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ARTICLE

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Evolution from a Natural Flavones Nucleus to Obtain 2-(4-Propoxyphenyl)quinoline Derivatives As Potent Inhibitors of the *S. aureus* NorA Efflux Pump

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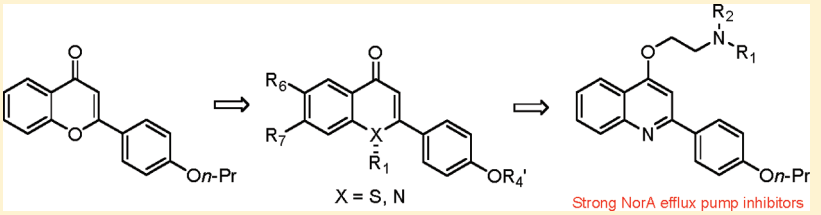
Supporting Information

ABSTRACT:



Overexpression of efflux pumps is an important mechanism by which bacteria evade the effects of substrate antimicrobial agents. Inhibition of such pumps is a promising strategy to circumvent this resistance mechanism. NorA is a *Staphylococcus aureus* efflux pump that confers reduced susceptibility to many structurally unrelated agents, including fluoroquinolones, resulting in a multidrug resistant phenotype. In this work, a series of 2-phenyl-4(1*H*)-quinolone and 2-phenyl-4-hydroxyquinoline derivatives, obtained by modifying the flavone nucleus of known efflux pump inhibitors (EPIs), were synthesized in an effort to identify more potent *S. aureus* NorA EPIs. The 2-phenyl-4-hydroxyquinoline derivatives **28f** and **29f** display potent EPI activity against SA-1199B, a strain that overexpresses *norA*, in an ethidium bromide efflux inhibition assay. The same compounds, in combination with ciprofloxacin, were able to completely restore its antibacterial activity against both *S. aureus* SA-K2378 and SA-1199B, *norA*-overexpressing strains.

INTRODUCTION

The emergence and spread of pathogens that have evolved mechanisms of resistance to multiple antibiotics is becoming a major threat to public health in the 21st century.[1] The seriousness of antibiotic resistance lies in the fact that today bacterial strains are not only resistant to commonly available antibacterials but also may have acquired augmented virulence.[2] Therefore, the discovery and development of new antibiotics is of crucial importance to counter the explosive growth of multidrug resistant pathogens.

Of particular concern among resistant microorganisms is the alarming rise of methicillin-resistant *Staphylococcus aureus* (MRSA) strains that are highly virulent.[3] The proportion of healthcare-associated staphylococcal infections that are due to MRSA has been increasing: 2% of *S. aureus* infections in U.S. intensive-care units were MRSA in 1974, 22% in 1995, and 64% in 2004.[4] Invasive MRSA infections occur in approximately 94000 persons each year and are associated with about 19000 deaths annually in U.S. Approximately 86% of these infections are healthcare-associated, and the remainder are community-associated.[5]

Although MRSA are characterized by the presence of β-lactam resistance,[6,7] these organisms also have the exceptional ability to acquire resistance to many antibacterials such as tetracyclines, macrolides, aminoglycosides, and fluoroquinolones.[8] It is, however, the ability of MRSA to acquire resistance to vancomycin, the main therapeutic agent for treatment of infections caused by this organism, which is a major source of concern. Fully vancomycin-resistant strains of *S. aureus* (MIC $\geq$16 μg/mL) were first isolated in 2002.[9,10]

Bacterial resistance that contributes to shorter drug life cycles is achieved by three main mechanisms: enzymatic inactivation,[11] modification of the drug target(s),[12,13] and reduction of intracellular drug concentration either by changes in membrane permeability[14] or overexpression of efflux pumps.[15−17] In recent years, many efforts aimed at overcoming antibacterial drug resistance have followed different approaches: (i) research on new antibacterials with novel mechanisms of action,[18,19] (ii) structural manipulation of existing antibacterials to increase the affinity with the target, even if mutated,[20,21] and/or to reduce the potential for the efflux without compromising antibacterial activity,[22] and (iii) identification of synthetic or natural non-antibiotic compounds that work as efflux pump inhibitors (EPIs),

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which can restore the susceptibility of resistant strains to coadministered antibacterials that are efflux pump substrates.[22] Efflux pumps may provide a self-defense mechanism by which antimicrobial substances made by other microbes, which fight for dominance in an environmental niche, or antibacterial drugs are actively removed from the cell. This activity results in sublethal drug concentrations at the active site that in turn may predispose the organism to the development of high-level target-based resistance.[23] Drug efflux is of major concern because of its key role in the selection of high level resistant strains.[24,25]

Efflux pumps fall into two categories based on their substrate specificity. Some pumps are specific for certain classes of substrates (e.g., the TetK system which extrudes certain tetracyclines), whereas others are multidrug resistance (MDR) pumps as they have a broader spectrum of substrate specificity and are capable of removing structurally unrelated antibacterials from the cell.[26] The proteins which make up these systems are divided into five distinct families primarily based on amino acid sequence homology.[27] These include the major facilitator superfamily (MFS),[28] the resistance-nodulation-division (RND),[29] small multidrug-resistance (SMR),[30] ATP-binding cassette (ABC),[31] and the multiple antibiotic and toxin extrusion (MATE) families.[32] Efflux of drugs from Gram-positive bacteria may be mediated by members of any of these families with the possible exception of RND proteins.[26] The most studied efflux pump of *S. aureus* is NorA, a transporter belonging to the MFS. MFS pumps such as NorA are capable of extruding multiple, structurally dissimilar substrates such as hydrophilic fluoroquinolones, various biocides, and dyes.[15]

Therefore, efflux pumps are viable antibacterial targets and identification and development of potent EPIs is a promising and valid strategy.[33] There are a number of reasons to pursue this area, including: (i) the EPI restores susceptibility to the antibacterial and an EPI−antibacterial combination has been shown to reduce the rate of emergence of antibiotic-resistant variants, (ii) this is a natural approach, as there are examples in which plants produce both antibacterials and EPIs that improve their antibacterial activity (e.g., berberine and the methoxyhydnocarpin, isoflavones and α-linolenic acid, and dalversinol A and a methoxychalcone),[34−36] (iii) EPIs used in combination with antibiotics may not only increase antibacterial potency[37] but also may expand the antibacterial spectrum and reduce the frequency of the emergence of target-based resistance,[38] (iv) EPIs have been shown to reduce biofilm formation and block the antibacterial tolerance of biofilms.[39]

In recent years, many EPIs capable of potentiating the activity of antimicrobial substrates have been identified. Early EPIs included reserpine and verapamil, but concentrations required for pump-inhibitory activity are too high to be clinically relevant.[40,41] Several other nonantibiotic compounds, such as omeprazole, paroxetine, chlorpromazine, and respective derivatives, and the synthetic acridone derivative GG918, have been shown to increase the antibacterial potency of pump substrates by inhibiting NorA of *S. aureus*.[42−46] The main efforts to find EPIs has been made by phytochemists, who have reported an extremely varied series of natural compounds in terms of their chemical class and shape including flavones, isoflavones, porphyrin phaeophorbide A, and acylated glycosides.[47,48]

To date, there are only a few examples of rationally designed inhibitors, and very little work has been done with respect to the structure−activity relationship (SAR) of NorA inhibitors.[49−53] Although the therapeutic utility of EPIs has yet to be validated in the clinical setting, this approach holds promise for improving the efficacy and/or extending the clinical utility of existing antibacterials, giving new life to old drugs[37] with secure economic benefit.

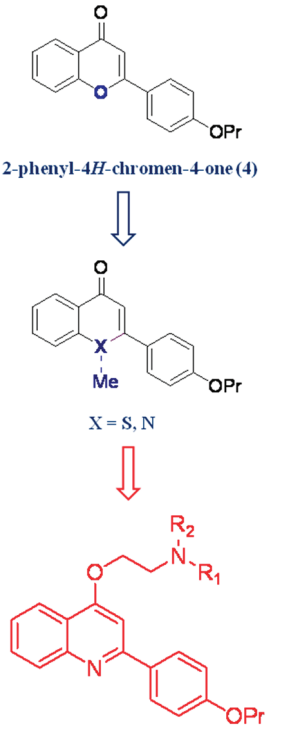

Figure 1. From the 2-phenyl-4*H*-chromen-4-ones to 2-(4-propoxyphenyl)-quinoline EPIs.

To our knowledge, the NorA binding pocket has not been defined; sequence homology and the sharing of a wide range of substrates and/or inhibitors with the *B. subtilis* Bmr MDR pump and the plasmid-encoded *S. aureus* QacA MDR pump have 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. This structural peculiarity could explain the broad substrate specificity of MDR pumps.[54] The lack of information regarding the mechanism(s) of interaction between substrates or EPIs and NorA makes it difficult to design new inhibitors via computational methods. In addition, the structural heterogeneity of known NorA EPIs do not allow a clear and unequivocal SAR for this category of compounds. For these reasons, we have chosen to undertake a classical medicinal chemistry approach to design new structures which could be more powerful EPIs with the goal being the identification of a small molecule chemotype capable of restoring ciprofloxacin (CPX) activity on *S. aureus* strains by inhibition of NorA.

Design Rationale. Our previous work in this field led to the identification of substituted 3-phenyl-1,4-benzothiazine EPIs by a minimization of the phenothiazine moiety, which were capable of completely restoring the antibacterial activity of CPX against NorA overexpressing strains.[51] We also identified 6-amino-8-methylquinolone esters, strong inhibitors of the *S. aureus* efflux pumps NorA (MFS) and MepA (MATE), by structural

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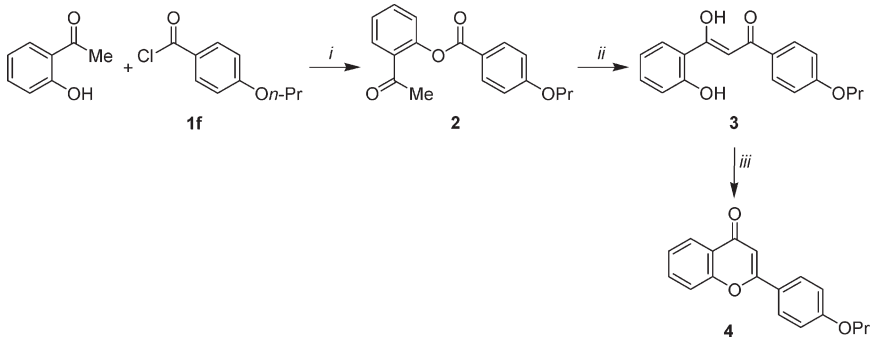
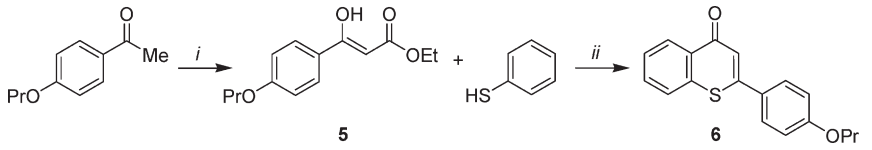
Scheme 1[a]



[a] Reagents and conditions: (i) dry pyridine, rt; (ii) K_2CO_3, acetone, reflux; (iii) Amberlist 15, *i*-PrOH/$(i\text{-Pr})_2O$, reflux.

Scheme 2[a]



[a] Reagents and conditions: (i) NaH, $(EtO)_2CO$, rt; (ii) PPA, 90 °C.

modifications of the 6-aminoquinolone antibacterial core.[52] In an attempt to obtain chemotypes with potent inhibitory activity against NorA, we have chosen to modify the 2-phenyl-4*H*-chromen-4-one moiety, a common feature of flavone and natural flavolignane EPIs.[47−49] This was performed by exchanging the endocyclic oxygen with an nitrogen atom to obtain the 2-phenylquinolone nucleus and then, after suitable substitutions, the 2-(4-propoxyphenyl)quinoline derivatives which are able to reduce transport of antibacterial quinolones by NorA (Figure 1).

The 2-phenylquinolone nucleus has all of the known requisites to provide favorable EPI activity, including a suitable large hydrophobic area (the two phenyl rings) and the capability of establishing an electrostatic interaction (by the N-1 and the ketone in C-4 position).[55] Moreover, in addition to being a mimic of the quinolone antibacterial core, possibly allowing for an positive interaction with NorA binding site(s), the 2-phenylquinolone nucleus is also a versatile skeleton suitable for relatively simple chemical modifications that could provide a large number of structurally different derivatives.

