δ 6.04 (1H, s, H-2), 7.40−7.60 (6H, m, Ar−H and N−H), 7.92−7.98 (1H, m, H-5), 8.05−8.15 (2H, m, H-7 and H-8). Anal. ($C_{15}H_{10}F_3NS$) C, H, N.

3-(4-Nitrophenyl)-6-(trifluoromethyl)-2*H*-1,4-benzothiazine (7j). Obtained from 2-bromo-1-(4-nitrophenyl)ethanone **5j** as orange solid in 52% yield; mp 138.5−139 °C. ^{1}H NMR ($CDCl_3$): δ 3.75 (2H, s, CH_2), 7.40−7.55 (2H, m, H-7 and H-8), 7.78−7.82 (1H, m, H-5), 8.23 (2H, d, $J = 6.94$ Hz, H-3′, H-5′), 8.38 (2H, d, $J = 6.92$ Hz, H-2′, H-6′). Anal. ($C_{15}H_9F_3N_2O_2S$) C, H, N.

3-(4-Methoxyphenyl)-6-(trifluoromethyl)-2*H*-1,4-benzothiazine Hydrochloride (7d). A suspension of 2-amino-4-(trifluoromethyl)benzenethiol hydrochloride **2** (0.37 g, 1.63 mmol) and triethylamine (0.45 mL, 3.27 mmol) in Et_2O/EtOH 50:50 (50 mL) was maintained at room temperature under nitrogen atmosphere and magnetic stirring for 15 min, then a solution of 2-bromo-1-[4-(methyloxy)phenyl]ethanone **5d** (0.25 g, 1.09 mmol) in the same solvent was added dropwise. After 90 min, the mixture was filtrated and organic solution was bubbled with HCl_g until the target compound was completely precipitated as hydrochloride. The solid was collected after crystallization with cycloesane/Et_2O to give 3-(4-methoxyphenyl)-6-(trifluoromethyl)-2*H*-1,4-benzothiazine hydrochloride **7d** (0.28 g) as a yellow solid in 79% yield; mp 171−172.5 °C. ^{1}H NMR ($CDCl_3$): δ 3.98 (3H, s, OCH_3), 4.05 (2H, s, CH_2), 7.17 (2H, d, $J = 8.93$ Hz, H-2′, H-6′), 7.50−7.55 (2H, m, H-7, H-8), 8.53 (2H, d, $J = 8.93$ Hz, H-3′, H-5′), 8.82 (1H, s, H-5). Anal. ($C_{16}H_{13}ClF_3NOS$) C, H, N.

1-(4-Chlorophenyl)-2-[(2-nitrophenyl)sulfonyl]ethanone (11c). To a solution of [(2-nitrophenyl)sulfonyl]sodium salt **10**[51] (1.00 g, 4.78 mmol) in DMF dry (8 mL) was added dropwise a solution of 2-bromo-1-(4-chlorophenyl)ethanone **5c** (1.12 g, 4.78 mmol) in the same solvent (7 mL) and the mixture was maintained at room temperature under nitrogen atmosphere and magnetic stirring for 6 h. The mixture was then poured in ice/water to obtain a yellow solid that was filtered and purified by flash column chromatography (cyclohexane:Et_2O 80:20) to give 0.91 g (yield 56%) of pure 1-(4-chlorophenyl)-2-[(2-nitrophenyl)sulfonyl]ethanone **11c** as pale-yellow solid; mp 153−154 °C. ^{1}H NMR ($CDCl_3$): δ 5.30 (2H, s, CH_2), 7.53 (2H, d, $J = 8.88$ Hz, H-3′, H-5′), 7.80−8.00 (5H, m, Ar−H), 8.17−8.24 (1H, m, H-5).

3-(4-Chlorophenyl)-4*H*-1,4-benzothiazine 1,1-Dioxide (12c). A stirred solution of 1-(4-chlorophenyl)-2-[(2-nitrophenyl)sulfonyl]ethanone **11c** (0.18 g, 0.54 mmol) in a mixture of EtOH:DMF (1:2) (25 mL) was hydrogenated over catalytic amount of Raney-Ni and under H_2 atmosphere at room temperature for 2 h. The mixture was then filtered over celite, and the filtrate was evaporated to dryness. The solid residue was crystallized by EtOH to give 3-(4-chlorophenyl)-4*H*-1,4-benzothiazine 1,1-dioxide **12c** (0.07 g, 44%) as white solid; mp 153−153.5 °C. ^{1}H NMR ($CDCl_3$): δ 6.32 (1H, s, H-2), 7.30 (1H, t, $J = 7.97$ Hz, H-6), 7.50−7.70 (4H, m, H-5, H-7, H-3′, H-5′), 7.77 (2H, d, $J = 8.67$ Hz, H-2′, H-6′), 7.83 (1H, dd, $J = 8.02$ and 1.18 Hz, H-8), 10.90 (1H, bs, NH). Anal. ($C_{14}H_{10}ClNO_2S$) C, H, N.

