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From 6-Aminoquinolone Antibacterials to 6-Amino-7-thiopyranopyridinylquinolone Ethyl Esters as Inhibitors of *Staphylococcus aureus* Multidrug Efflux Pumps

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The thiopyranopyridine moiety was synthesized as a new heterocyclic base to be inserted at the C-7 position of selected quinolone nuclei followed by a determination of antibacterial activity against strains of *Staphylococcus aureus*. Selected thiopyranopyridinylquinolones showed significant antimicrobial activity, including strains having mutations in *gyrA* and *grlA* as well as other strains overexpressing the NorA multidrug (MDR) efflux pump. Most derivatives did not appear to be NorA substrates. The effect of the thiopyranopyridinyl substituent on making these quinolones poor substrates for NorA was investigated further. Several quinolone ester intermediates, devoid of any intrinsic antibacterial activity, were tested for their abilities to inhibit the activities of NorA (MFS family) and MepA (MATE family) *S. aureus* MDR efflux pumps. Selected quinolone esters were capable of inhibiting both MDR pumps more efficiently than the reference compound reserpine. Moreover, they also were able to restore, and even enhance, the activity of ciprofloxacin toward some genetically modified resistant *S. aureus* strains.

Introduction

The emergence and spread of pathogens that have evolved mechanisms of resistance to multiple antibiotics are becoming paramount public health threats in the 21st century.[1] The seriousness of antibiotic resistance lies in the fact that today bacterial strains not only are resistant to commonly available antibiotics but also may have acquired augmented virulence.[2] Therefore, the discovery and development of new antibiotics are of crucial importance to counter the explosive growth of multidrug resistant (MDR)[a] 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. Approximately 86% of these infections are healthcare-associated, and the remainder are community-associated.[5]

Bacterial resistance that contributes to shorter drug life cycles is achieved by three main mechanisms: enzymatic inactivation,[6] modification of the drug target(s),[7,8] and reduction of intracellular drug concentration by either changes in membrane permeability[9] or overexpression of efflux pumps.[10] 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 (unfortunately, this is a long and difficult road with many years between the introduction of novel antibacterial classes);[11] (ii) structural manipulation of existing antibiotics to reduce the potential for efflux without compromising antibacterial activity;[12,13] and (iii) identification of synthetic or natural nonantibiotic compounds, including current drugs, that work as efflux pump inhibitors (EPIs), which can restore the susceptibility of resistant strains to coadministered antibiotics that are efflux pump substrates.[13]

Resistance to quinolone antibacterials is principally due to chromosomal mutations in genes that encode the subunits of target enzymes, namely, DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*grlA* and *grlB*), and in other genes that regulate the expression of multidrug-resistant efflux systems.[13,14] With regard to the former, the first approach used to combat resistance to quinolones was focused on increasing the affinity for the target enzymes, even if mutated.[14] With respect to efflux pumps, these membrane-based proteins may provide a self-defense mechanism by which antibiotics, made by other microbes that fight for dominance in an environmental niche, or antibacterial drugs are actively removed from the cell. For antibacterials, this 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.[15] Although both mechanisms contribute to resistance, increased active efflux of the drugs is a major concern because of its key role on selection for high-level resistance strains.[16,17]

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[a] Abbreviations: MDR, multidrug resistance; MFS, major facilitator superfamily; MATE, multidrug and toxic extrusion family; EPI, efflux pump inhibitor; EtBr, ethidium bromide; QRDR, quinolone resistance-determining region.

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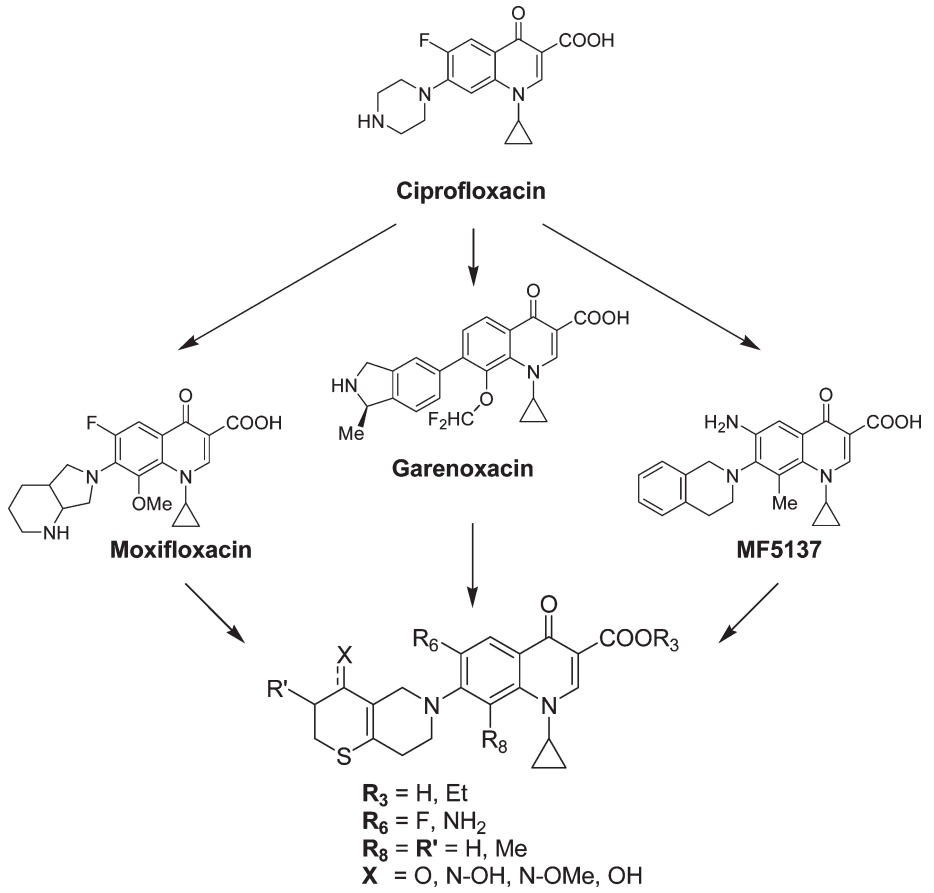


Figure 1. Structural features of CPX, selected antistaphylococcal quinolones, and general structural features of thiopyranopyridinylquinolones.

For quinolone antibacterials, efforts to bypass MDR are focused mainly on overcoming efflux-mediated resistance by identifying new compounds that are not substrates for efflux pumps. This goal can be achieved by the appropriate functionalization of the quinolone core so as to reduce or abolish the affinity for efflux pumps. For example, susceptibility to members of the latest generation of these compounds including moxifloxacin (MFX), garenoxacin, gemifloxacin, and gatifloxacin, is only slightly affected by *S. aureus* efflux pumps.[18]

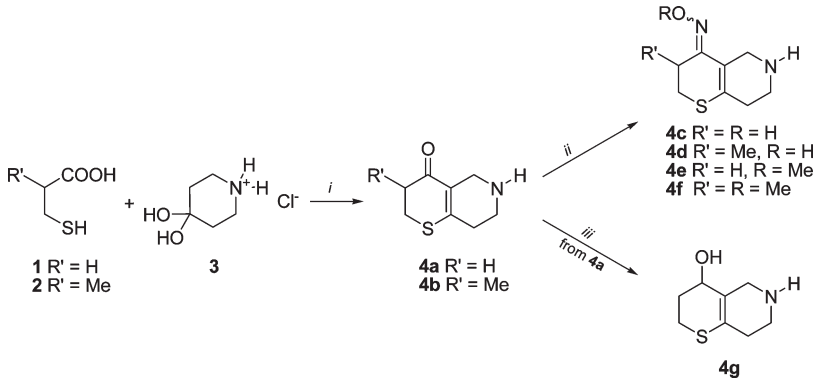
An alternative strategy is the development of new chemical entities that, although devoid of intrinsic antimicrobial activity, can restore the activity of substrate antibiotics that are prone to efflux by acting as EPIs. Therefore, the development of EPIs is a reasonable goal because when coadministered with antibiotics they not only can increase antibiotic activity but also may result in activity against previously nonsusceptible efflux pump overexpressing strains.[19−22] 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 antibiotics, giving new life to old drugs[21] with secure economic benefit.