Chemistry. To investigate if the quinolone nucleus was a good replacement of the chromen-4-one on the inhibition of NorA, we have resynthesized the flavone compound **4**[49] (Scheme 1) and its 2-(4-propoxyphenyl)-4*H*-thiochromen-4-one strict analogue **6** (Scheme 2) to compare with the corresponding 2-(4-propoxyphenyl)quinolin-4(1*H*)-one derivatives obtained, introducing a nitrogen atom instead of the endocyclic oxygen of the 2-(4-propoxyphenyl)-4*H*-chromen-4-one **4**[49] (Figure 1).

2-(4-Propoxyphenyl)-4*H*-chromen-4-one **4**[49] was obtained starting from the 1-(2-hydroxyphenyl)ethanone following the procedure reported from Patonay et al.[56] for the synthesis of the flavone nucleus (Scheme 1).

The initial acylation of the 2-phenol group with 4-propoxybenzoyl chloride **1f**,[57] obtained by treating the corresponding 4-propoxy benzoic acid with $SOCl_2$, in dry pyridine at rt, provided the 2-acetylphenyl 4-propoxybenzoate **2** in good yield (72%). Then, a base-catalyzed transposition of the acyl group to the α-methylketone provided the diphenyl-1,3-ketoenol **3** in 60% yield that was then cyclized, in acid condition, with Amberlist 15, to the 2-(4-propoxyphenyl)-4*H*-chromen-4-one **4**[49] (yield 32%) (Scheme 1).

The synthetic route giving rise to the 2-(4-propoxyphenyl)-4*H*-thiochromen-4-one **6** (Scheme 2) entailed the synthesis of the (2*Z*)-3-hydroxy-3-(4-propoxyphenyl)prop-2-enoate **5**,[58] obtained by reaction of 1-(4-propoxyphenyl)ethanone[59] with $(EtO)_2CO$ and NaH in good yields (76%), which in turn reacted with thiophenol in polyphosphoric acid (PPA) to give the target compound **6** in 46%yield.

Attempting to synthesize the 2-[4-(propyloxy)phenyl]quinolin-4(1*H*)-one analogue of flavone **4**,[49] we observed that if the N-1 nitrogen atom of the quinolone nucleus was unalkylated, the only tautomer that we have obtained and detected by ^{1}H NMR was the 2-(4-propoxyphenyl)-4-hydroxyquinoline **20f** instead of the desired C-4 ketone form. To resolve this limitation, we decided to make a direct methylation of compound **20f** to obtain the desired *N*-alkylated 1-methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one **22f** as well as the *O*-methyl derivative 4-methoxy-2-(4-propoxyphenyl)quinoline **21f** as a byproduct (Scheme 3).

Flavone compound **4**[49] and its strict sulfur-analogue **6** were preliminarily compared with 2-(4-propoxyphenyl)-4-hydroxyquinoline **20f** and 1-methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one **22f** for their ability to reduce the efflux of the well-known NorA substrate ethidium bromide (EtBr) against the well-described *norA*-overexpressing strain SA-1199B.[60,61] Data confirm that 2-phenylquinolone **22f** displays the same, even if weak (about 30%), inhibition of EtBr efflux with respect to the reference flavone compound **4**.[49] This weak but interesting activity led us to deepen our study subjecting compound **22f** to a series of chemical manipulations with the goal of obtaining more powerful derivatives (Table 1).

Modifications initially involved the propoxy group of the C-2 phenyl ring of compound **22f**, in which the propyl chain

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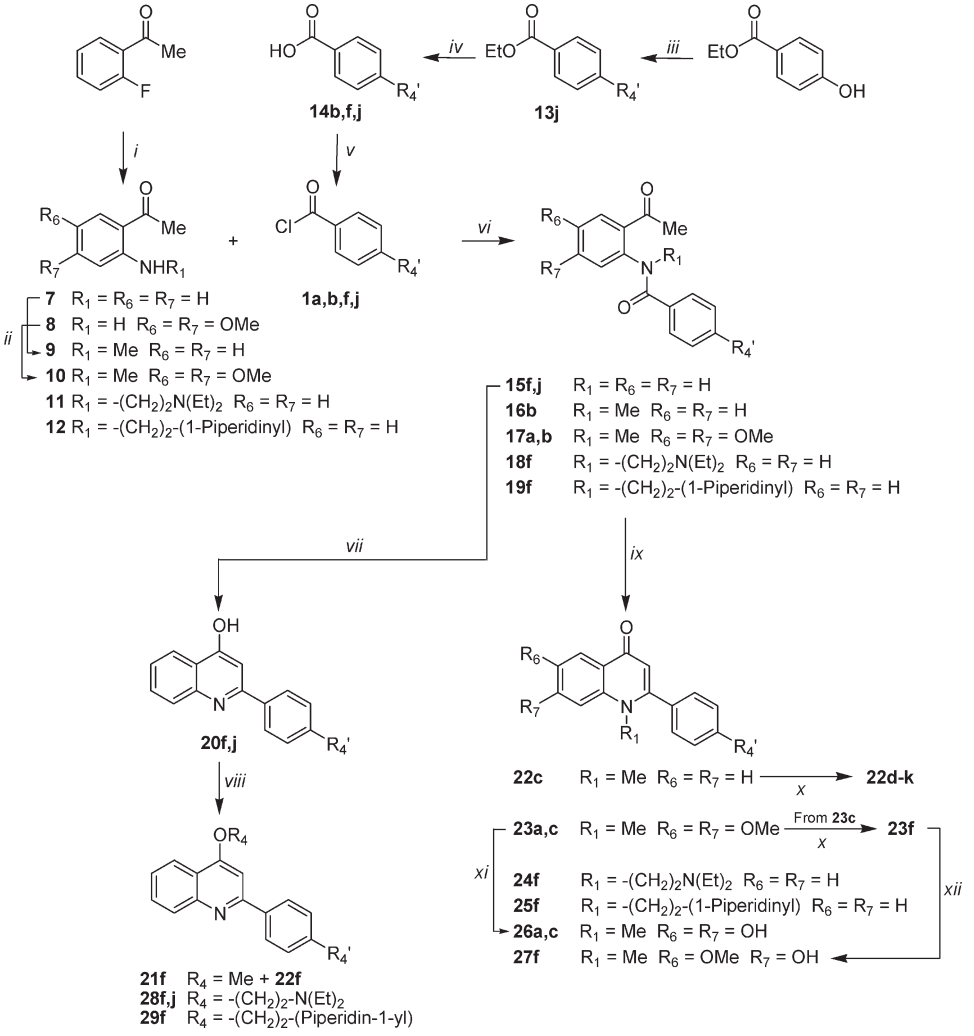
Scheme 3[a]



[a] Reagents and conditions: (i) *N*,*N*-diethylethane-1,2-diamine or 2-(1-piperidinyl)ethanamine, DMF, 100 °C; (ii) MeI, toluene, reflux; (iii) 1-(2-chloroethyl)-piperidine hydrochloride, K_2CO_3, dry DMF, 100 °C; (iv) NaOH 5N, MeOH, rt; (v) $SOCl_2$, reflux; (vi) Et_3N, THF, 70 °C; (vii) *t*-BuOK, *t*-BuOH, heating; (viii) MeI, K_2CO_3, dry DMF, 100 °C or (2-chloroethyl)diethylamine hydrochloride, K_2CO_3, dry DMF, reflux or 1-(2-chloroethyl)piperidine hydrochloride, *t*-BuOK, dry DMF, 100 °C; (ix) NaH, dry DMF, rt or *t*-BuOK/*t*-BuOH, 60 °C; (x) R-X, K_2CO_3, dry DMF, 70 °C; (xi) CH_2Cl_2, BBr_3, rt; (xii) LiCl, dry DMF, reflux.

was deleted (**22c**),[62] replaced with shorter alkyl chains (**22d**,[63] **22e**, and **22g**) or with *O*-ethylamino chains (**22h**−**k**). Further modifications involved the C-6 and C-7 positions of the quinolone nucleus of compound **22f** by introducing 6,7-dimethoxy (**23a**,[64] **23c**, and **23f**), 6,7-dihydroxy (**26a** and **26c**), or 6,7-methoxyhydroxy (**27f**) groups, also present on natural and synthetic EPIs (Chart 1).[40−42,46−48] Encouraging results were obtained by introducing an ethyl-2-diethylamine or an ethyl-2-piperidine at the N-1 position of the 2-phenylquinolone nucleus (compounds **24f** and **25f**) (Scheme 3).

It was then decided to explore the chemical space around the C-4 position of the quinolone nucleus by modifying the hydroxyl group in the C-4 position of the 2-phenyl-4-hydroxyquinoline nucleus of compound **20f** through a simple alkylation reaction, and this permitted us to obtain compounds **28f,j** and **29f** (Table 1).

The synthetic strategy was established to obtain both the 2-phenylquinoline (**20**, **21**, **28**, **29**) and 2-pheniquinolone (**22**−**27**) nuclei (Scheme 3) needed to provide the appropriate starting 1-(2-aminophenyl)ethanones (**7**−**12**) and the suitable benzoyl chlorides (**1a**, **1b**, **1f**,[57] and **1j**) to give the key intermediates *N*-(2-acetylphenyl)benzamides (**15**−**19**). Starting from the 1-(2-fluorophenyl)ethanone, the 1-{2-[(2-aminoethyl)amino]phenyl}ethanones

Chart 1. R_4' Substituents

	R_4'		R_4'
a	H	g	O*i*-Pr
b	OAc	h	O-$(CH_2)_2$-$N(Me)_2$
c	OH	i	O-$(CH_2)_2$-$N(Et)_2$
d	OMe	j	O-$(CH_2)_2$-(Piperidin-1-yl)
e	OEt	k	O-$(CH_2)_2$-(Morpholin-4-yl)
f	O*n*-Pr		

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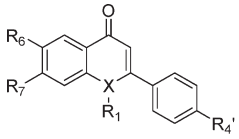
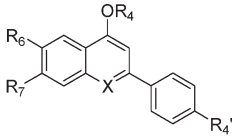
Table 1. EtBr Efflux Inhibition (%) on SA-1199B of Synthesized Compounds at 50 μM Concentration



4,6,22-27



20,21,28,29

								MIC	
compd	X	R_1	R_4	R_6	R_7	R_4'	EtBr efflux inhib (%)	μM	μg/mL
4	O			H	H	O*n*-Pr	32.4	*a*	*a*
6	S			H	H	O*n*-Pr	6.3		
20f	N		H	H	H	O*n*-Pr	0.0		
22f	N	Me		H	H	O*n*-Pr	33.9		
22c[62]	N	Me		H	H	OH	0.0		
22d[63]	N	Me		H	H	OMe	0.0		
22e	N	Me		H	H	OEt	10.8		
22g	N	Me		H	H	O*i*-Pr	10.3		
22h	N	Me		H	H	$O(CH_2)_2N(Me)_2$	4.4		
22i	N	Me		H	H	$O(CH_2)_2N(Et)_2$	1.7		
22j	N	Me		H	H	$O(CH_2)_2$-(piperidin-1-yl)	19.8		
22k	N	Me		H	H	$O(CH_2)_2$-(morpholin-4-yl)	0.0		
23a[64]	N	Me		OMe	OMe	H	9.4		
23c	N	Me		OMe	OMe	OH	0.0		
23f	N	Me		OMe	OMe	O*n*-Pr	0.0		
26a	N	Me		OH	OH	H	0.0		
26c	N	Me		OH	OH	OH	0.0		
27f	N	Me		OMe	OH	O*n*-Pr	9.1		
24f	N	$-(CH_2)_2N(Et)_2$		H	H	O*n*-Pr	57.3		
25f	N	$-(CH_2)_2$-(piperidin-1-yl)		H	H	O*n*-Pr	75.1	>256	>100
21f	N		Me	H	H	O*n*-Pr	63.7		
28f	N		$-(CH_2)_2N(Et)_2$	H	H	O*n*-Pr	93.4	>241	>100
28j	N		$-(CH_2)_2N(Et)_2$	H	H	$O(CH_2)_2$-(piperidin-1-yl)	65.6	>223	>100
29f	N		$-(CH_2)_2$-(piperidin-1-yl)	H	H	O*n*-Pr	88.5	256	100
reserpine							84.8	>164	>100
paroxetine							89.7	>303	>100

[a] Not determined for those compounds that have shown an EtBr inhibition efflux less than 65%.

11[65] and **12** were obtained by aromatic nucleophilic substitution of the fluorine atom with *N*,*N*-diethylethane-1,2-diamine and (2-piperidin-1-ylethyl)amine, in dry DMF at 100 °C, with yields of 70 and 46%, respectively. The 1-[2-(Methylamino)phenyl]-ethanones **9**[66] and **10**[67] were synthesized by methylation with MeI in toluene at reflux of 1-(2-aminophenyl)ethanone **7** and 1-(2-amino-4,5-dimethoxyphenyl)ethanone **8** in 42 and 48% yield, respectively.