1-[4-(Methylthio)phenyl]-2-[(2-nitrophenyl)thio]ethanone (14m). To a solution of 2-nitrobenzenesulfenyl chloride **13** (2.00 g, 10.55 mmol) in CH_3CN dry (20 mL) was added dropwise a solution of 1-[4-(methylthio)phenyl]ethanone **4m** (1.16 g, 7.04 mmol) in the same solvent (5 mL), and the mixture was maintained at reflux under nitrogen atmosphere and magnetic stirring for 90 min. After this time, a yellow solid was separated from the mixture by filtration and purified by flash column chromatography ($CHCl_3$:MeOH 95:5) to give 1.50 g (yield 67%) of pure 1-[4-(methylthio)phenyl]-2-[(2-nitrophenyl)thio]ethanone **14m** as pale-yellow solid; mp 113.5−114.5 °C. ^{1}H NMR ($CDCl_3$): δ 2.5 (3H, s, SCH_3), 4.30 (2H, s, CH_2), 7.53 (2H, d, $J = 8.88$ Hz, H-3′, H-5′), 7.80−8.00 (5H, m, Ar−H), 8.17−8.24 (1H, m, H-5).

3-(4-Methoxyphenyl)-3,4-dihydro-2*H*-1,4-benzothiazine (15d). To a suspension of $LiAlH_4$ (0.03 g, 0.83 mmol) in THF dry (10 mL) was added dropwise a solution of 1-(4-methoxyphenyl)-2-[(2-nitrophenyl)thio]ethanone **14d**[44] (0.10 g, 0.33 mmol) in the same solvent (30 mL). The reaction mixture was maintained at room temperature under nitrogen atmosphere and magnetic stirring for 40 min. Then the mixture was added of EtOAc (10 mL), poured in water, and extracted with EtOAc (3 × 30 mL). The organic extracts were dried with Na_2SO_4, and the solvent was removed under reduced pressure to obtain a residue that was purified by flash column chromatography (EP:Et_2O 90:10 → 80:20) to give 0.04 g (yield 47%) of pure 3-(4-methoxyphenyl)-3,4-dihydro-2*H*-1,4-benzothiazine **15d** as colorless oil. ^{1}H NMR ($CDCl_3$): δ 2.48 (1H, d, $J = 3.00$ Hz, N−H), 3.26 (2H, d, $J = 5.70$ Hz, CH_2), 3.80 (3H, s, OCH_3), 4.85−5.00 (1H, m, CH), 6.90 (2H, d, $J = 8.78$ Hz, H-3′, H-5′), 7.25−7.30 (1H, m, H-6), 7.35 (2H, d, $J = 8.78$ Hz, H-2′, H-6′), 7.45−7.60 (2H, m, H-5, H-7), 8.20 (1H, dd, $J = 8.18$ and 1.34 Hz, H-8). Anal. ($C_{15}H_{15}NOS$) C, H, N.

3-[4-(Methylthio)phenyl]-3,4-dihydro-2*H*-1,4-benzothiazine (15m). Obtained by the procedure described for compound **15d**, from 1-[4-(methylthio)phenyl]-2-[(2-nitrophenyl)thio]ethanone **14m** as a colorless oil in 57% yield. ^{1}H NMR ($CDCl_3$): δ 2.39 (1H, bs, N−H), 2.52 (3H, s, SCH_3), 3.25−3.35 (2H, m, CH_2), 4.85−5.00 (1H, m, CH), 7.20−7.35 (5H, m, H-5, H-2′, H-3′, H-5′, H-6′), 7.45−7.65 (2H, m, H-6, H-7), 8.20 (1H, d, $J = 8.18$ Hz, H-8). Anal. ($C_{15}H_{15}ClNS_2$) C, H, N.

Supporting Information Available: Elemental analysis data for final compounds and figure of PLS loading plot for properties reported in Table 3 and percent of inhibition of EtBr efflux against SA1199B (Activity). This material is available free of charge via the Internet at http://pubs.acs.org.

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