In a previous paper, we reported that 6-amino-8-methyl quinolones show potent antibacterial activity against Gram-positive bacteria. For *S. aureus*, the most active compound identified was MF-5137, which had a bulky bicyclic 5,6,7,8-tetrahydro-isoquinoline at the C-7 position.[23] Selected substituents at the C-7 and C-8 positions result in better penetration through the thicker Gram-positive cell wall and could be crucial for reaching target enzymes and/or avoiding efflux.[18] This was confirmed in MFX[24] and garenoxacin,[15] which have bulky, lipophilic, bicyclic substituents (octahydro-1*H*-pyrrolo-[3,4-*b*]pyridine and 1-methyl-2,3-dihydro-1*H*-isoindole, respectively) at the C-7 position along with groups at C-8 that increase lipophilicity. Both are very active against Gram-positive bacteria, including some resistant strains. Moreover, replacement of the C-7 substituent in ofloxacin by a bulky biaryl urea gives a potent inhibitor of major facilitator superfamily (MFS) (NorA) and multidrug and toxic extrusion family (MATE) (MepA) efflux pumps in *S. aureus*.[25] In an attempt to obtain new quinolones with potent activity against Gram-positive bacteria, a new bicyclic bulky 2,3,5,6,7,8-hexahydro-4*H*-thiopyran[3,2-*c*]pyridin-4-one was designed and synthesized to be used, as is or suitably functionalized, as a lipophilic substituent at the C-7 position (Figure 1).

To evaluate the impact of a new bicyclic base at the C-7 position of the quinolone scaffold, a series of 6-fluoro-7-thiopyranopyridinequinolones (**9a**−**g**) were prepared. For comparative purposes, a 6-amino (**14a**−**g**) and 6-amino-8-methylquinolone (**15a**−**g**) analogue series also were synthesized and tested for antibacterial activity.

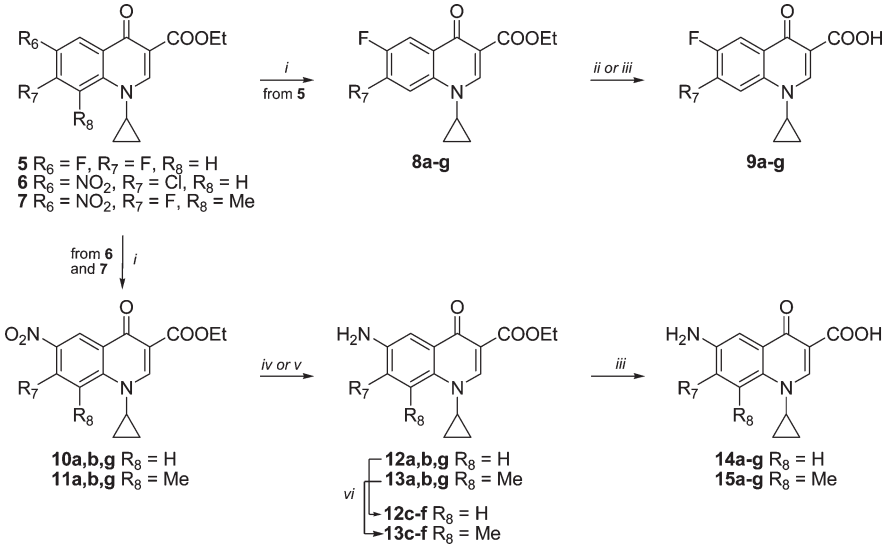
The new fluoroquinolones (**9a**−**g**), which differ from their closest analogue ciprofloxacin (CPX) only by the presence of the new hetero bicycle at the C-7 position, were first tested for their antibacterial activity against *Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. aureus* 25A-MRSA CPX^R. Although the compounds showed disappointing activity against the *E. coli* strain and variable activity against *S. aureus* ATCC 25923, almost all of them displayed interesting activity against the CPX and methicillin-resistant test strain 25A-MRSA CPX^R. This activity was better than that of CPX and, in some cases (**9c** and **9e**), comparable to that of

Scheme 1[a]



[a] Reagents and conditions: (i) H_2SO_4, 70 °C. (ii) $NH_2OR \cdot HCl$, pyridine, MeOH, 60 °C. (iii) $NaBH_4$, MeOH.

Scheme 2[a]



[a] Reagents and conditions: (i) Thiopyranopyridine **4a**−**g** (see Chart 1), DMSO, Et_3N, 70−100 °C. (ii) NaOH (4%) or 6 N HCl. (iii) $LiOH \cdot H_2O$, dioxane/H_2O. (iv) Fe, AcOH. (v) H_2, Raney-Ni, DMF/EtOH. (vi) $NH_2OH \cdot HCl$ or $NH_2OMe \cdot HCl$, pyridine, MeOH, 60 °C.

MFX and MF-5137. These preliminary results encouraged further investigations of this series of new heterocyclic substituents (**4a**−**g**) by linking them at the C-7 position of our previously reported 6-amino[26] and 6-amino-8-methyl[23] quinolone nuclei (derivatives **14a**−**g** and **15a**−**f**, respectively).

To determine whether these compounds were substrates for the NorA MDR efflux pump, all of the synthesized derivatives were tested against two pairs of *S. aureus* strains, SA-K1902 (*norA*−)/SA-K1904 (*norA*++) and SA-1199 (*norA* wild-type)/SA-1199B (*norA*++ and A116E GrlA). Comparing the antibacterial activity of synthesized compounds against both SA-K1902 and SA-K1904, it was clearly demonstrated that overexpression of the NorA pump does not affect the activity of 6-amino-8-methylquinolones **15a**−**g** and 6-fluoroquinolones **9b**,**c**,**e**,**f**, whereas for 6-aminoquinolones, **14a**−**g**, and some other 6-fluoroquinolones, it results in decreased activity. The same tendency was observed when comparing the antibacterial activity of these compounds against both SA-1199 and SA-1199B, even if in this case the GrlA mutation could be responsible for the slight decrease of activity observed. These tests show a trend in which the 6-aminoquinolones (**14a**−**g**) could be poor substrates for the NorA pump, while some of the 6-fluoro and all of the 6-amino-8-methyl (**15a**−**g**) derivatives seem not to be substrates at all (Table 1).

The more interesting compounds **9b**,**d**,**g** and **15a**,**b**,**e** were then tested against five *S. aureus* strains carrying point mutations resulting in amino acid substitutions in one or both of the quinolone targets, topoisomerase IV, and DNA gyrase. The tested compounds displayed an activity that was comparable to that of CPX; a single substitution in the topoisomerase IV A subunit produced a minimum inhibitory concentration (MIC) increase of 4−16-fold, whereas the activity disappears when a mutation in the DNA gyrase A subunit is also present (Table 2).

Comparing the microbiological data against the two pairs of *S. aureus* strains, in which there is or is not upregulation of NorA efflux pump (SA-K1902/SA-K1904 and SA-1199/SA-1199B), it was shown that many compounds having the bulky thiopyranopyridine moiety at C-7 were very poor or not substrates for NorA. On the basis of these results, the ester analogues were evaluated as EPIs. The esters were selected because they did not show any intrinsic antimicrobial activity that may make data interpretation difficult.

When tested for their ethidium bromide (EtBr) efflux inhibitory activity against SA-1199B, quinolone esters **8f**, **12d**, and **13d** displayed inhibitory activity better than that of the reference compound reserpine. Moreover, when these compounds were tested for their synergism with CPX against

resistant *S. aureus* strains using checkerboard assays, they were able to significantly reduce MICs of the fluoroquinolone. More active compounds **8f**, **12d**, **12e**, and **13d** were also tested against SA-K2886 (*mepA*++) using checkerboard assays to characterize their combined activity with EtBr. Good potency was observed, with MIC reductions from 4- (**12d**) to 32-fold (**12e** and **13d**).