With the exception of **1a**, the benzoyl chlorides **1b**, **1f**,[57] and **1j** were obtained in quantitative yields from the respective carboxylic acids **14b**, **14f**, and **14j**,[68] which were treated with $SOCl_2$ at reflux for 1 h. The 4-(2-piperidin-1-ylethoxy)benzoic acid **14j**[68] was obtained from ethyl 4-hydroxybenzoate by alkylation with 1-(2-chloroethyl)piperidine hydrochloride in dry DMF and K_2CO_3 at 100 °C to give the ethyl 4-(2-piperidin-1-ylethoxy)-benzoate **13j**,[68] which was hydrolyzed by NaOH 5% to furnish the corresponding acid in good yields.

The 1-(2-aminophenyl)ethanones **7**, **8**, **9**,[66] **10**,[67] **11**,[65] and **12** gave a nucleophilic substitution to the suitable benzoyl chlorides **1a**, **1b**, **1f**,[57] and **1j** in dry THF at 70 °C, using Et_3N as HCl scavenger, to obtain the corresponding *N*-(2-acetylphenyl)benzamides (**15**−**19**). The *N*-(2-acetylphenyl)benzamides **15f** and **15j** were then cyclized with *t*-BuOK in *t*-BuOH to obtain the corresponding 4′-substituted 2-phenyl-4-hydroxyquinolines **20f**

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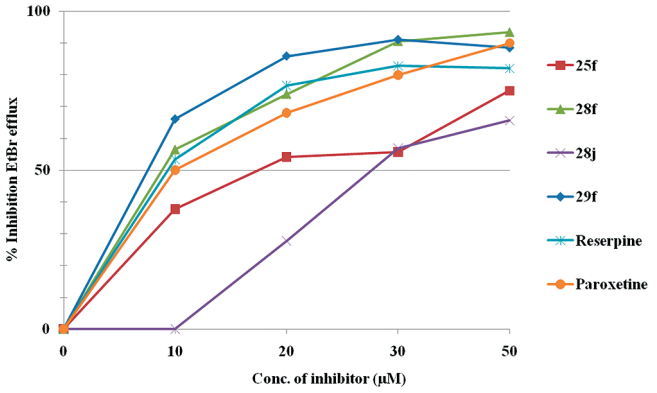

Figure 2. Effect of compounds **25f**, **28f**, **28j**, **29f**, reserpine, and paroxetine on EtBr efflux of SA-1199B.

and **20j**. Methylation of **20f** with MeI in dry DMF/K_2CO_3 gave the *O*-Me derivative **21f** and *N*-Me derivative **22f**. Alkylation of hydroxyquinolines **20f** and **20j** with the appropriate 2-chloroethylamines gave only the *O*-substituted compounds **28f**, **28j**, and **29f**.

Cyclization of the *N*-substituted-(2-acetylphenyl)benzamides **16**−**19** in NaH/dry DMF or *t*-BuOK/*t*-BuOH gave the corresponding 1-alkyl-2-phenyl-4(1*H*)-quinolinones **22**−**25**. Under these conditions, the 4′-acethoxy group of compounds **16b** and **17b** were also hydrolyzed, resulting in 2-(4′-hydroxyphenyl)-1-methyl-4(1*H*)-quinolinone **22c**[62] and its 6,7-dimethoxy analogue **23c**. Starting from 2-(4′-hydroxyphenyl)-1-methyl-4(1*H*)-quinolinones **22c**[62] and **23c**, the 4′-substituted compounds **22d**[63]−**k** and **23f** were obtained in moderate to quite good yields (14−65%) by an alkylation procedure in dry DMF/K_2CO_3 using the appropriate alkyl halide.

6,7-Dihydroxy derivatives **26a,c** were obtained in good yields (82 and 99%, respectively) by *O*-demethylation of the corresponding 6,7-dimethoxy derivatives **23a**,[64]**c** with 1 M BBr_3 in CH_2Cl_2 at rt. Under the same conditions, or in 48% HBr, the 6,7-dimethoxy-1-methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one **23f** does not give the corresponding 6,7-dihydroxy derivative but only the trihydroxy compound **26c**. Milder conditions such as LiCl in dry DMF at reflux were used to avoid C-4′ *O*-depropylation, and only 7-hydroxy-6-methoxy-1-methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one **27f** was obtained in low yields (13%) after 4 days. A confirmation that *O*-demethylation was carried out to the OMe in the C-7 position was achieved by an $^1H-^1H$ 2D-NOESY NMR experiment (see Supporting Information). This clearly showed that the OMe group in C-6 position got a NOE interaction with H-5 and thus indicated that the demethylation happened to the OMe group in position C-7.

RESULTS AND DISCUSSION

In this study, a series of 2-phenyl-4(1*H*)-quinolone (**22**−**27**) and 2-phenyl-4-hydroxyquinoline derivatives (**20**, **21**, **28**, **29**) were designed by exchanging the endocyclic oxygen of the 2-phenyl-4*H*-chromen-4-one nucleus, a common feature of flavone and natural flavolignane EPIs,[47−49] with a nitrogen atom, to obtain more effective *S. aureus* NorA EPIs. A microbiological approach was used to assess EPI function by determining their EtBr efflux inhibition using SA-1199B, a well characterized strain that overexpresses *norA*,[60,61] at 50 μM concentration (Table 1).

For the more promising compounds (**25f**, **28f**, **28j**, and **29f**) demonstrating inhibition of EtBr efflux by SA-1199B by >65% at a 50 μM concentration, dose−response curves were built to assess their activity at lower concentrations in comparison with the reference compounds reserpine and paroxetine (Figure 2). Moreover, the same active compounds (**25f**, **28f**, **28j**, and **29f**) were tested for their synergism with CPX against two pairs of *S. aureus* strains, SA-K1902 (*norA*−)/SA-K2378 (*norA*++) and SA-1199 (*norA* wild-type)/SA-1199B (*norA*++ and A116E GrlA) as well as against *S. aureus* ATCC 25923 (wild-type strain), using checkerboard assays,[69] evaluating their ability to reduce the MICs of the fluoroquinolone in comparison with the above cited reference compounds (Figure 3).

To validate the hypothesis that the quinolone nucleus could be a suitable scaffold for a new class of EPIs able to confer a good inhibitory activity against NorA, preliminary screening was performed by assessing inhibition of EtBr efflux by SA-1199B. Compounds evaluated included the 2-(4-propoxyphenyl)-4-hydroxyquinoline **20f**, the 1-methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one **22f**, the reference compound 2-(4-propoxyphenyl)-4*H*-chromen-4-one **4**,[49] and 2-(4-propoxyphenyl)-4*H*-thiochromen-4-one **6**, its strict sulfur-analogue. The screening concentration employed was 50 μM. The data in Table 1 confirm that 1-methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one **22f** displays the same, even if low (about to 30%), inhibitory activity as the reference flavone compound **4**.[49] This activity was too weak to permit the quinolone compound **22f** to display a synergistic activity with CPX against *norA*-overexpressing strains of *S. aureus*, but it was enough to make this compound a hit to subject to a series of chemical manipulations in an effort to obtain more powerful derivatives (Table 1).

Initially, we modified the propoxy group of the phenyl ring in the C-2 position of the quinolone scaffold of compound **22f** by removing the propyl chain (compound **22c**),[62] replacing that with shorter alky chains (compounds **22d**,[63] **22e**, and **22g**) or different ethylamino chains (compounds **22h**−**k**). None of these modifications gave more active compounds in terms of EtBr efflux inhibition activity, suggesting that the best substituent in this position is the propoxy group (Table 1). The same unsatisfactory results were obtained by introducing 6,7-dimethoxy

Table 2. Evaluation of Intrinsic Antibacterial Activity (MICs, μg/mL) of Compounds 25f, 28f, 28j, 29f, Reserpine, Paroxetine, and CPX against the Five *S. aureus* Strains Included in the Test of Synergism with CPX

	modified strains				
	MIC (μg/mL)				
compd	*S. aureus* ATCC 25923 (WT)	SA-K1902 (*norA*−)	SA-K2378 (*norA*++)	SA-1199 (*norA* WT)	SA-1199B (*norA*++/ *A116E GrlA*)
25f	>100	>100	>100	>100	>100
28f	100	50	50	>100	>100
28j	100	100	100	>100	>100
29f	100	50	50	100	100
reserpine	>100	>100	>100	>100	>100
paroxetine	>100	>100	100	>100	>100
CPX	0.31	0.63	2.50	0.63	10

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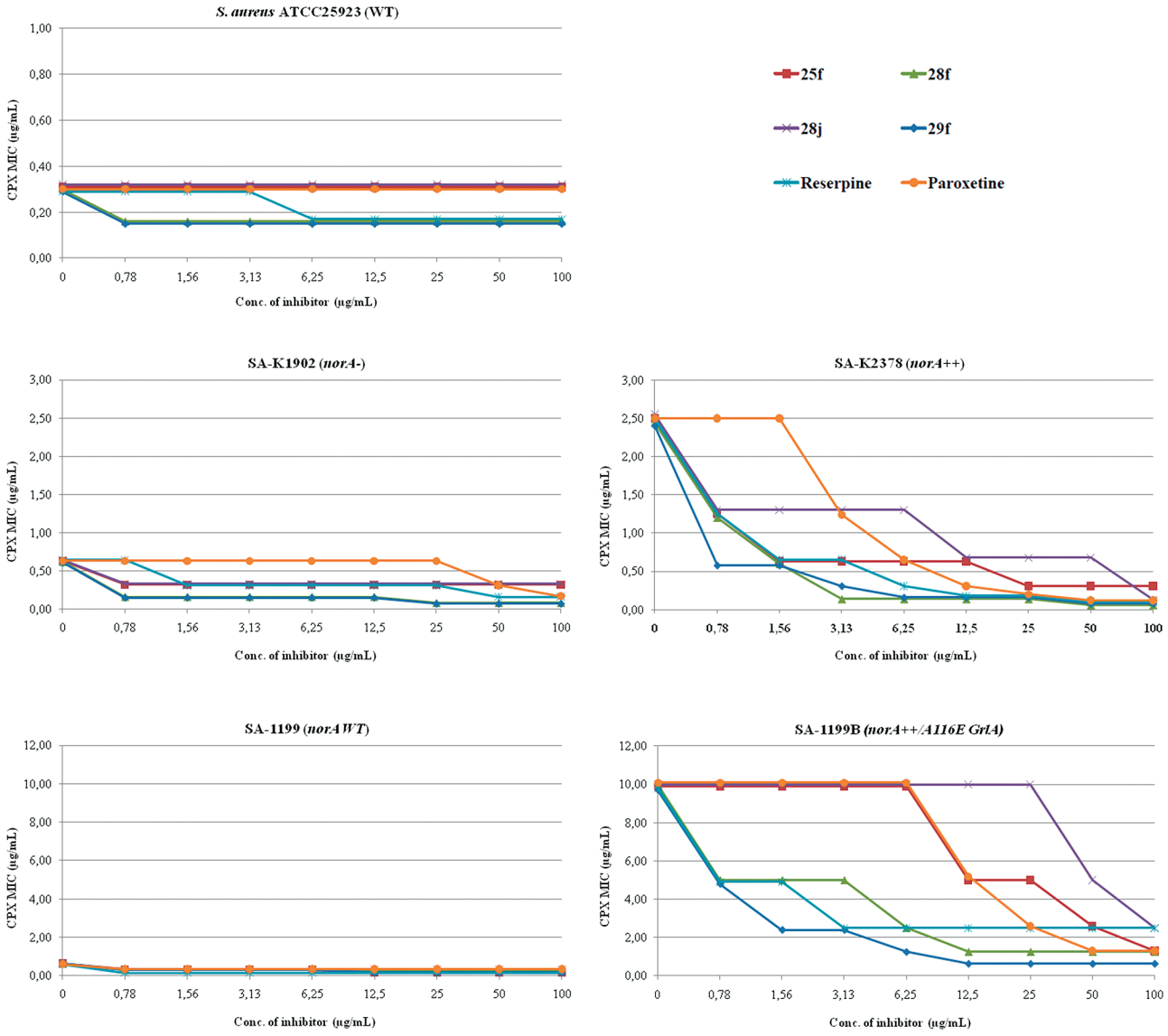

Figure 3. Effect of compounds **25f**, **28f**, **28j**, **29f**, reserpine, and paroxetine on the MIC of ciprofloxacin against *S. aureus* ATCC25923, SA-K1902, SA-K2378, SA-1199, and SA-1199B.