Chemistry

The synthesis of the thiopyranopyridines **4a**−**g** was achieved by a one-pot reaction of 3-mercaptopropionic acid (**1**) or 3-mercapto-2-methylpropionic acid (**2**)[27] with 4-piperidone monohydrate hydrochloride (**3**) (Scheme 1), in concentrated sulphuric acid, with yields from 30 to 50%. The moderate yields likely are the result of a polycyclization phenomenon, which has been observed previously in the synthesis of a thiopyranobenzothiazine moiety.[28] In this case, we can hypothesize that the 4-keto group of 2,3,5,6,7,8-hexahydro-4*H*-thiopyrano[3,2-*c*]pyridine **4a**, more than its 3-methyl analogue **4b**, further reacts with mercaptoacids **1** or **2**,[27] respectively, to give polycyclic compounds as byproducts.

Ketones **4a** and **4b** then were derivatized to thiopyranopyridin-4-one oximes **4c**,**d** and thiopyranopyridin-4-one *O*-methyloximes **4e**,**f** by reaction with hydroxylamine hydrochloride or *O*-methylhydroxylamine hydrochloride, respectively, in MeOH and in the presence of pyridine with yields ranging

Chart 1. R_7 Substituent for Compounds **8**−**15**

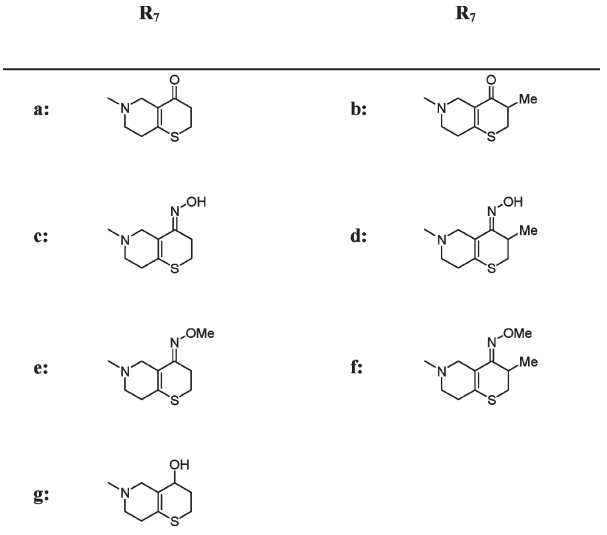


Table 2. Antibacterial Activity of Selected Compounds against *S. aureus* Strains with Target Mutation

		MICs (μg/mL)					
		wild-type	*S. aureus* mutants				
compd		SA-1199	K1035	K1134	K1305	K1628	K1640
mutation	GyrA	WT	WT	WT	WT	S84L	S84L
	GrlA	WT	S80F	S80Y	E84K	S80F	S80Y
9b		0.08	1.25	1.25	1.25	> 10	> 10
9d		0.16	1.25	1.25	1.25	> 10	> 10
9g		0.04	0.63	1.25	0.63	> 10	> 10
15a		0.31	1.25	1.25	1.25	> 10	> 10
15b		0.16	1.25	1.25	1.25	> 10	> 10
15e		0.31	1.25	2.50	1.25	> 10	> 10
CPX		0.31	2.50	2.50	2.50	> 10	> 10
MFX		0.08	0.31	0.31	0.31	5.0	2.5
MF5137		≤0.02	≤0.02	≤0.02	≤0.02	1.25	0.63

Table 1. Antibacterial Activity of Synthesized Compounds against Selected Bacterial Strains

	MICs (μg/mL)						
				modified *S. aureus* strains			
compd	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*S. aureus* 25 A MRSA CPX^R	SA-K1902 (norA−)	SA-K1904 (norA++)	SA-1199 (norA WT)	SA-1199B (norA++/A116E GrlA)
9a	8	0.08	> 128	0.31	0.63 (2)[a]	1.56	3.13 (2)
9b	8	0.31	32	0.16	0.16	0.08	0.16 (2)
9c	1	2	2	0.63	0.63	0.63	2.50 (4)
9d	32	0.31	4	0.16	0.31 (2)	0.16	0.31 (2)
9e	32	0.25	1	1.25	1.25	1.25	2.50 (2)
9f	64	2	8	2.50	2.50	2.50	5.00 (2)
9g	64	0.08	8	0.04	0.16 (4)	0.04	0.16 (4)
14a	128	6.25	> 128	3.13	6.25 (2)	1.56	25.00 (16)
14b	64	6.25	16	1.56	6.25 (4)	3.13	12.50 (4)
14c	2	2	16	1.56	6.25 (4)	1.56	12.50 (8)
14d	32	6.25	32	1.56	3.13 (2)	1.56	6.25 (4)
14e	16	2	8	1.56	3.13 (2)	3.13	12.50 (4)
14f	128	4	4	3.13	3.13	6.25	6.25
14g	128	16	128	12.50	25.00 (2)	25.00	> 100
15a	1	0.25	4	0.31	0.31	0.31	0.63 (2)
15b	64	0.08	32	0.16	0.16	0.16	0.31 (2)
15c	0.5	0.31	0,5	0.63	0.63	0.63	2.50 (4)
15d	0.5	0.16	8	0.31	0.31	0.31	1.25 (4)
15e	16	0.16	32	0.31	0.31	0.31	0.31
15f	8	16	8	6.25	6.25	3.13	3.13
15g	> 128	> 128	> 128	> 100	> 100	> 100	> 100
CPX	≤0.12	0.50	32	0.31	2.50 (8)	0.31	10.00 (32)
MFX	< 0.08	< 0.08	2	0.08	0.08	0.08	0.25 (4)
MF5137	≤0.12	≤0.12	≤0.12	≤0.02	≤0.02	≤0.02	≤0.02

[a] (*n*-Fold) increase of antibacterial MIC.

from 49 to 77%. Surprisingly, for all of the hydroxyimino/methoxyimino derivatives, both thin-layer chromatography (TLC) and ^{1}H NMR spectral data highlight that only one of the two possible oxime geometric isomers is formed. It has been demonstrated previously that the geometry of the oxime is very sensitive to its steric environment. For example, when a cyclic oxime has an R substituent, the OR group of the oxime tends to orient itself in the opposite direction to avoid an unfavorable steric interaction.[29] The determination of the *E* and/or *Z* configuration for the more hindered 3-methylthiopyranopyridin-4-methoxyimine **4f** as well as the less hindered thiopyranopyridin-4-methoxyimine **4e** was achieved by ^{1}H−^{1}H two-dimensional nuclear Overhauser effect spectroscopy (2D NOESY) NMR experiments (see the Supporting Information). Interestingly, methoxyimines **4e**,**f** result clearly in the *E* configuration. Because hydroxyimines **4c**,**d** do not have the bulky methoxy group to the oxime moiety, we believe that compounds adopt the *E* configuration. For these reasons, it is plausible to assume that all of the hydroxy/methoxyimine derivatives synthesized in this study are in the *E* configuration. Reduction of compound **4a** with $NaBH_4$ in MeOH gave 4-hydroxy derivative **4g** with a 56% yield.

The target 6-fluoroquinolones **9a**−**g** were obtained by nucleophilic substitution of the C-7 fluorine of ethyl 1-cyclopropyl-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate

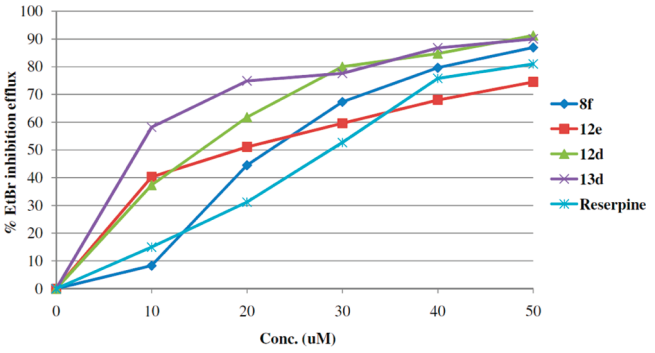


Figure 2. Effect of quinolone esters **8f**, **12d**, **12e**, **13d**, and reserpine on EtBr efflux of SA-1199B.

Table 3. Percent Reduction in EtBr Efflux of SA-1199B by 50 μM Test Compounds

compd	MIC (μg/mL)	% reduction
8d	> 100	22.5
8f	> 100	86.9
12a	> 100	35.7
12b	> 100	63.5
12d	> 100	91.2
12e	> 100	74.5
12f	> 100	19.7
12g	> 100	26.3
13a	> 100	36.8
13b	> 100	50.5
13c	> 100	36.2
13d	> 100	90.0
13e	> 100	54.4
13f	> 100	21.4
13g	> 100	47.3
CPXE	> 100	20.8
reserpine		84.8

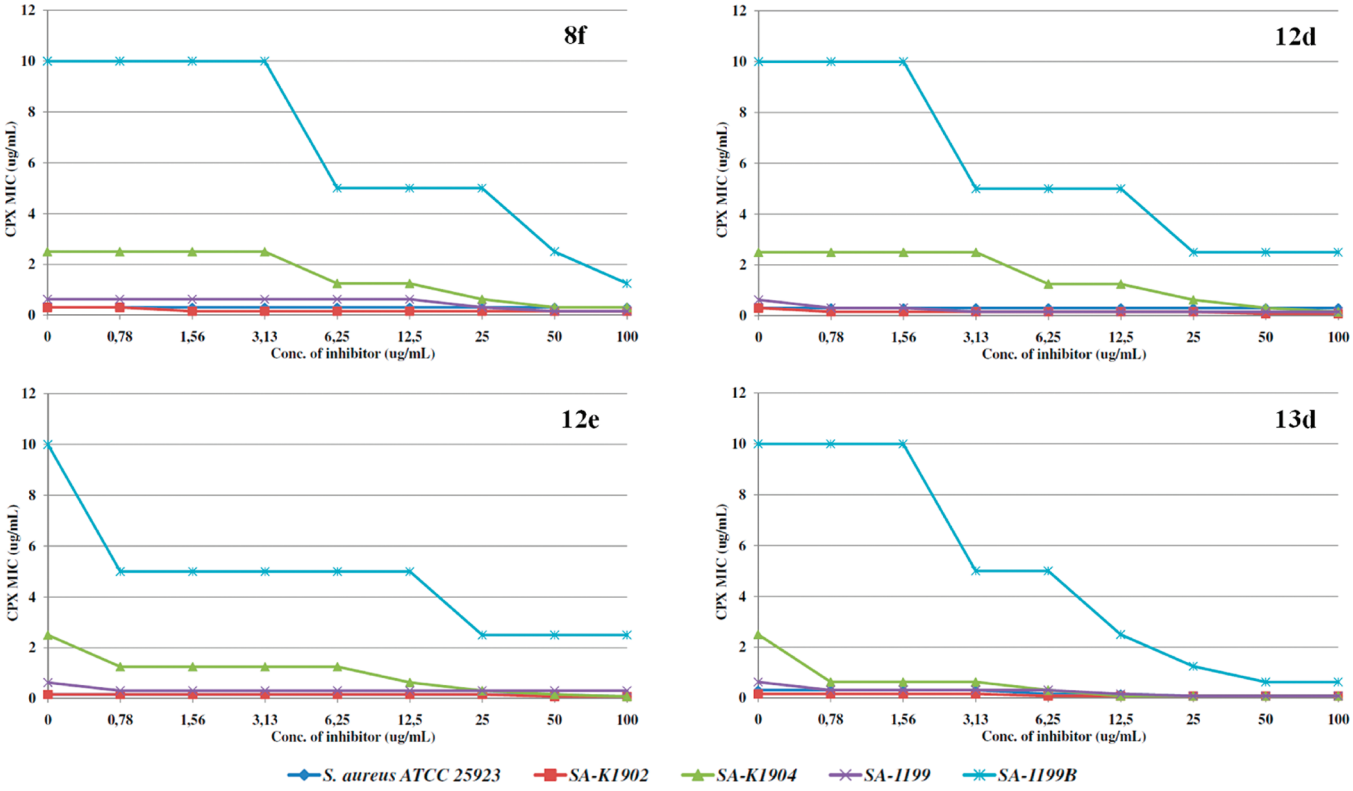


Figure 3. Effect of combining **8f**, **12d**, **12e**, and **13d** on ciprofloxacin MICs against *S. aureus* ATCC 25923, SA-K1902, SA-K1904, SA-1199, and SA-1199B.

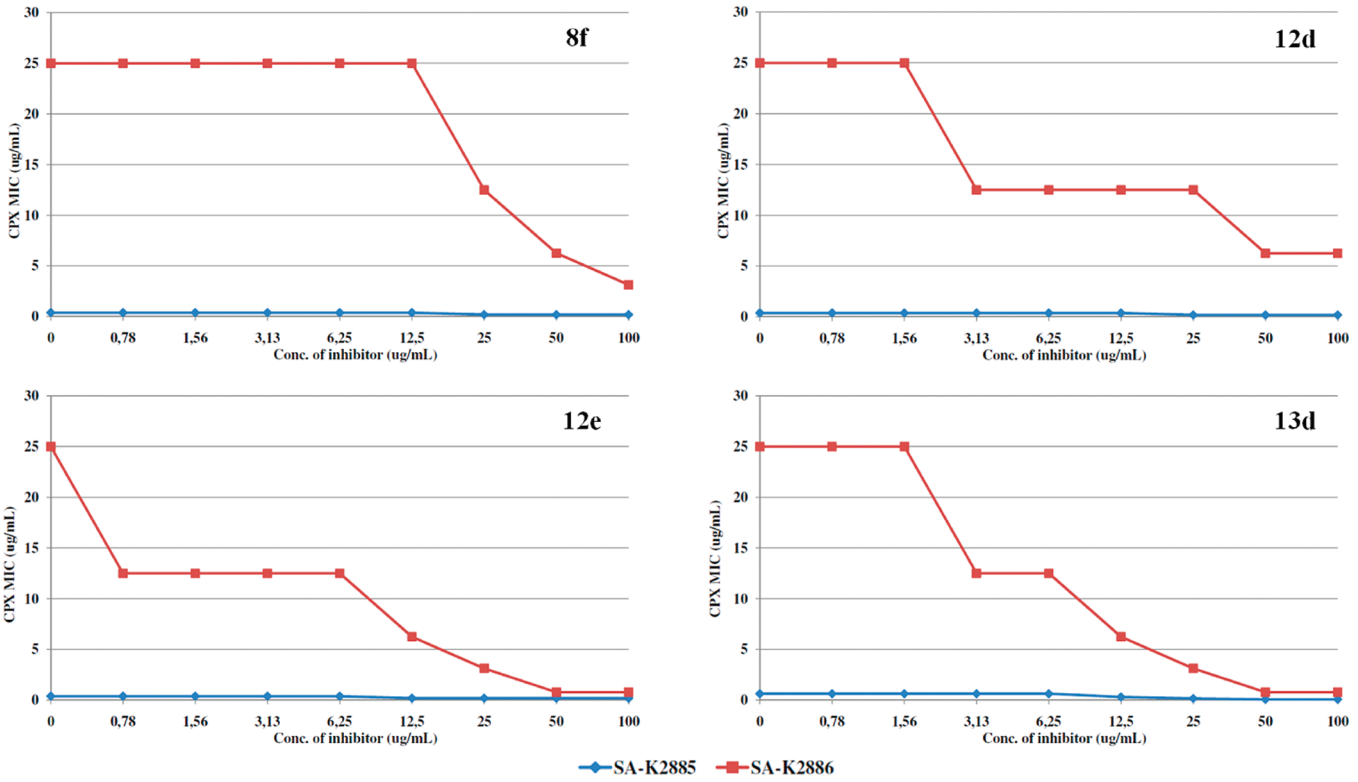


Figure 4. Effect of combining **8f**, **12d**, **12e**, and **13d** on EtBr MICs against SA-K2885 (*mepA*−) and SA-K2886 (*mepA*+).