(**23a**,[64] **23c**, and **23f**), 6,7-dihydroxy (**26a** and **26c**), or 6,7-methoxy-hydroxy (**27f**) groups, also present on natural and synthetic EPIs,[40−42,46−48] in the C-6 and C-7 positions of the quinolone nucleus of compound **22f** (Table 1).

Modification of the substituent in the N-1 position of the 2-phenylquinolone nucleus of compound **22f** provided improved results. By introducing an ethyl-2-diethylamine or an ethyl-2-piperidine in that position, compounds **24f** and **25f** were obtained which display EtBr efflux inhibitory activity of 57.3 and 75.1% at 50 μM concentration, respectively (Table 1).

A turning point in this work is represented from the test of 4-methoxy-2-(4-propoxyphenyl)quinoline **21f**, previously obtained as a byproduct, that displays reasonably potent inhibition of EtBr efflux (63.7%). These results led us to switch our attention to the *O*-substituted 2-phenyl-4-hydroxyquinoline nucleus of compound **21f** instead of the *N*-substituted 2-phenylquinolone moiety of compound **22f**, synthesizing the corresponding C-4 *O*-ethyl-2-diethylamine and *O*-ethyl-2-piperidine derivatives (compounds **28f** and **29f**). These compounds were the most potent NorA inhibitors, demonstrating 93.4 and 88.5% inhibition of EtBr efflux, respectively. These results were slightly better than that of the reference compound reserpine (84.8%) and comparable to that of paroxetine (89.7%) (Table 1). To deepen our studies on the SAR of this new class of EPIs and to better comprehend the key role played by the ethylamino substituent, at least for compounds **24f**, **25f**, **28f**, and **29f**, a further ethylpiperidine chain was introduced instead of the propoxy group of the phenyl ring in the C-2 position of the quinoline scaffold of compound **28f** to obtain the derivative **28j**. This modification produces, for compound **28j**, a decreased EtBr efflux inhibition activity (65.6%) when compared with its strict analogue **28f** (Table 1).

For compounds **25f**, **28f**, **28j**, and **29f**, which overcome the threshold of 65% EtBr efflux inhibition against SA-1199B at a concentration of 50 μM, a dose−response curve was built to investigate their potency at different concentrations in comparison with the reference compounds reserpine and paroxetine (Figure 2).

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The dose−response curves shown in Figure 2 confirm that compounds **25f** and **28j** are inferior to both reference compounds. The activity of compound **28f** is approximately equipotent to reserpine and paroxetine, whereas compound **29f** seems to be slightly better than the reference compounds at concentrations less than 50 μM. These results were confirmed by comparing the IC_{50} values, which were between 7 and 10 μM for both reference compounds as well as **28f** and **29f**.

To establish whether the efflux inhibitory activity of compounds **25f**, **28f**, **28j**, and **29f** results in a synergistic interaction with ciprofloxacin (CPX), a fluoroquinolone antibacterial substrate of the NorA efflux pump, it was necessary to initially evaluate the intrinsic antibacterial activity of the new EPIs against the *S. aureus* strains included in the test (*S. aureus* ATCC 25923, SA-K1902, SA-K2378, SA-1199, and SA-1199B), to avoid a misleading interpretation in assessing NorA inhibitory activity. Reserpine, paroxetine, and CPX were included for comparative purposes (Table 2).

Data collected in Table 2 display that compounds **25f**, **28f**, **28j**, and **29f** show weak (50−100 μg/mL) or no antibacterial activity up to the top concentration tested, a stark contrast in comparison with the marketed antibacterial quinolone CPX. The effect of combining **25f**, **28f**, **28j**, **29f**, reserpine, and paroxetine on CPX MICs was assessed by checkerboard assays,[69] which were performed using two pairs of *S. aureus* strains, SA-K1902 (*norA*−)/SA-K2378 (*norA*++) and SA-1199 (*norA* wild-type)/SA-1199B (*norA*++ and A116E GrlA), as well as *S. aureus* ATCC25923 (control, wild-type strain) (Figure 3).

Isobolograms shown in Figure 3 reveal no synergistic activity between any of the tested compounds and CPX against wild-type strain *S. aureus* ATCC25923. These data are in agreement with a low level of expression of the NorA efflux pump in this strain. For SA-K1902, in which the *norA* gene is deleted, compounds **25f** and **28j**, as well as reserpine and paroxetine, do not show any significant synergistic activity with CPX. However, compounds **28f** and **29f** demonstrate a modest synergistic effect manifest by a 4-fold reduction in CPX MIC at 0.78 μg/mL concentration to an 8-fold reduction for concentrations above 25 μg/mL. This synergistic activity may be the result of weak antibacterial activity of both compounds **that may** be observed at concentrations greater than $^1/_4$ of the MIC of the EPIs for this strain (50 μg/mL) or with an interaction with some undefined off target (Figure 3 and Table 2).

With respect to SA-K2378, which overexpresses *norA* from a multicopy plasmid, there is a gradual reduction of CPX MICs across all tested concentrations of compounds **25f**, **28f**, **28j**, and **29f**. Although **25f** and **28j** were the least active compounds, with a 4-fold reduction of CPX MICs at 1.56 and 12.5 μg/mL concentrations respectively, compounds **28f** and **29f** display a strong synergistic activity with CPX in that they are able to reduce MICs 16-fold at 3.13 and 6.25 μg/mL concentrations, respectively, which are below of $^1/_4$ of their MICs against this strain (50 μg/mL). Against SA-K2378, the synergistic activity with CPX of the compounds **28f** and **29f** results are better than that of paroxetine and comparable to that of reserpine. Comparing the synergistic activities with CPX against strains SA-K1902 (*norA*−) and SA-K2378 (*norA*++), compounds **28f**, **29f**, and reserpine are able to completely restore the antibacterial activity of CPX against the resistant strain (Figure 3).

For SA-1199 (*norA* wild-type), none of the tested compounds nor reserpine or paroxetine have any significant synergistic activity with CPX (Figure 3). For SA-1199B (CPX MIC 10 μg/mL), which overexpresses *norA* and also has a A116E GrlA mutation, all tested compounds display a different level of synergism with CPX. For the least active compounds **25f**, **28j**, and paroxetine, this effect was observed only at high concentrations (50, 100, and 25 μg/mL, respectively). Compound **28f** displays good synergism with CPX, resulting in an 8-fold reduction in MIC at concentrations above 12.5 μg/mL. These results were superior to the reference compound reserpine. Compound **29f** was most active, showing a synergism with CPX superior to reserpine at all concentrations above 6.25 μg/mL. MIC reductions of 16-fold (10 to 0.63 μg/mL) were seen at concentrations ≥12.5 μg/mL. Compound **29f** completely restored the antibacterial activity of CPX against this highly resistant strain (Figure 3). This observation suggests that efflux pump inhibition by **29f** is so efficacious as to block all NorA activity in this strain. Considering that the MIC of compound **29f** against SA-1199B was 100 μg/mL and the best synergistic activity with CPX was achieved at 12.5 μg/mL, it is likely that there was minimal interaction between the intrinsic antibacterial activities of the two compounds. On the other hand, we cannot exclude the involvement of some off-target activities (i.e., inhibition of some non NorA efflux pumps which have a basal expression in this strain) both for compound **29f** and reserpine.

Data collected in this study highlight that 2-{[2-(4-propoxyphenyl)-4-quinolinyl]oxy}ethanamine derivatives **28f** and **29f**, obtained by modification of the simplest flavone EPI **4**,[49] can be effective inhibitors of NorA. For the more active compounds, a good agreement between the inhibitory activity of EtBr efflux against SA-1199B and the synergistic activity with CPX against NorA overexpressing *S. aureus* strains was observed. In an attempt to delineate a preliminary SAR for this new class of EPIs, it is possible to state that the best activities were displayed by compounds carrying the 2-phenyl-4-hydroxyquinoline nucleus instead of the quinolone core, but only if the C-4 hydroxyl group was alkylated. A key role was played by the 2-ethylamino chains, which when inserted at the N-1 position of the quinolone nucleus or at the C-4 hydroxyl of the quinoline moiety, gave compounds with activity better than those carrying the same chains in the C-2 phenyl ring. The C-4′ propoxy group appears to be the best substituent for the C-2 phenyl ring, while neither the methoxy nor the hydroxyl groups are tolerated in the C-6 and C-7 positions of the quinolone nucleus.

■ CONCLUSION

In conclusion, the 2-{[2-(4-propoxyphenyl)-4-quinolinyl]-oxy}ethanamine derivatives **28f** and **29f** show modest or no intrinsic antistaphylococcal activity up to the highest concentration tested (100 ug/mL) and were able to restore, in a concentration-dependent manner, the antibacterial activity of CPX against *norA*-overexpressing *S. aureus* strains. Although the EPI activity of these derivatives in the EtBr efflux inhibition assay provide results similar to that of reserpine, their synergistic activity with CPX was superior to that shown by the reference compound against the norA-overexpressing strain SA-1199B. Actually, we cannot exclude the involvement of some off target activities, which will be the subject of further studies.

■ EXPERIMENTAL SECTION

Bacterial Strains. The strains of *S. aureus* employed were ATCC 25923 (wild-type), SA-K1902 (*norA*-deleted), SA-1199B (overexpressing *norA* and also possesses an A116E GrlA substitution), and its

isogenic parent SA-1199.[60,61] In addition, SA-K2378, which overexpresses *norA* from a multicopy plasmid, was also used. This strain was produced by cloning *norA* and its promoter into plasmid pCU1 and then introducing the construct into SA-K1902.[70]

Microbiologic Procedures. MICs were determined by microdilution techniques according to CLSI guidelines.[71] The effect of combining reserpine or various test compounds, with scalar dilutions of freshly prepared solutions of each selected compound, on the MICs of CPX was also determined. Checkerboard combination studies using CPX and **25f**, **28f**, **28j**, and **29f** were performed as described previously.[69]

EtBr Efflux. The loss of EtBr from *S. aureus* SA-1199B was determined fluorometrically as previously described.[72] Experiments were performed in duplicate, and the results were expressed as mean total efflux over a 5 min time course. The effect of increasing concentrations of reserpine, **25f**, **28f**, **28j**, and **29f** on the EtBr efflux of SA-1199B was compared to that in their absence, allowing the calculation of the percentage reduction in efflux.

Synthesis. All reactions were routinely checked by thin-layer chromatography (TLC) on silica gel $60F_{254}$ (Merck) and visualized using UV illumination. Flash column chromatography was performed on Merck Silica Gel 60 (mesh 230−400) using the indicated solvents. Yields were of purified product and were not optimized. Melting points were determined in capillary tubes (Mettler PF62 apparatus) and are uncorrected. Elemental analyses were performed by a Fisons elemental analyzer (model EA1108CHN), and the data for C, H, and N are within 0.4% of the theoretical values. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100.62 MHz, respectively, with a Bruker Advance-DRX 400 instrument 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-Acetylphenyl 4-propoxybenzoate (2). 4-Propoxybenzoyl chloride **1f**[57] was obtained by adding $SOCl_2$ (2 mL) to 4-propoxy benzoic acid (0.50 g, 2.78 mmol) at reflux under magnetic stirring for 1 h, distilling the exceeding $SOCl_2$, and washing with dry toluene for three times to obtain a yellow semisolid, to which was added dry pyridine (3 mL) and 1-(2-hydroxyphenyl)ethanone (0.38 g, 2.78 mmol) at 0 °C. The reaction mixture was maintained at rt for 40 h under magnetic stirring and then was poured in water, basified with NaOH 2N and extracted with EtOAc (3 × 20 mL). The collected organic extracts were washed with HCl 2N and then dried with Na_2SO_4. The solvent was removed under reduced pressure to obtain a white semisolid that was purified by column chormatography with a mixture of EP:Et_2O 90:10 to have 0.60 g of the title compound **2** as a white solid (yield 72%, mp 67.2−68.4 °C). ^{1}H NMR ($CDCl_3$): δ 1.06 (3H, t, J = 7.40 Hz, $OCH_2CH_2CH_3$), 1.80−1.90 (2H, m, $OCH_2CH_2CH_3$), 2.54 (3H, s, $COCH_3$), 4.01 (2H, t, J = 6.55 Hz, $OCH_2CH_2CH_3$), 7.00 (2H, d, J = 9.10 Hz, H-3′ H-5′), 7.24 (1H, dd, J = 6.86 and 1.21 Hz, H-5), 7.35 (1H, dt, J = 6.51 and 1.15 Hz, H-4), 7.57 (1H, dt, J = 6,87 and 1.74 Hz, H-3), 7.85 (1H, dd, J = 7.76 and 2.05 Hz, H-2), 8.16 (2H, d, J = 9.93 Hz, H-2′, H-6′).