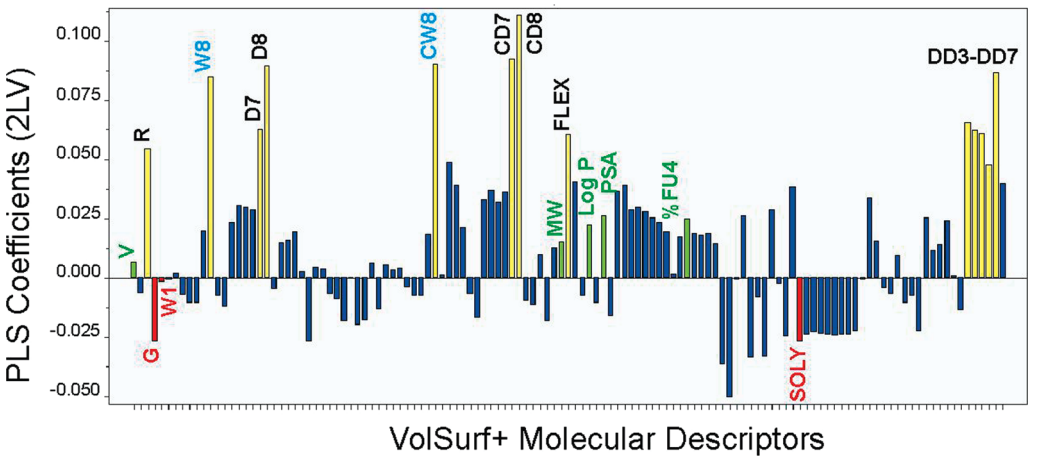


Figure 5. PLS coefficients for the model derived for the 16 quinolone esters, reporting the relevance of the variables to the model. The higher the bar is, the more important the corresponding variable is. "Positive" bars are directly correlated, while "negative" bars are inversely correlated with the inhibitory activity of EtBr efflux. The more relevant variables to the previous model[33] are green- or red-colored, whereas yellow-colored variables are the most relevant to the current model (based on the 16 ester molecules). Among them, the variables related to the "eighth descriptors" of both hydrophilic (W8 and CW8) and hydrophobic (D7, D8, CD7, CD8, and DD3−DD7) volumes[34] refer to interactions with "significant" strength that, in the scale of the GRID force -field, is −1.6 kcal/mol for the interaction with the DRY probe and −6.0 kcal/mol for the interaction with the OH2 probe.[35]

(**5**)[30] with the appropriate thiopyranopyridine **4a**−**g**, in DMSO and Et_3N; the corresponding esters **8a**−**g** were then hydrolyzed in either basic (4% NaOH or $LiOH \cdot H_2O$) or acid (6 N HCl) conditions to give the acid derivatives **9a**−**g** (Scheme 2).

The synthetic route that gives the 6-amino and 6-amino-8-methyl quinolones (**14a**−**g** and **15a**−**g**, respectively) first entailed the nucleophilic substitution of the C-7 position of ethyl 7-chloro-1-cyclopropyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**6**)[26] or ethyl 1-cyclopropyl-7-fluoro-8-methyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**7**)[23] with the appropriate thiopyranopyridine **4a**,**b**,**g**. Although in aromatic nucleophilic substitution the C-7 chlorine atom of synthone **6**[26] is a poorer leaving group in comparison with the C-7 fluorine of **7**,[23] nitro esters **10a**,**b**,**g** were obtained in yields of about 80%, while derivatives **11a**,**b**,**g** were obtained with poor yields (10−30% after some days). It is plausible that the methyl group at C-8 of synthone **7**[23] has a negative influence for both the steric hindrance and the inductive effect. The following reduction step of the C-6 nitro group, for compounds **10a** and **10b**, was performed with iron powder in acetic acid, while for **10g** and **11a**,**b**,**g** with Raney-Ni in a mixture of DMF/EtOH to obtain amino derivatives **12a**,**b**,**g** and **13a**,**b**,**g**. The

6-amino and 6-amino-8-methyl esters **12c−f** and **13c−f** were obtained in moderate to good yields (from 46 to 79%) by oxymation of ketones **12a**,**b** and **13a**,**b** with hydroxylamine hydrochloride or *O*-methylhydroxylamine hydrochloride in MeOH and in the presence of pyridine at 60 °C. Classical hydrolysis in basic or acid conditions of esters **12a−g** and **13a−g** gave only the decomposition products; the correspondent quinolone acids **14a−g** and **15a−g** were obtained with $LiOH \cdot H_2O$ in a mixture of dioxane/H_2O at room temperature, although in low yields (Scheme 2). CPX ester (CPXE)[31] was synthesized starting from 1-cyclopropyl-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**5**)[30] by reaction with piperazine in a mixture of N-methyl pyrrolidinone/*t*-butanol, using Et_3N as proton scavenger, in 70% yield.

Results and Discussion

Three series of 6-fluoro-, 6-amino-, and 6-amino-8-methylquinolones bearing the new thiopyranopyridine moiety at the C-7 position were synthesized and tested against one Gram-negative (*E. coli* ATCC 25922) and two Gram-positive bacteria (*S. aureus* ATCC 25923 and *S. aureus* 25A-MRSA CPX^R) in comparison with CPX, MFX, and the previously reported 6-amino-8-methylquinolone MF-5137 (Table 1). All of the tested compounds (**9a−g**, **14a−g**, and **15a−g**) showed poor antibacterial activity against *E. coli* ATCC 25922, while, in some cases, they displayed quite good activity against *S. aureus* strains. In general, 6-fluoroquinolones **9** had an antibacterial activity against *S. aureus* ATCC 25923 comparable to that of CPX, with the exception of derivatives **9a** and **9g**, which were similar to those of MFX and MF-5137. Interestingly, both the hydroxyimines **9c**,**d** and the methoxyimine **9e** exhibited superior activity to that of CPX (MICs 8−32-fold lower) and fairly comparable activity to MFX against the resistant strain *S. aureus* 25A MRSA CPX^R. For the 6-aminoquinolones (**14a−g**), the MIC values against ATCC 25923 and 25A MRSA CPX^R strains were generally higher than 6-fluoro analogues (**9a−g**), CPX, MFX, and MF-5137.

All of the 6-amino-8-methylquinolones, with the exception of derivatives **15f** and **15g**, displayed good antibacterial activity against *S. aureus* ATCC 25923, with MICs slightly lower than those of CPX and comparable to MFX and MF-5137. Confirming that the hydroxyimine moiety represents a highly suitable substituent, derivative **15c** was very active against the resistant strain 25A MRSA-CPX^R with MIC values 64-fold lower than CPX and 4-fold lower than MFX. On the basis of these results, an in-depth study was carried out with the aim of understanding how efflux and target mutations, the two main mechanisms of resistance to quinolones in *S. aureus*, may affect the antibacterial activity of these new series of quinolones.

To evaluate the effect of the NorA efflux pump on the antibacterial activity of derivatives (**9a−g**) and the analogues modified at the C-6 position (**14a−g** and **15a−g**), MICs were determined using two different *S. aureus* strains. These strains included SA-K1904, which overexpresses *norA*, and SA-1199B, which also overexpresses *norA* but also has a mutation of *grlA*, encoding the A subunit of topoisomerase IV, resulting in an A116E substitution. The appropriate parent strains also were included (SA-K1902 and SA-1199, respectively). CPX, MFX, and MF-5137 were used as reference compounds (Table 1). All 6-fluoroquinolone compounds (**9a−g**) exhibited the same or lower antibacterial activity than that of CPX against SA-K1902, with the only exception being **9g** (MIC 8-fold lower). While the activity of CPX was significantly decreased against SA-K1904 as compared to its cognate parent strain SA-K1902 (8-fold), the 6-fluoroquinolones **9a−g** were generally only slightly, or not at all, affected by the overexpression of the NorA pump. Against SA-1199, two of the seven tested compounds (**9b** and **9g**) displayed MICs 4−8-fold lower than that of CPX and comparable to that of MFX and MF-5137. When tested against SA-1199B, a slight rise of MIC values was observed, probably as a result of its topoisomerase IV substitution. The antibacterial activity of the 6-fluoroquinolones indicates that all, with the possible exception of **9g**, may not be NorA substrates.

The antibacterial activities of the 6-aminoquinolones **14a−g** were less than that of CPX against parent strains not expressing any resistance mechanisms (SA-K1902 and SA-1199). Small to moderate MIC increases were observed against the resistant strains SA-K1904 and SA-1199B (no change to 16-fold increase **14a**), whereas 8−32-fold MIC increases occurred for CPX. Although both the poor activity against unmodified strains and the increased MICs on SA-1199B could be attributed to a weak affinity with topoisomerase IV even if mutated, it appears that the thiopyranopyridine moiety at C-7 reduces the efficiency of efflux by NorA also on 6-aminoquinolones **14a−g**.