(2*Z*)-3-Hydroxy-3-(2-hydroxyphenyl)-1-(4-propoxyphenyl)prop-2-en-1-one (3). To a suspension of K_2CO_3 (2.76 g, 20.0 mmol) in acetone (3 mL), a solution of 2-acetylphenyl 4-propoxybenzoate **2** (0.30 g, 1.0 mmol) dissolved in acetone (3 mL) was added dropwise. The mixture was maintained at reflux under magnetic stirring for 40 h and then poured in ice/water and filtrated to obtain 0.18 g of the title compound **3** as a yellow solid (yield 60%, mp 100.9−102.2 °C). ^{1}H NMR ($CDCl_3$): δ 1.07 (3H, t, J = 7.43 Hz, $OCH_2CH_2CH_3$), 1.80−1.91 (2H, m, $OCH_2CH_2CH_3$), 4.01 (2H, t J = 6.54 Hz, $OCH_2CH_2CH_3$), 6.77 (1H, s, vinyl *H*, H-3′, H-5′), 6.88−7.02 (4H, m, H-3′, H-5′, H-3, H-5), 7.45 (1H, t, J = 7.68 Hz, H-4), 7.77 (1H, d, J = 7.98 Hz, H-6), 7.92 (2H, d, J = 8.28 Hz, H-2′, H-6′), 12.20 (1H, s, O*H* phenolic), 15.80 (1H, s, O*H* enol).

2-(4-Propoxyphenyl)-4*H*-chromen-4-one (4).[49] To a solution of (2*Z*)-3-hydroxy-3-(2-hydroxyphenyl)-1-(4-propoxyphenyl)prop-2-en-1-one **3** (0.10 g, 0.34 mmol) in a mixture of *i*-PrOH (5 mL) and $(i\text{-Pr})_2O$ (5 mL), 0.10 g of Amberlist 15 was added. The mixture was maintained at reflux under magnetic stirring for 7 h, then the Amberlist 15 was filtered, and after cooling from the solution, a white solid was obtained and this was filtrated and purified by column chromatography with a mixture of EP:Et_2O 80:20 to give 0.03 g of the title compound **4**[49] as a white solid (yield 32%, mp 131.1−132.9 °C). ^{1}H NMR ($CDCl_3$): δ 1.06 (3H, t, J = 7.39 Hz, $OCH_2CH_2CH_3$), 1.81−1.91 (2H, m, $OCH_2CH_2CH_3$), 4.02 (2H, t J = 6.56 Hz, $OCH_2CH_2CH_3$), 6.86 (1H, s, H-3), 7.02 (2H, d, J = 6.01 Hz, H −3′, H-5′), 7.44 (1H, dt, J = 7.45 and 1.25 Hz, H-6), 7.58 (1H, dd, J = 8.88 and 1.15 Hz, H-5), 7.71 (1H, dt, J = 7.71 and 1.73 Hz, H-7), 7.91 (1H, d, J = 6.88 Hz, H-2,H-6), 9.30 (1H, dd, J = 7.94 and 1.31 Hz, H-8). Anal. ($C_{18}H_{16}O_3$) C, H, N.

Ethyl (2*Z*)-3-hydroxy-3-(4-propoxyphenyl)acrylate (5).[58] To a suspension of NaH (0.24 g, 10.0 mmol) in $(EtO)_2CO$ (10 mL), 1-(4-propoxyphenyl)ethanone[59] (1.50 g, 8.43 mmol) diluted in $(EtO)_2CO$ (3 mL) was added dropwise. The mixture was maintained under magnetic stirring for 1 h, at rt and then was poured in water and extracted with EtOAc. The organic solvent was removed under reduced pressure to obtain 1.60 g of the title compound **5**[58] as a yellow oil (yield 76%). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J = 7.35 Hz, $OCH_2CH_2CH_3$), 1.30 (3H, t, J = 7.15 Hz, OCH_2CH_3), 1.75−1.90 (2H, m, $OCH_2CH_2CH_3$), 3.90 (2H, s, $COCH_2CO$), 4.00 (2H, t, J = 6.53 Hz, $OCH_2CH_2CH_3$), 4.19 (2H, q, J = 7.15 Hz, OCH_2CH_3), 6.85 (2H, d, J = 6.85 Hz, H-3, H-5), 7.90 (2H, d, J = 6.85 Hz, H-2, H-6).

2-(4-Propoxyphenyl)-4*H*-thiochromen-4-one (6). To 15.0 g of poliphosphoric acid (PPA) kept at 90 °C in an open vessel under manual stirring, thiophenol (0.44 g, 4.0 mmol) and ethyl (2*Z*)-3-hydroxy-3-(4-propoxyphenyl)acrylate **5**[58] (1.0 g, 4.0 mmol) were slowly added. The mixture was maintained to that temperature for 3 h and then was poured in ice/water to obtain a greenish solid, that was filtrated, solubilized in EtOAc, and washed with Na_2CO_3 (solution 20%) to remove the unreacted thiophenol. The organic solution was evaporated to dryness under reduced pressure, and the residue was crystallized by a mixture of Et_2O/cyclohexane to obtain 0.54 g of the title compound **6** as a greenish solid (yield 46%, mp 105.3−106.0 °C). ^{1}H NMR ($CDCl_3$): δ 1.20 (3H, t, J = 7.40 Hz, $OCH_2CH_2CH_3$), 1.80−2.00 (2H, m, $OCH_2CH_2CH_3$), 4.00 (2H, t, J = 6.57 Hz, $OCH_2CH_2CH_3$), 7.00 (2H, d, J = 8.83 Hz H-3′,H-5′), 7.20 (1H, s, H-3), 7.50−7.70 (5H, m, H-6, H-7, H-8, H-2′, H-6′), 8.55 (1H, d, J = 7.00 Hz, H-5). Anal. ($C_{18}H_{16}O_2S$) C, H, N.

1-[2-(Methylamino)phenyl]ethanone (9).[66] To a solution of 1-(2-aminophenyl)ethanone **7** (5.0 g, 37.0 mmol) in dry toluene (35 mL), MeI (10.53 g, 74.0 mmol) was added. The reaction mixture was maintained under magnetic stirring for 48 h at reflux, then it was filtrated and evaporated to dryness to obtain a residue that was purified by flash column chromatography (EP:Et_2O 98:2) to obtain 2.30 g of the title compound **9**[66] as a colorless oil (yield 42%). ^{1}H NMR ($CDCl_3$): δ 2.58 (3H, s, $COCH_3$), 2.90 (3H, d, NCH_3), 6.59 (1H, dd, J = 6.98 and 1.08 Hz, H-3), 7.39 (1H, dt, J = 8.57 and 1.15 Hz, H-5), 7.39 (2H, d, J = 7.80 and 1.01 Hz, H-4), 7.74 (1H, dt, J = 8.06 and 1.52 Hz, H-6), 8.80 (1H, s, N*H*).

1-[4,5-Dimethoxy-2-(methylamino)phenyl]ethanone (10).[67] With the same procedure described reported for compound **9**,[66] the title compound **10**[67] was obtained starting from 1-(2-amino-4,5

-dimethoxyphenyl)ethanone **8** as a yellow solid (yield 48%, mp 126.8−127.7 °C). ^{1}H NMR ($CDCl_3$): δ 2.51 (3H, s, $COCH_3$), 2.91 (3H, d, NCH_3), 3.83 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.11 (1H, s, H-3), 7.16 (1H, s, H-6), 8.90 (1H, s, NH).

1-(2-{[2-(Diethylamino)ethyl]amino}phenyl)ethanone (11).[65] 1-(2-Fluorophenyl)ethanone (2.0 g, 14.5 mmol) and *N*,*N*-diethylethane-1,2-diamine (2.11 g, 18.1 mmol) were solubilized in dry DMF (7.5 mL), and the resulting mixture was kept at 100 °C under magnetic stirring for 33 h. The mixture was poured in water, acidified with HCl 2N until pH = 3, and washed with Et_2O (2 × 50 mL). The water layer was then basified with NaOH 2.5N until pH = 12 and extracted with Et_2O (2 × 50 mL) to obtain, after the evaporation of the organic solvent under reduced pressure, 2.40 g of the title compound **11**[65] as an orange oil (yield 70%). ^{1}H NMR ($CDCl_3$): δ 1.08 (6H, t, J = 14.03 Hz, NCH_2CH_3), 2.52 (3H, s, CH_3), 2.65 (4H, q, J = 13.6 Hz, NCH_2CH_3), 3.34 (2H, t, J = 9.71 Hz, NCH_2CH_2), 4.01−4.12 (2H, m, NCH_2CH_2), 6.54 (1H, t, J = 15.06 Hz, NH), 6.70 (1H, d, J = 8.64 Hz, H-2), 7.31 (2H, t, J = 15.49 Hz, H-3, H-4), 7.69 (1H, d, J = 8.06 Hz, H-5).

1-{2-[(2-Piperidin-1-ylethyl)amino]phenyl}ethanone (12). With the same procedure reported for compound **11**[65] using (2-piperidin-1-ylethyl)amine instead of *N*,*N*-diethylethane-1,2-diamine, the title compound **12** was obtained as a brown oil (yield 46%). ^{1}H NMR ($CDCl_3$): δ 1.39−1.45 (2H, m, $NCH_2CH_2CH_2$), 1.52−1.59 (4H, m, $NCH_2CH_2CH_2$), 1.78 (4H, t, J = 9.67 Hz, $NCH_2CH_2CH_2$), 2.53 (3H, s, CH_3), 2.57 (2H, t, J = 9.93 Hz, NCH_2CH_2), 3.22−3.32 (2H, m, NCH_2CH_2), 6.52 (1H, t, J = 16.21 Hz, NH), 6.66 (1H, d, J = 8.36 Hz, H-2), 7.30 (2H, t, J = 17.07 Hz, H-3, H-4), 7.69 (1H, d, J = 9.68 Hz, H-5).

Ethyl 4-(2-Piperidin-1-ylethoxy)benzoate (13j).[68] A mixture of ethyl 4-hydroxybenzoate (2.00 g, 12.05 mmol) and K_2CO_3 (5.82 g, 42.18 mmol) in dry DMF (15 mL) was added dropwise of a solution of 1-(2-chloroethyl)piperidine hydrochloride (3.75 g, 20.49 mmol) in dry DMF (10 mL) and maintained at 100 °C under magnetic stirring for 17 h. The reaction mixture was poured in water, and the solid obtained was filtered, solubilized with EtOAc, and anhydrified with Na_2SO_4. From the evaporation of the organic solvent under reduced pressure were obtained 2.30 g of the title compound **13j**[68] as brownish oil (yield 68%). ^{1}H NMR ($CDCl_3$): δ 1.32 (3H, t, J = 6.97 Hz, CH_2CH_3), 1.36−1.46 (2H, m, $NCH_2CH_2CH_2$), 1.47−1.69 (4H, m, NCH_2CH_2-CH_2), 2.58−2.75 (4H, m, $NCH_2CH_2CH_2$), 2.76−2.98 (2H, m, NCH_2-CH_2O), 4.24−4.34 (4H, m, NCH_2CH_2O, OCH_2CH_3), 6.86 (2H, d, J = 8.63 Hz, H-2, H-6), 7.94 (2H, d, J = 8.59 Hz, H-3, H-5).

4-(2-Piperidin-1-ylethoxy)benzoic Acid (14j).[68] A solution of ethyl 4-(2-piperidin-1-ylethoxy)benzoate **13j**[68] (0.77 g, 2.78 mmol) in MeOH (12 mL) was added of 5% NaOH (4 mL) and maintained for 15 h under magnetic stirring at rt. The mixture was poured in water and acidified with HCl 6N to obtain a white precipitate that was filtered to give 0.58 g of the title compound **14j**[68] as a white solid (yield 84%, mp 275.5−277.1 °C). ^{1}H NMR ($CDCl_3$): δ 1.30−1.90 (6H, m, NCH_2-CH_2CH_2 and $NCH_2CH_2CH_2$), 3.02−3.05 (2H, m, NCH_2CH_2O), 3.50 (4H, t, J = 4.63 Hz, NCH_2CH_2O), 7.11 (2H, d, J = 8.89 Hz, H-2, H-6), 7.95 (2H, d, J = 8.85 Hz, H-3, H-5), 10.7 (1H, s, OH).

***N*-(2-Acetylphenyl)-4-propoxybenzamide (15f).** A solution of 1-(2-aminophenyl)ethanone **7** (1.52 g, 12.59 mmol) and Et_3N (6.37 g, 8.76 mL, 62.95 mmol) in 50 mL of dry THF was added dropwise to 4-propoxybenzoyl chloride **1f**[57] (2.0 g, 9.60 mmol), prepared as reported above, and maintained at 70 °C for 3 h. The mixture was poured in water and extracted with EtOAc (3 × 50 mL). From the evaporation of the organic solvent under reduced pressure, a solid was obtained, which after crystallization with a mixture of Et_2O/EtOH, gave 2.70 g of the title compound **15f** as a white solid (yield 72%, mp 105.7−106.2 °C). ^{1}H NMR ($CDCl_3$): δ 1.10 (3 H, t, J = 7.41 Hz, $OCH_2CH_2CH_3$), 1.75−2.00 (2 H, m, $OCH_2CH_2CH_3$), 2.75 (3 H, s, $COCH_3$), 4.00 (2 H, t, J = 6.56 Hz, $OCH_2CH_2CH_3$), 7.00 (2 H, d, J = 7.90 Hz, H-3′, H-5′), 7.20 (1 H, t, J = 7.60 Hz, H-4), 7.60 (1 H, t, J = 7.60 Hz, H-5), 7.90 (1 H, d, J = 7.60 Hz, H-3), 8.10 (2 H, d, J = 7.90 Hz, H-2′, H-6′), 9.00 (1 H, d, J = 7.60 Hz, H-6), 11.50 (1 H, bs, NH).

***N*-(2-Acetylphenyl)-4-(2-piperidin-1-ylethoxy)benzamide (15j).** With the same procedure reported for compound **15f** using 4-(2-piperidin-1-ylethoxy)benzoyl chloride **1j**, obtained from the respective acid as previously reported for 4-propoxybenzoyl chloride **1f**,[57] the title compound **15j** was obtained as a yellow oil (yield 84%). ^{1}H NMR ($CDCl_3$): δ 1.40−1.55 (2H, m, piperidinic CH_2), 1.72−2.07 (4H, m, piperidinic CH_2), 2.48 (3H, s, CH_3), 3.05−3.25 (4H, m, piperidinic CH_2), 3.30−3.45 (2H, m, NCH_2), 4.32−4.45 (2H, m, OCH_2CH_2N), 6.60 (2H, d, J = 8.40 Hz, H-3′, H-5′), 7.12 (2H, d, J = 8.40 Hz, H-2′, H-6′), 7.18−7.25 (2H, m, Ar−H), 7.40−7.55 (2H, m, Ar−H).

4-{[(2-Acetylphenyl)(methyl)amino]carbonyl}phenyl Acetate (16b). With the same procedure described for compound **15f** using 4-acetyl benzoyl chloride **1b**, obtained from the respective acid as previously reported for 4-propoxybenzoyl chloride **1f**[57] and 1-[2-(methylamino)phenyl]ethanone **9**,[66] the title compound **16b** was obtained as a brownish solid (yield 90%, mp 137.2−137.9 °C). ^{1}H NMR ($CDCl_3$): δ 2.20 (6H, s, $OCOCH_3$, $COCH_3$), 3.40 (3H, s, $N-CH_3$), 6.85 (2H, d, J = 8.50 Hz, H-3′, H-5′), 7.15−7.30 (4H, m, H-2′, H-6′, H-5, H-4), 7.45−7.55 (2H, m, H-3, H-6).

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-*N*-methylbenzamide (17a).** With the same procedure described for compound **15f** using benzoyl chloride **1a** and 1-[4,5-dimethoxy-2-(methylamino)phenyl]-ethanone **10**,[67] the title compound **17a** was obtained as a white solid (yield 60%, mp 134.2−134.5 °C). ^{1}H NMR ($CDCl_3$): δ 2.30 (3H, s, $COCH_3$), 3.50 (3H, s, NCH_3), 3.85 (6H, s, OCH_3), 6.65 (1H, s, H-3), 7.00 (1H, s, H-6), 7.10−7.25 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′).

4-{[(2-Acetyl-4,5-dimethoxyphenyl)(methyl)amino]carbonyl}phenyl Acetate (17b). With the same procedure described for compound **15f** using 4-acety benzoyl chloride **1b**, obtained from the respective acid as previously reported for 4-propoxybenzoyl chloride **1f**[57] and 1-[4,5-dimethoxy-2-(methylamino)phenyl]ethanone **10**,[67] the title compound **17b** was obtained, after crystallization with EtOH, as a white solid (yield 59%, mp 176.2−177.1 °C). ^{1}H NMR ($CDCl_3$): δ 2.30 (3H, s, $OCOCH_3$), 2.40 (3H, s, $COCH_3$), 3.50 (3H, s, NCH_3), 4.00 (6H, s, OCH_3), 6.75 (1H, s, H-3), 7.00 (2H, d, J = 8.70 Hz, H-3′, H-5′), 7.10 (1H, s, H-6), 7.35 (2H, d, J = 8.70 Hz, H-2′, H-6′).

***N*-(2-Acetylphenyl)-*N*-[2-(diethylamino)ethyl]-4-propoxybenzamide (18f).** Under nitrogen atmosphere, a solution of 1-(2-{[2-(diethylamino)ethyl]amino}phenyl)ethanone **11**[65] (0.65 g, 2.77 mmol) and Et_3N (1.40 g, 1.90 mL, 13.85 mmol) in 10 mL of THF dry was added dropwise of 4-propoxybenzoyl chloride **1f**[57] (0.55 g, 2.77 mmol), prepared as reported above, and was maintained for 3 h, at rt, under magnetic stirring. The reaction mixture was poured in water, basified in NaOH 2.5N, and extracted with EtOAc (3 × 50 mL). After anhydrification with Na_2SO_4, from the evaporation of the organic extracts, a residue was obtained, and this was purified by flash chromatography with $CHCl_3$:MeOH (97:3) to give 0.39 g of the title compound **18f** as a yellowish oil (yield 40%). ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 7.32 Hz, $OCH_2CH_2CH_3$), 1.30−1.50 (6H, m, NCH_2CH_3), 1.54−1.68 (2H, m, $OCH_2CH_2CH_3$), 1.97 (3H, s, CH_3), 3.03−3.70 (6H, m, NCH_2), 3.75 (2H, t, J = 7.27 Hz, $OCH_2CH_2CH_3$), 4.06−4.45 (2H, m, NCH_2CH_2N), 6.78 (2H, d, J = 8.81 Hz, H-3′, H-5′), 7.09 (2H, d, J = 8.84 Hz, H-2′, H-6′), 7.30−7.40 (1H, m, H-3), 7.35−7.65 (3H, m, Ar−H).

***N*-(2-Acetylphenyl)-*N*-(2-piperidin-1-ylethyl)-4-propoxybenzamide (19f).** With the same procedure described for compound **18f** using 4-propoxybenzoyl chloride **1f**,[57] obtained from the respective acid as reported above, and 1-{2-[(2-piperidin-1-ylethyl)-amino]phenyl}ethanone **12**, the title compound **19f** was obtained, after purification by flash chromatography column CH_2Cl_2:MeOH (85:15), as a brown oil (yield 28%). ^{1}H NMR ($CDCl_3$): δ 0.93 (3H, t, J = 7.32 Hz, $OCH_2CH_2CH_3$), 1.32−1.47 (6H, m, piperidinic CH_2), 1.51−1.63

(2H, m, $OCH_2CH_2CH_3$), 1.94 (3H, s, CH_3), 3.05−3.68 (6H, m, piperidinic CH_2 and NCH_2), 3.75 (2H, t, J = 7.27 Hz, $OCH_2CH_2CH_3$), 4.13−4.38 (2H, m, NCH_2CH_2N), 6.91 (2H, d, J = 8.81 Hz, H-3′, H-5′), 7.14 (2H, d, J = 8.84 Hz, H-2′, H-6′), 7.35−7.43 (1H, m, H-3), 7.48−7.69 (3H, m, Ar−H).

2-(4-Propoxyphenyl)quinolin-4-ol (20f). A solution of *N*-(2-acetylphenyl)-4-propoxybenzamide **15f** (2.70 g, 9.09 mmol) in *t*-BuOH (30 mL) was added of *t*-BuOK (5.10 g, 45.41 mmol) and warmed at 60 °C for 3 h under magnetic stirring. The mixture was poured in water, acidified with HCl 2N to obtain a solid that was filtrated and purified by a flash chromatographic column $CHCl_3$:MeOH (80:20) to give 1.96 g of the title compound **20f** as a white solid (yield 77%, mp 271.0−273.0 °C). ^{1}H NMR (DMSO-d_6): δ 1.00 (3H, t, J = 7.38 $OCH_2CH_2CH_3$), 1.65−1.75 (2H, m, $OCH_2CH_2CH_3$), 4.00 (2H, t, J = 6.60 Hz, $OCH_2CH_2CH_3$), 6.20 (1H, s, H-3), 7.10 (2H, d, J = 8.76 Hz, H-3′, H-5′), 7.30 (1H, t, J = 7.20 Hz, H-6), 7.65 (1H, t, J = 7.20 Hz, H-7), 7.75 (3H, m, H-8, H-2′, H-6′), 8.10 (1H, d, J = 7.82 Hz, H-5), 11.50 (1H, bs, OH). Anal. ($C_{18}H_{17}NO_2$) C, H, N.

2-[4-(2-Piperidin-1-ylethoxy)phenyl]quinolin-4-ol (20j). With the same procedure described for compound **20f** starting from *N*-(2-acetylphenyl)-4-(2-piperidin-1-ylethoxy)benzamide **15j**, stirring for 72 h at 100 °C, the title compound **20j** was obtained as a brownish solid (yield 81%, mp 215.0−217.2 °C). ^{1}H NMR ($CDCl_3$): δ 1.45−1.65 (6H, m, piperidinic CH_2), 2.55−2.70 (4H, m, piperidinic CH_2), 2.87 (2H, t, J = 5.99 Hz, NCH_2), 4.21 (2H, t, J = 5.46 Hz, OCH_2CH_2N), 6.53 (1H, s, H-3), 7.04 (2H, d, J = 8.92 Hz, H-3′, H-5′), 7.38−7.42 (2H, m, Ar−H), 7.45−7.55 (2H, m, Ar−H), 7.58−7.70 (4H, m, H-2′, H-6′ and Ar−H).

4-Methoxy-2-(4-propoxyphenyl)quinoline (21f) and 1-Methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one (22f). To a mixture of 2-(4-propoxyphenyl)quinolin-4-ol **20f** (0.20 g, 0.72 mmol) and K_2CO_3 (0.30 g, 2.16 mmol) in dry DMF (5 mL) was added MeI (0.21 g, 0.09 mL, 1.44 mmol) and maintained at 60 °C for 2 h under magnetic stirring. The mixture was poured in ice/water and extracted with EtOAc (3 × 50 mL). From the evaporation of the organic extracts, a crude product was obtained, and this was purified by flash chromatography with $CHCl_3$:MeOH (90:10) to give 0.03 g of the compound **21f** as a white solid (yield 14%, mp 115.8−117.1 °C) and 0.04 g of the compound **22f** as a white solid (yield 19%, mp 147.7−148.5 °C).

21f: ^{1}H NMR ($CDCl_3$): δ 1.11 (3H, t, J = 7.34 Hz, $OCH_2CH_2CH_3$), 1.70−1.95 (2H, m, $OCH_2CH_2CH_3$), 4.05 (2H, t, J = 6.57 Hz, $OCH_2CH_2CH_3$), 4.17 (3H, s, OCH_3), 7.08 (2H, d, J = 8.69 Hz, H-3′, H-5′), 7.20 (1H, s, H-3), 7.51 (1H, t, J = 7.15 Hz, H-6), 7.75 (1H, t, J = 6.86 Hz, H-7), 8.05−8.25 (4H, m, H-2′, H-6′, H-5, H-8). Anal. ($C_{19}H_{19}NO_2$) C, H, N.

22f: ^{1}H NMR ($CDCl_3$): δ 1.12 (3H, t, J = 7.37 Hz, $OCH_2CH_2CH_3$), 1.75−2.00 (2H, m, $OCH_2CH_2CH_3$), 3.86 (3H, s, NCH_3), 4.04 (2H, t, J = 6.62 Hz, $OCH_2CH_2CH_3$), 6.30 (1H, s, H-3), 7.05 (2H, d, J = 8.53 Hz, H-3′, H-5′), 7.41 (2H, d, J = 8.53 Hz, H-2′, H-6′), 7.57 (1H, t, J = 7.27 Hz, H-6), 7.74 (1H, d, J = 9.09 Hz, H-5), 7.87 (1H, t, J = 7.27 Hz, H-7), 8.52 (1H, d, J = 8.00 Hz, H-8). Anal. ($C_{19}H_{19}NO_2$) C, H, N.