Results for the 6-amino-8-methylquinolones **15a−e** showed that these compounds performed better than the 6-fluoro and 6-amino analogues against all test strains, regardless of overexpression of *norA* or the presence of a topoisomerase substitution. The antibacterial activity for **15a−e** was generally equivalent to CPX against the parent strains SA-K1902 and SA-1199 (Table 1). Pure *norA* overexpression (SA-K1904) had no effect on susceptibility of any of these compounds, indicating that they are not NorA substrates. Small to no MIC increases occurred between SA-1199 and SA-1199B, indicating a probable minor effect of the topoisomerase substitution on activity. Compound **15f** was less active across all test strains but still appeared to not be a NorA substrate. Compound **15g** was inactive.

It is possible that MIC increases observed for SA-1199B for 6-amino-8-methyquinolones **15a−d** could be the result of overexpression of other known *S. aureus* efflux pumps (NorB, NorC, MepA, and MdeA). A TaqMan real-time polymerase chain reaction-based approach was used to assess expression of these other pump genes and showed that none were overexpressed in this strain (data not shown). These data support the conclusion that the topoisomerase substitution present in this strain is the reason for these MIC increases.

The last observations prompted us to investigate the activity of compounds **9b**,**d**,**g** and **15a**,**b**,**e** against five *S. aureus*-resistant strains carrying one (K1035, K1134, and K1305) or more mutations (K1628 and K1640) in the quinolone resistance-determining region (QRDR) regions of the A subunits of topoisomerase IV and DNA gyrase to assess how alterations of quinolone target affect the antibacterial activity. Against strains K1035, K1134, and K1305, characterized by a single amino acid substitution in the topoisomerase IV A subunit, all of the tested compounds showed an antibacterial activity comparable to that of CPX, although less than that of MFX and MF-5137. However, a sharp drop in activity was observed when the compounds were tested against *S. aureus* K1628 and K1640, strains characterized by QRDR region substitutions in both the A subunits of topoisomerase IV and the DNA gyrase (Table 2).

These data confirm that our compounds are not only minimally affected by NorA-mediated efflux but also retain

good activity in the presence of a single QRDR region mutation. Unfortunately, a combination of mutations in both target enzymes abrogates the activity of these derivatives. This observation led us to hypothesize that, as reported for MFX,[32] in *S. aureus* strains, the preferential target for those compounds could be topoisomerase IV, even if we observe a drop of activity when either of the targets was mutated.

We wished to clarify the role of the thiopyranopyridine moiety in conferring activity to 6-amino-, 6-amino-8-methyl-, and 6-fluoroquinolones against NorA overexpressing strains with respect to inhibition of EtBr efflux using SA-1199B. However, the antibacterial activity shown against this strain (see Table 1) could have led to a misleading interpretation in assessing NorA inhibitory activity. Thereby, taking into the account the well-established structure−activity relationship (SAR) of fluoroquinolone antibacterials, at least with regard to the importance of the key β-ketocarboxylic moiety that confers antibacterial activity, we decided to perform this screening with selected intermediate esters of the three series of quinolone synthesized (**8d**,**f**, **12a**,**b**, **12d**−**g**, and **13a**−**g**), which bore the thiopyranopyridine moiety while being devoid of any antibacterial activity (Table 3). The selection of quinolone esters has been done considering the antibacterial activity of corresponding quinolone acids against modified *S. aureus* strains. In our opinion, 6-amino-8-methylquinolone acids are the most interesting, hence the testing of all of the corresponding esters. Among 6-aminoquinolones esters, only compound **12c** is missing, although the antibacterial activity of the corresponding acid **14c** is similar to that of compound **14b** (and ester **12b** has been included in the test). For the 6-fluoroquinolone series, only the corresponding esters of the most active and the least active quinolone acids are considered in the test. For comparative purposes, the ethyl ester of ciprofloxacin (CPXE)[31] and reserpine, a well-known NorA EPI, were included in the analysis.

The ester intermediates used in this analysis variably inhibited EtBr efflux by SA-1199B. Of the tested derivatives, compounds **8f**, **12d**, and **13d** demonstrated strong inhibition (86.9, 91.2, and 90.0%, respectively) and were slightly superior to the effect of reserpine (Table 3). CPXE was not effective in EtBr efflux inhibition, confirming the key role of the thiopyranopyridine moiety in the C-7 position on inhibition of the NorA efflux pump. Comparing the antibacterial activity of quinolone acids **9**, **14**, and **15** with the EtBr efflux inhibitory activity of the selected corresponding quinolone esters **8**, **12**, and **13**, no parallelisms could be observed. Active EPIs were identified among all of the three series of tested quinolone esters (**8f**, **12d**, **12e**, and **13d**). From a direct comparison of the EtBr inhibitory activity of 6-aminoquinolone esters **12a**,**b**,**d-g** and 6-amino-8-methylquinolone esters **13a**−**g**, it is possible to identify a trend in which the most active compounds were derivatives **12d** and **13d**, carrying either a 3-methyl-2,3,5,6,7,8-hexahydro-4*H*-thiopyrano[3,2-*c*]pyridin-4-one oxime in C-7, followed by compounds **12e** and **13e**, characterized by a 2,3,5,6,7,8-hexahydro-4*H*-thiopyrano[3,2-*c*]pyridin-4-one *O*-methyloxime substituent on the C-7, and the less active couples **12b** and **13b** > **12a** and **13a** > **12 g** and **13g** > **12f** and **13f**. For the 6-fluoroquinolone esters included in the assays, the same trend was not observed. A tentative explanation could be that the presence of the C-6 amino substituent causes steric hindrance that does not allow all of the orientations of the C-7 thiopyranopyridine substituents that are possible with the smaller C-6 fluorine atom.

For those compounds having an inhibitory activity ≥70% (**8f**, **12d**, **12e**, and **13d**), a dose−response curve was generated (Figure 2). Reserpine was included as a reference. The dose−response curves confirmed that the tested quinolone esters displayed a good inhibitory activity of EtBr efflux on SA-1199B. At the lowest tested concentration (10 μM), compounds **12d**, **12e**, and **13d** were from 3- to 4-fold more potent than reserpine.

These same compounds (**8f**, **12d**, **12e**, and **13d**) were evaluated against five *S. aureus* strains having varying levels of susceptibility to fluoroquinolone antibacterials using combination plates with CPX. The *S. aureus* strains included in this test were *S. aureus* ATCC 25923, SA-K1902, SA-K1904, SA-1199, and SA-1199B. MIC data for CPX for these strains are presented in Table 1. Isobolograms shown in Figure 3 reveal little synergistic activity between any of the test compounds and CPX against *S. aureus* ATCC 25923, SA-K1902, and SA-1199. With regard to compound **8f**, significant synergy with CPX occurred against SA-K1904 and SA-1199B (8- fold MIC reductions for both) (Figure 3).

The synergistic activity displayed by compounds **12d** and **12e** resulted in a reduction of the MIC of CPX by 16- and 32-fold, respectively, against SA-K1904 and only 4-fold against SA-1199B (Figure 3). Compound **13d** was the most potent compound as it was able to restore the MICs of CPX against SA-K1904 and SA-1199B at 6.25 and 50 μg/mL concentrations, respectively (Figure 3). This observation lead us to hypothesize that NorA inhibition by **13d** is so efficacious that, although in SA-1199B the primary target (topoisomerase IV A subunit) was mutated, the high intracellular concentration achieved by CPX resulted in an interaction with the secondary target (DNA-gyrase) and a MIC of 0.63 μg/mL.

Finally, compounds **8f**, **12d**, **12e**, and **13d** were tested against a *S. aureus* strain overexpressing the MepA MATE family multidrug efflux pump (SA-K2886 − *mepA*++) and its cognate parent strain that expresses *mepA* at a wild-type level (SA-K2885) to evaluate their synergistic activity with EtBr (Figure 4). All tested compounds had no antibacterial activity (data not shown), nor did they result in any synergistic activity against the parent strain SA-K2885. Compounds **8f** and **12d** were able to reduce the EtBr MIC from 4- to 8-fold, respectively, against SA-K2886 (Figure 4). Interestingly, both **12e** and **13d** derivatives were able to reduce the EtBr MIC 32-fold, restoring its antibacterial activity against the *mepA*-overexpressing strain (Figure 4). These data indicate that thiopyranopyridinylquinolone esters **8f**, **12d**, **12e**, and **13d** are inhibitors of both the NorA and the MepA MDR efflux pumps. In particular, compound **13d** was able to completely restore the activity of CPX and EtBr on *S. aureus* strains overexpressing either pump.