2-(4-Hydroxyphenyl)-1-methylquinolin-4(1*H*)-one (22c).[62] To a suspension of NaH (0.93 g, 38.6 mmol) in dry DMF (25 mL) was added dropwise a solution of 4-{[(2-acetylphenyl)(methyl)amino]-carbonyl}phenyl acetate **16b** (2.40 g, 7.70 mmol) in dry DMF (30 mL) and maintained for 1 h under magnetic stirring at rt. After decomposition of the excess of NaH with EtOAc, the mixture was poured in water and extracted with EtOAc (3 × 50 mL). From the evaporation under reduced pressure of the organic solvent and after purification with flash column chromatography $CHCl_3$:MeOH (99:1 →80:20), the title compound **22c**[62] was obtained as a white solid (yield 52%, mp 335.3−336.5 °C). ^{1}H NMR (DMSO-d_6): δ 3.50 (3H, s, N−CH_3), 5.90 (1 H, s, H-3), 6.90 (2H, d, J = 8.53 Hz, H-3′, H-5′), 7.30−7.50 (3H, m, H-2′, H-6′, H-6), 7.70−7.80 (2H, m, H-7, H-5), 8.20 (2H, d, J = 7.86 Hz, H-8), 9.50−10.50 (1H, bs, OH). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

1-[2-(Diethylamino)ethyl]-2-(4-propoxyphenyl)quinolin-4(1*H*)-one (24f). With the same procedure described for compound **22c**[62] starting from *N*-(2-acetylphenyl)-*N*-[2-(diethylamino)ethyl]-4-propoxybenzamide **18f**, the title compound **24f** was obtained, after purification with column chromatography CH_2Cl_2:MeOH (99:1), as a white solid (yield 85%, mp 212.2−213.2 °C). ^{1}H NMR ($CDCl_3$): δ 0.88 (6H, t, J = 7.14 Hz, NCH_2CH_3), 1.11 (3H, t, J = 7.45 Hz, $OCH_2CH_2CH_3$), 1.78−2.00 (2H, m, $OCH_2CH_2CH_3$), 2.39 (4H, q, J = 7.06 Hz, NCH_2CH_3), 2.66 (2H, t, J = 7.27 Hz, NCH_2CH_2N), 4.06 (2H, t, J = 6.58 Hz, $OCH_2CH_2CH_3$), 4.27 (2H, t, J = 7.28 Hz, NCH_2CH_2N), 6.28 (1H, s, H-3), 7.04 (2H, d, J = 11.45 Hz, H-3′, H-5′), 7.39 (2H, d, J = 8.75 Hz, H-2′, H-6′), 7.40−7.50 (1H, m, H-6), 7.72 (1H, d, J = 10.23 Hz, H-5), 7.70−7.77 (1H, m, H-7), 8.53 (1H, dd, J = 10.26 and 3.29 Hz, H-8). Anal. ($C_{24}H_{30}N_2O_2$) C, H, N.

1-(2-Piperidin-1-ylethyl)-2-(4-propoxyphenyl)quinolin-4(1*H*)-one (25f). With the same procedure described for compound **22c** starting from *N*-(2-acetylphenyl)-*N*-(2-piperidin-1-ylethyl)-4-propoxybenzamide **19f**, the title compound **25f** was obtained, after purification by flash column chromatography $CHCl_3$:MeOH (99:1), as a yellow solid (yield 55%, mp 83.7−84.5 °C). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J = 7.35 Hz, $OCH_2CH_2CH_3$), 1.30−1.60 (6H, m, piperidinic CH_2), 1.87−2.00 (2H, m, $OCH_2CH_2CH_3$), 2.10−2.29 (4H, m, piperidinic CH_2), 2.55 (2H, t, J = 7,40 Hz, NCH_2CH_2N), 4.06 (2H, t, J = 6.58 Hz, $OCH_2CH_2CH_3$), 4.24 (2H, t, J = 6.51 Hz, NCH_2CH_2N), 6.27 (1H, s, H-3),7.03 (2H, d, J = 8.13 Hz, H-3′, H-5′), 7.32 (2H, d, J = 5.67 Hz, H-2′, H-6′), 7.37−7.49 (1H, m, H-6), 7.68 (1H, d, J = 8.74 Hz, H-5), 7.70−7.79 (1H, m, H-7), 8.54 (1-H, dd, J = 8.77 and 1.29 Hz, H-8). Anal. ($C_{25}H_{30}N_2O_2$) C, H, N.

6,7-Dimethoxy-1-methyl-2-phenylquinolin-4(1*H*)-one (23a).[64] To a solution of *N*-(2-acetyl-4,5-dimethoxyphenyl)-*N*-methylbenzamide **17a** (0.30 g, 1.10 mmol) in *t*-BuOH (15 mL), *t*-BuOK was added (0.58 g, 4.80 mmol) and warmed to 60 °C for 3 h. After cooling, the reaction mixture was filtered, and the resulting filtrate was evaporated under reduced pressure to obtain a crude product that was crystallized by EtOH to give 0.11 g of the title compound **23a**[64] as a white solid (yield 38%, mp 263.3−265.4 °C). ^{1}H NMR ($CDCl_3$): δ 3.65 (3H, s, NCH_3), 4.00 (6H, s, OCH_3), 6.25 (1H, s, H-3), 6.85 (1H, s, H-8), 7.35−7.55 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 7.85 (1 H, s, H-5). Anal. ($C_{18}H_{17}NO_3$) C, H, N.

2-(4-Hydroxyphenyl)-6,7-dimethoxy-1-methylquinolin-4(1*H*)-one (23c). With the same procedure described for compound **23a**[64] starting from 4-{[(2-acetyl-4,5-dimethoxyphenyl)(methyl)-amino]carbonyl}phenyl acetate **17b**, the title compound **23c** was obtained, after crystallization with EtOH/DMF, as a white solid (yield 35%, mp 337.3−337.9 °C). ^{1}H NMR (DMSO-d_6): δ 3.60 (3H, s, NCH_3), 3.85 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 5.85 (1H, s, H-3), 6.85 (2H, d, J = 8.55 Hz, H-3′, H-5′), 7.10 (1 H, s, H-8), 7.30 (2H, d, J = 8.55 Hz, H-2′, H-6′), 7.55 (1H, s, H-5), 10.00 (1H, bs, OH). Anal. ($C_{18}H_{17}NO_4$) C, H, N.

2-(4-Methoxyphenyl)-1-methylquinolin-4(1*H*)-one (22d).[63] To a suspension of 2-(4-hydroxyphenyl)-1-methylquinolin-4(1*H*)-one **22c**[62] (0.20 g, 0.80 mmol) and K_2CO_3 (0.22 g, 1.60 mmol) in dry DMF (10 mL) was added dropwise of MeI (0.34 g, 0.15 mL, 2.40 mmol) and warmed to 70 °C for 1.5 h under magnetic stirring. The mixture was poured in water and the solid obtained, was filtrated, dried and purified by column chromatography $CHCl_3$:MeOH (99:1) to give 0.10 g of the title compound **22d**[63] as a yellow solid (yield 47%, mp 135.5−138.0 °C). ^{1}H NMR ($CDCl_3$): δ 3.60 (3H, s, NCH_3), 3.85 (3H, s, OCH_3), 6.25 (1H, s, H-3), 7.00 (2H, d, J = 8.85 Hz, H-3′, H-5′), 7.30−7.45 (3H, m, H-2′, H-6′, H-6), 7.55 (1H, d, J = 8.60 Hz, H-5) 8.00 (1H, dt, J = 8.62 Hz and J = 1.71, H-7), 8.50 (1H, dd, J = 8.00 and 1.70 Hz, H-8). Anal. ($C_{17}H_{15}NO_2$) C, H, N.

2-(4-Ethoxyphenyl)-1-methylquinolin-4(1*H*)-one (22e). With the same procedure described for compound **22d**,[63] using EtI instead of MeI, the title compound **22e** was obtained as a white solid (yield 65%, mp 200.0−201.8 °C). ^{1}H NMR ($CDCl_3$): δ 1.45 (3H, t, J = 7 Hz, OCH_2CH_3), 3.68 (3H, s, NCH_3), 4.10 (2H, q, J = 7 Hz, OCH_2CH_3), 6.45 (1H, s, H-3), 7.00 (2H, d, J = 8.50 Hz, H-3′, H-5′), 7.35 (2H, d, J = 8.50 Hz, H-2′, H-6′), 7.00 (1H, t, J = 7.27 Hz, H-6), 7.58 (1H, d, J = 8.80 Hz, H-5), 7.74 (1H, d, J = 7.80 Hz, H-7), 8.48 (1H, d, J = 7.80 Hz, H-8). Anal. ($C_{18}H_{17}NO_2$) C, H, N.

1-Methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one (22f). With the same procedure described for compound **22d**,[63] using *n*-PrI instead of MeI, the title compound **22f** was obtained as a white solid (yield 38%, mp 147.7−148.5 °C). This compound was also obtained in mixture with compound **21f** following the procedure described above (see the procedure for spectral data).

2-(4-Isopropoxyphenyl)-1-methylquinolin-4(1*H*)-one (22g). With the same procedure described for compound **22d**,[63] using *i*-PrI instead of MeI, the title compound **22g** was obtained as a white solid (yield 55%, mp 173.2−175.1 °C). ^{1}H NMR ($CDCl_3$): δ 1.40 (6H, d, J = 6.00 Hz, $OCH(CH_3)_2$), 3.75 (3H, s, NCH_3), 4.52−4.72 (1H, m, $OCH(CH_3)_2$), 6.58 (1H, s, H-3), 6.98 (2,H, d, J = 8.70 Hz, H-3′, H-5′), 7.33 (2H, d, J = 8.70 Hz, H-2′, H-6′), 7.47 (1H, t, J = 7.10 Hz, H-6), 7.62 (1H, d, J = 8.50 Hz, H-5), 7.75 (1H, dd, J = 6.90 and 1.60 Hz, H-7), 8.48 (1H, dd, J = 8.00 and 1.30 Hz, H-8). Anal. ($C_{19}H_{19}NO_2$) C, H, N.

2-{4-[2-(Dimethylamino)ethoxy]phenyl}-1-methylquinolin-4(1*H*)-one (22h). With the same procedure described for compound **22d**,[63] using (2-chloroethyl)dimethylamine hydrochloride instead of MeI, the title compound **22h** was obtained, after purification with flash column chromatography CH_2Cl_2:MeOH (95:5), as a yellowish solid (yield 16%, mp 122.0−125.0 °C). ^{1}H NMR ($CDCl_3$): δ 2.44 (6H, s, NCH_3), 2.85 (2H, t, J = 5.63 Hz, CH_2N), 3.67 (3H, s, CH_2N), 4.20 (2H, t J = 5.67 Hz, OCH_2), 6.35 (1H, s, H-3), 7.08 (2H, d, J = 8.74 Hz, H-3′, H-5′), 7.44 (2H, d, J = 11.86 Hz, H-2′, H-6′), 7.40−7.53 (1H, m, H-6), 7.59 (1H, d, J = 8.52 Hz, H-5), 7.69−7.81 (1H, m, H-7), 8.54 (1-H, d, J = 8.00 Hz, H-8). Anal. ($C_{20}H_{22}N_2O_2$) C, H, N.

2-{4-[2-(Diethylamino)ethoxy]phenyl}-1-methylquinolin-4(1*H*)-one (22i). With the same procedure described for compound **22d**,[63] using (2-chloroethyl)diethylamine hydrochloride instead of MeI, the title compound **22i** was obtained, after purification with flash chromatography column CH_2Cl_2:MeOH (99:1), as brownish solid (yield 25%, mp 104.0−106.0 °C). ^{1}H NMR ($CDCl_3$): δ 1.15 (6H, t, J = 7.13 Hz, NCH_2CH_3), 2.71 (4H, q, J = 7.13 Hz, NCH_2CH_3), 2.96 (2H, t, J = 6.27 Hz, CH_2N), 3.67 (3H, s NCH_3), 4.16 (2H, t, J = 6.25 Hz, OCH_2), 6.34 (1H, s, H-3), 7.07 (2H, d, J = 9.71 Hz, H-3′, H-5′), 7.39 (2H, d, J = 6.68 Hz, H-2′, H-6′), 7.40−7.50 (1H, m, H-6), 7.59 (1H, d, J = 8.33 Hz, H-5), 7.72−7.79 (1H, m, H-7), 8.55 (1H, dd, J = 798 and 1.55 Hz, H-8). Anal. ($C_{22}H_{26}N_2O_2$) C, H, N.