An attempt to delineate a preliminary SAR for both the antibacterial activity of synthesized quinolones and the NorA efflux pump inhibition of quinolone esters was carried out. In general, these new quinolone acids exhibit selective in vitro activity toward Gram-positive bacteria. The presence of a C-6 fluorine atom was confirmed to be not necessary for the antibacterial activity of these thiopyranopyridinylquinolones. In fact, 6-amino derivatives **14a**−**g** display a slightly lower activity when compared to the corresponding 6-fluoroquinolones **9a**−**g** against tested bacterial strains. Good results were obtained by introducing a methyl group in the C-8 position of 6-aminoderivatives **14a**−**g** to obtain 6-amino-8-methylquinolones **15a**−**g**, confirming the role of the C-8 substituent for activity against Gram-positive bacteria.

A pivotal role in the antibacterial activity against NorA overexpressing *S. aureus* strains was played by the thiopyranopyridine moiety in the C-7 position. The presence of this moiety is sufficient to make the synthesized 6-fluoro and 6-aminoquinolones poor substrates for the NorA MDR efflux pump. Only slight differences in the antibacterial activity were observed when the C-4 keto group of thiopyrane side was converted in the corresponding hydroxyimine or methoxyimine, while a drop of activity was generally obtained by its reduction to the hydroxyl group.

Regarding the inhibition of the NorA efflux pump by quinolone esters, the same strong activity can be obtained with each of the three quinolones core functionalized at the C-7 position with the appropriate thiopyranopyridinyl moiety (**8f**, **12d**, **12e**, and **13d**). Generally, the best results were displayed with 3-methylthiopyranopyridinil-4-one hydroxyimines (compounds **12d** and **13d**), but also, thiopyranopyridinil-4-one methoxyimine (compounds **12e** and **13e**) was able to confer good activity.

Data collected in this study clearly show that a thiopyranopyridine moiety is a key feature capable of conferring good antibacterial activity against *S. aureus* strains, including those overexpressing MDR efflux pumps, to quinolone acids as well as a strong synergistic activity with CPX against NorA overproducing *S. aureus*-resistant strains to quinolone esters.

Molecular Modeling

As previously reported for 3-phenyl-1,4-benzothiazine derivatives,[33] for 15 quinolone esters, as well as for CPXE, some physicochemical parameters were calculated in silico to investigate whether they correlate with NorA inhibitory activity. This was done by subjecting this set of compounds to PLS modeling using Volsurf descriptors[34] from the GRID forcefield[35] as the X-matrix and the EtBr efflux inhibitory activity as the dependent variable. All of the compounds of Table 3 were used for modeling with the exception of reserpine, which is macroscopically too different from all of the other compounds of the series and whose inclusion in the set would bias any modeling approach. Among these compounds, four exhibited an EtBr efflux inhibitory activity higher than 70% (**8f**, **12d**, **12e**, and **13d**), eight were almost inactive, that is, with inhibitory values lower than 40% (**8d**, **12a**, **12f**, **12g**, **13a**, **13c**, **13f**, and CPXE), while the other four exhibited intermediate activity (40−70% inhibition) (**12b**, **13b**, **13e**, and **13g**). According to spectral data, all of the hydroxy/methoxyimine derivatives were modeled in their *E* forms.

It is noteworthy to mention that since the previously reported 3-phenyl-1,4-benzothiazines[33] and these 16 quinolone esters belong to two different chemical series, any kind of modeling attempt considering all of them together would necessarily describe the macroscopic difference between the two series and would fail in bringing out the differences within the same series. This is why only the series of esters was considered for modeling. However, different considerations obtained for the individual series (concerning lipophilicity, size, hydrogen bonding, etc.) can be compared as a first analysis.

In the previous model,[33] the molecular descriptors that better correlated with efflux inhibition were both hydrophobic properties and molecular dimensions. In that PLS model, Log P was directly correlated with efflux inhibition, water solubility (SOLY) and the percentage of uncharged molecules at pH 4 (%FU4) were strongly but inversely correlated with NorA inhibition, whereas polarity (PSA), molecular dimension (MW, *V*, and *G*), and hydrophilic accessible volume (W1) were less correlated with the inhibition of EtBr efflux. The model suggested that within the series of 3-phenyl-1,4-benzothiazines, the H-bond pattern is more relevant than molecular size and shape with respect to efflux.

From the analysis of the coefficients of the PLS model obtained for this series of quinolone esters (Figure 5), it seems that the capability of forming strong interactions is related to the inhibitory activity. Among the variables cited in the previous work, Log P maintains a positive coefficient, meaning a positive effect on the inhibitory activity of EtBr efflux, while water solubility (SOLY) has a negative coefficient (inversely correlated with inhibition). Molecular dimensions (MW and *V*) seem to be less important. In addition, polarity (PSA), molecular charge (% FU4), and hydrophilic accessible volume (W1) are also less correlated with the inhibition of EtBr efflux, whereas the globularity (*G*) of the compounds is inversely related with the activity (Figure 5). Although analysis of this local series of compounds can help in the understanding of what modifications can be performed on these molecular structures to develop more potent compounds, a deeper analysis of a larger data set is required to be able to study molecules belonging to different chemical series together.

Conclusions

We have shown that the thiopyranopyridine moiety is an effective C-7 substituent for both 6-fluoro-, 6-amino-, and 6-amino-8-methylquinolone antibacterials. It was able to confer good antibacterial activity against resistant *S. aureus* strains because for the most part these compounds were able to avoid NorA-mediated efflux. Suitable modifications of the quinolone antibacterial core, such as the substitution of the acid function with an ethyl ester group, plus the addition of a bulky thiopyranopyridinyl moiety at C-7, result in potent NorA EPIs that also reduce MepA efflux activity. The activity of some of these thiopyranopyridinylquinolone esters was superior to that of the reference compound reserpine against NorA overexpressing strain SA-1199B.

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.[36,37] In addition, SA-K1904, 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.[38] SA-K2885 and K2886 are *norA*-deleted strains containing the empty expression vector pALC2073 and pALC2073-*mepA*, respectively.[39] Genes cloned into pALC2073 are under control of a *xyl*/*tetO* promoter, which is inducible by 0.05 μg/mL tetracycline.[40] This concentration of tetracycline was included in all experiments utilizing these strains.

Microbiologic Procedures. MICs were determined in duplicate by microdilution techniques according to CLSI guidelines.[41] 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 also was determined. Checkerboard combination studies using CPX and **8f**, **12d**, **12e**, and **13d** were performed as described previously.[42]

EtBr Efflux. The loss of EtBr from *S. aureus* SA-1199B was determined fluorometrically as previously described.[43] 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, **8f**, **12d**, **12e**, and **13d** on the EtBr efflux of SA-1199B was also determined and efflux in the presence of test compounds was compared to that determined in their absence, allowing the calculation of percent reduction in efflux.

Molecular Modeling. The molecules were built using the Sybyl 8.0[44] Molecular Modeling package; the structures were minimized using the Powell force field with default settings. The Volsurf+ descriptors were generated using the version 1.0.5 of Volsurf+.[45]

Synthesis. All reactions were routinely checked by 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; and 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.