1-Methyl-2-[4-(2-piperidin-1-ylethoxy)phenyl]quinolin-4(1*H*)-one (22j). With the same procedure described for compound **22d**,[63] using 1-(2-chloroethyl)piperidine hydrochloride instead of MeI, the title compound **22j** was obtained, after purification with flash column chromatography CH_2Cl_2:MeOH (90:10), as a brownish solid (yield 35%, mp 144.0−146.0 °C). ^{1}H NMR ($CDCl_3$): δ 1.43−1.51 (2H, m, $NCH_2CH_2CH_2$), 1.52−1.75 (4H, m, $NCH_2CH_2CH_2$), 2.61 (4H, t, J = 5.03 Hz, $NCH_2CH_2CH_2$), 2.86 (2H, t, J = 6.00 Hz, CH_2N), 3.68 (3H, s, NCH_3), 4.22 (2H, t, J = 6.09 Hz, OCH_2), 6.35 (1H, s, H-3), 7.07 (2H, d, J = 8.74 Hz, H-3′, H-5′), 7.38 (2H, d, J = 6.74 Hz, H-2′, H-6′), 7.42−7.53 (1H, m, H-6), 7.58 (1H, d, J = 10.66 Hz, H-5), 7.70−7.82 (1H, m, H-7), 8.55 (1-H, dd, J = 7.98 and 1.62 Hz, H-8). Anal. ($C_{23}H_{26}N_2O_2$) C, H, N.

1-Methyl-2-[4-(2-morpholin-4-ylethoxy)phenyl]quinolin-4(1*H*)-one (22k). With the same procedure described for compound **22d**,[63] using 4-(2-chloroethyl)morpholine instead of MeI, the title compound **22k** was obtained, after purification with flash column chromatography CH_2Cl_2:MeOH (90:10), as a white solid (yield 14%, mp 132.2−132.9 °C). ^{1}H NMR ($CDCl_3$): δ 2.56 (4H, t, J = 4.84 Hz, NCH_2), 2.81 (2H, t, J = 5.83 Hz, CH_2N), 3.59 (3H, s, NCH_3), 3.71 (4H, t, J = 4.75 Hz, CH_2O), 4.14 (2H, t, J = 5.55 Hz, OCH_2), 6.25 (1H, s, H-3), 6.97 (2H, d, J = 8.84 Hz, H-3′, H-5′), 7.30 (2H, d, J = 8.87 Hz, H-2′, H-6′), 7.30−7.48 (1H, m, H-6), 7.58 (1H, d, J = 11.68 Hz, H-5), 7.60−7.70 (1H, m, H-7), 8.46 (1-H, dd, J = 7.91 and 1.79 Hz, H-8). Anal. ($C_{22}H_{24}N_2O_3$) C, H, N.

6,7-Dimethoxy-1-methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one (23f). With the same procedure described for compound **22d**,[63] starting from 2-(4-hydroxyphenyl)-6,7-dimethoxy-1-methylquinolin-4(1*H*)-one **23c**, and using *n*-PrI instead of MeI, the title compound **23f** was obtained, after purification with a flash chromatographic column CH_2Cl_2:MeOH (99:1), as a white solid (yield 24%, mp 258.2−259.5 °C). ^{1}H NMR ($CDCl_3$): δ 1.05 (3 H, t, J = 7.43, $OCH_2CH_2CH_3$), 1.54−1.57 (2 H, m, $OCH_2CH_2CH_3$), 3.55−3.65 (2 H, m, $OCH_2CH_2CH_3$), 3.90−4.05 (9 H, m, two OCH_3, NCH_3), 6.25 (1 H, s, H-3), 6.85 (1 H, s, H-8), 6.95 (2 H, d, J = 8.67 Hz, H-3′, H-5′), 7.30 (2 H, d, J = 8.67 Hz, H-2′, H-6′), 7.85 (1 H, s, H-5). Anal. ($C_{21}H_{23}NO_4$) C, H, N.

6,7-Dihydroxy-1-methyl-2-phenylquinolin-4(1*H*)-one (26a). To a solution of 6,7-dimethoxy-1-methyl-2-phenylquinolin-4(1*H*)-one **23a**[64] (0.20 g, 0.60 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise of a solution 1 M of BBr_3 (8.5 mL, 8.50 mmol) in CH_2Cl_2 and maintained for 3 h at room temperature under magnetic stirring. The mixture was poured in water and the obtained precipitate, after crystallization in a mixture of MeOH/CH_2Cl_2, gave 0.12 g of the title compound **26a** as a white solid (yield 82%, mp 350.0−352.2 °C). ^{1}H NMR (MeOD): δ 3.70 (3H, s, NCH_3), 6.20 (1H, s, H-3), 7.20 (1H, s, H-8), 7.40−7.65 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 7.80 (1 H, s, H-5). Anal. ($C_{16}H_{13}NO_3$) C, H, N.

6,7-Dihydroxy-2-(4-hydroxyphenyl)-1-methylquinolin-4(1*H*)-one (26c). With the same procedure described for compound **26a**, starting from 2-(4-hydroxyphenyl)-6,7-dimethoxy-1-methylquinolin-4(1*H*)-one **23c**, the title compound **26c** was obtained as a white solid (yield 99%, mp 368.5−369.6 °C). ^{1}H NMR (DMSO-d_6): δ 3.50 (3H, s, NCH_3), 5.75 (1H, s, H-3), 6.80 (2H, d, J = 8.52 Hz, H-3′, H-5′), 7.00 (1H, s, H-8), 7.25 (2H, d, J = 8.52 Hz, H-2′, H-6′), 7.50 (1H, s, H-5), 9.75−10.00 (3H, bs, 3 OH). Anal. ($C_{16}H_{13}NO_4$) C, H, N.

7-Hydroxy-6-methoxy-1-methyl-2-(4-propoxyphenyl)-quinolin-4(1*H*)-one (27f). To a solution of 6,7-dimethoxy-1-methyl-2-(4-propoxyphenyl)quinolin-4(1*H*)-one **23f** (0.10 g, 0.30 mmol) in dry DMF (10 mL) was added of LiCl (0.06 g, 1.40 mmol) and maintained for 4 days at reflux. The mixture was poured in water, acidified with HCl 2N at pH = 6, and the obtained precipitate, after filtration and purification by a chromatographic column $CHCl_3$:MeOH (95:5), gave 0.012 g of the title compound **27f** as a white solid (yield 13%, mp 271.3−273.0 °C). ^{1}H NMR (DMSO-d_6): δ 1.02 (3H, t, J = 7.50 Hz, CH_3), 1.70−1.85 (2H, m, CH_2CH_3), 3.50 (3H, s, NCH_3), 3.88 (3H, s, OCH_3), 4.00 (2H, t, J = 6.50 Hz, OCH_2), 5.82 (1H, s, H-3), 7.00−7.15 (3H, m, H-3′, H-5′, H-8),7.40 (2H, d, J = 8.85 Hz, H-2′, H-6′), 7.55 (1H, s, H-5), 10.20 (1H, bs, OH). Anal. ($C_{20}H_{21}NO_4$) C, H, N.

***N*,*N*-Diethyl-2-{[2-(4-propoxyphenyl)quinolin-4-yl]oxy}-ethanamine Hydrochloride (28f).** A suspension of 2-(4-propoxyphenyl)quinolin-4-ol **20f** (0.15 g, 0.54 mmol) and K_2CO_3 (0.22 g, 1.62 mmol) in dry DMF (10 mL) was maintained for 30 min under magnetic stirring at room temperature. Then was added dropwise a solution of (2-chloroethyl)diethylamine hydrochloride (0.15 g, 1.08 mmol) in dry DMF (5 mL) and warmed at 110 °C for 5 h. The mixture was poured in water and extracted with Et_2O (5 × 30 mL). The organic phase was dried with Na_2SO_4 and bubbled with HCl g to obtain 0.12 g of a white solid that resulted in the title compound **28f** as hydrochloride (yield 54%, mp 243.8−245.0 °C). ^{1}H NMR ($CDCl_3$): δ 1.00−1.25 (9H, m, $OCH_2CH_2CH_3$ and both NCH_2CH_3), 1.75−2.00 (2H,

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m, $OCH_2CH_2CH_3$), 2.70 (4H, q, J = 7.16 Hz, NCH_2CH_3), 3.05 (2H, t, J = 6.10 Hz, OCH_2CH_2N), 4.00 (2H, t, J = 6.65 Hz, $OCH_2CH_2CH_3$), 4.35 (2H, t, J = 6.10 Hz, OCH_2CH_2N), 7.00 (2H, d, J = 6.80 Hz, H-3′, H-5′), 7.15 (1H, s, H-3), 7.45 (1H, t, J = 8.20 Hz, H-6), 7.70 (1H, t, J = 8.22 Hz, H-7), 8.00−8.10 (3H, m, H-2′, H-6′, H-8), 8.25 (1H, d, J = 7.34 Hz, H-5). Anal. ($C_{24}H_{31}ClN_2O_2$) C, H, N.

***N*,*N*-Diethyl-2-({2-[4-(2-piperidin-1-ylethoxy)phenyl]-quinolin-4-yl}oxy)ethanamine (28j).** With the same procedure described for compound **28f**, starting from 2-[4-(2-piperidin-1-ylethoxy)-phenyl]quinolin-4-ol **20j**, without bubbling HCl g, the title compound **28j** was obtained as the free base, after purification by a flash chromatographic column CH_2Cl_2:MeOH (95:5), as a brown oil (yield 47%). ^{1}H NMR ($CDCl_3$): δ 1.05 (6H, t, J = 7.10 Hz, NCH_2CH_3), 1.30−1.45 (2H, m, piperidinic CH_2), 1.50−1.70 (4H, m, piperidinic CH_2), 2.40−2.55 (4H, m, piperidinic CH_2), 2.67 (4H, q, J = 7.16 Hz, NCH_2CH_3), 2.77 (2H, t, J = 5.98 Hz, CH_2N), 3.04 (2H, t, J = 6.06 Hz, CH_2N), 4.15 (2H, t, J = 3.32 Hz, OCH_2), 4.32 (2H, t, J = 2.43 Hz, OCH_2), 6.99 (2H, d, J = 8.70 Hz, H-3′, H-5′), 7.11 (1H, s, H-3), 7.36−7.43 (1H, m, H-7), 7.58−7.67 (1H, m, H-6), 7.99−8.14 (4H, m, Ar−H). Anal. ($C_{28}H_{37}N_3O_2$) C, H, N.

4-(2-Piperidin-1-ylethoxy)-2-(4-propoxyphenyl)quinoline (29f). A mixture of 2-(4-propoxyphenyl)quinolin-4-ol **20f** (0.15 g, 0.54 mmol), *t*-BuOK (0.18 g, 1.60 mmol), and 1-(2-chloroethyl)-piperidine hydrochloride (0.20 g, 1.10 mmol) in dry DMF (5 mL) was maintained for 8 h at 100 °C under magnetic stirring. After cooling, the mixture was poured in water and the obtained precipitate was filtered, dried, and crystallized from EtOH to obtain 0.10 g of the title compound **29f** as a white solid (yield 51%, mp 95.2−97.6 °C). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J = 7.00 Hz, $OCH_2CH_2CH_3$), 1.50−1.65 (2H, m, piperidinic CH_2), 1.75−2.00 (4H, m, $OCH_2CH_2CH_3$ and piperidinic CH_2), 2.70−2.80 (4H, m, piperidinic CH_2), 3.20 (2H, t, J = 5.30 Hz, OCH_2CH_2N), 4.00 (2H, t, J = 6.70 Hz, $OCH_2CH_2CH_3$), 4.55 (2H, t, J = 5.60 Hz, OCH_2CH_2N), 7.05 (2H, d, J = 8.70 Hz, H-3′,H-5′), 7.20 (1H, s, H-3), 7.45 (1H, t, J = 8.15 Hz, H-6), 7.70 (1H, t, J = 8.15 Hz, H-7), 8.00−8.15 (4H, m, H-2′, H-6′, H-5,H-8). Anal. ($C_{25}H_{30}N_2O_2$) C, H, N.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^{1}H−^{1}H 2D-NOESY NMR spectral data of compound **27f** and elemental analysis data for target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

MDR, multidrug resistance; MFS, major facilitator superfamily; EPI, efflux pump inhibitor; EtBr, ethidium bromide

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