2,3,5,6,7,8-Hexahydro-4*H*-thiopyrano[3,2-*c*]pyridin-4-one (4a). 3-Mercaptopropanoic acid (**1**) (16.9 mL, 195.0 mmol) was added dropwise to a solution of 4-piperidone monohydrate hydrochloride (**3**) (20.0 g, 130.0 mmol) in concentrated H_2SO_4 (60 mL) maintained at 70 °C under vigorous stirring. An evolvement of HCl_g was observed, and after 5 h, the reaction mixture was cooled, poured into H_2O/ice, then basified with NaOH pellets until pH ≅ 12, and extracted several times with EtOAc. 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 CH_2Cl_2/MeOH (90:10 → 85:15), to give 6.2 g (26.4%) of pure 2,3,5,6,7,8-hexahydro-4*H*-thiopyrano[3,2-*c*]pyridin-4-one (**4a**) as a yellow-orange semisolid. ^{1}H NMR ($CDCl_3$): δ 2.20−2.32 (2H, m, CH_2,C-7), 2.52−2.63 (2H, m, CH_2,C-3), 2.81 (2H, t, J = 5.7 Hz, CH_2,C-8), 2.94−3.06 (2H, m, CH_2,C-2), 3.46 (2H, t, J = 1.9 Hz, CH_2,C-5). ^{13}C NMR ($CDCl_3$): δ 192.15, 153.45, 126.12, 43.13, 41.83, 37.58, 30.57, 26.66. Anal. ($C_8H_{11}NOS$) C, H, N.

3-Methyl-2,3,5,6,7,8-hexahydro-4*H*-thiopyrano[3,2-*c*]pyridin-4-one (4b). Compound **4b** was prepared according to the procedure for **4a** above, except that intermediate 3-mercapto-2-methylpropanoic acid (**2**)[27] was used instead of **1** to give the title compound **4b** as a yellow oil; yield, 49%. ^{1}H NMR ($CDCl_3$): δ 1.20 (3H, d, J = 6.8 Hz, CH_3), 2.25−2.75 (3H, m, CH and CH_2, C-7), 2.80−3.10 (4H, m, CH_2, C-2 and C-8), 3.25 (1H, s, NH), 3.55 and 3.65 (each 1H, d, J = 16.6 Hz, CH_2, C-5). ^{13}C NMR ($CDCl_3$): δ 194.61, 152.25, 124.25, 42.92, 41.43, 40.26, 33.23, 29.92, 14.69. Anal. ($C_9H_{13}NOS$) C, H, N.

(4*E*)-2,3,5,6,7,8-Hexahydro-4*H*-thiopyrano[3,2-*c*]pyridin-4-one Oxime (4c). Thiopyranopyridin-4-one (**4a**) (2.00 g, 12.0 mmol) dissolved in the smallest quantity of MeOH was added dropwise to a solution of $NH_2OH\cdot HCl$ (1.32 g, 19.0 mmol) and pyridine (2.10 mL, 26.0 mmol) in 12 mL of MeOH, maintained under stirring, and then warmed until 60 °C. After 6 h, the reaction mixture was evaporated to dryness under reduced pressure, and the residue was treated with H_2O/ice and extracted several times with CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4, and concentrated to obtain 1.16 g (52.7%) of desired compound **4c** as a yellow oil that was used without further purification in subsequent reactions. ^{1}H NMR (DMSO-d_6): δ 2.00−2.20 (2H, m, CH_2, C-3), 2.70−2.90 (6H, m, CH_2, C-2, C-7 and C-8), 3.40 (2H, s, CH_2, C-5), 11.00 (1H, bs, NOH). ^{13}C NMR (DMSO-d_6): δ 149.63, 148.87, 111.72, 46.16, 42.91, 32.24, 28.09, 25.64. Anal. ($C_8H_{12}N_2OS$) C, H, N.

(4*E*)-3-Methyl-2,3,5,6,7,8-hexahydro-4*H*-thiopyrano[3,2-*c*]pyridin-4-one Oxime (4d). Compound **4d** was prepared according to the procedure for **4c** above, except that intermediate **4b** was used instead of **4a** to give the title compound **4d** after 48 h as white solid; yield, 49%: mp 74.5−76.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.10 (3H, d, J = 7.2 Hz, CH_3), 2.30−2.75 (3H, m, CH, CH_2, C-8), 3.00−3.25 (4H, m, CH_2, C-2 and C-7), 3.25 (1H, s, NH), 3.50−3.75 (2H, m, CH_2, C-5), 11.25 (1H, s, NOH). ^{13}C NMR (DMSO-d_6): δ 158.06, 148.87, 110.87, 46.67, 42.91, 36.15, 32.53, 28.09, 13.15. Anal. ($C_9H_{14}N_2OS$) C, H, N.

(4*E*)-2,3,5,6,7,8-Hexahydro-4*H*-thiopyrano[3,2-*c*]pyridin-4-one *O*-methyloxime (4e). Compound **4e** was prepared according to the procedure for **4c** above, except that $NH_2OMe\cdot HCl$ was used instead of $NH_2OH\cdot HCl$ to give the title compound **4e** after 24 h as white solid; yield, 77%; mp 275.0−276.5 °C. ^{1}H NMR (DMSO-d_6): δ 2.70−2.80 (2H, m, CH_2, C-3), 2.80−2.90 (2H, m, CH_2, C-8), 3.05−3.20 (2H, m, CH_2, C-7), 3.25−3.55 (2H, m, CH_2, C-2), 3.70 (2H, s, CH_2, C-5), 4.80 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6): δ 147.41, 146.91, 112.48, 61.12, 46.98, 41.91, 32.57, 28.09, 26.46. Anal. ($C_9H_{14}N_2OS$) C, H, N.

(4*E*)-3-Methyl-2,3,5,6,7,8-hexahydro-4*H*-thiopyrano[3,2-*c*]-pyridin-4-one *O*-methyloxime (4f). Compound **4f** was prepared according to the procedure for **4c** above, except that intermediate **4b** was used instead of **4a** and $NH_2OMe\cdot HCl$ instead of $NH_2OH\cdot HCl$ to give the title compound **4f** after 24 h as white solid; yield, 52%; mp 244.5−245.7 °C. ^{1}H NMR (DMSO-d_6): δ 1.20 (3H, d, J = 6.8 Hz, CH_3), 1.90−2.30 (2H, m, CH_2, C-7), 2.55−2.70 (2H, m, CH_2, C-8), 2.80−2.95 (1H, m, CH), 3.10−3.30 (2H, m, CH_2, C-2), 3.35−3.65 (2H, m, CH_2, C-5), 3.80 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6): δ 155.34, 147.41, 111.63, 61.01, 47.49, 42.91, 36.90, 33.35, 28.09, 13.79. Anal. ($C_{10}H_{16}N_2OS$) C, H, N.

3,4,5,6,7,8-Hexahydro-2*H*-thiopyrano[3,2-*c*]pyridin-4-ol (4g). $NaBH_4$ (0.13 g, 3.5 mmol) was added to a solution of compound **4a** (0.50 g, 2.9 mmol) in dry MeOH (5 mL), and the mixture was maintained at −5 °C under stirring. After 1 h, some drops of water were added to the reaction mixture to disrupt the excess of $NaBH_4$, then the mixture was evaporated to dryness, and the residue was purified by flash column chromatography ($CHCl_3$/MeOH, 80:20) to obtain 0.28 g (56.4%) of desired compound **4g** as a yellow-orange semisolid. ^{1}H NMR (MeOD-d_4): δ 1.80−2.40 (4H, m, CH_2, C-3 and C-8), 2.60−3.20 (4H, m, CH_2, C-2 and C-7), 3.30 and 3.65 (each 1H, d, J = 16.6 Hz, CH_2, C-5), 4.00 (1H, bs, CH, C-4). ^{13}C NMR (DMSO-d_6): δ 124.72, 121.17, 61.91, 43.81, 42.91, 31.63, 26.23, 22.14. Anal. ($C_8H_{13}NOS$) C, H, N.

General Procedure for Nucleophilic Substitution of the C-7 Position of Synthones 5,[30] 6[26] and 7.[23] A mixture of appropriate synthone **5**,[30] **6**,[26] or **7**[23] (1.0 equiv), the selected thiopyranopyridine (4.0 equiv), and Et_3N (from 2.0 to 8.0 equiv) in dry DMSO was warmed to 70−100 °C until the starting synthon was disappeared (TLC: from 3 h to 10 days). Successively, the reaction mixture was poured into ice/H_2O, extracted with CH_2Cl_2, and then purified by flash column chromatography (CH_2Cl_2/MeOH 95:5 unless otherwise indicated) to obtain the desired product in reported yields.

Ethyl 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-1,4-dihydroquinoline-3-carboxylate (8a). Compound **8a** was obtained from **5**[30] and thiopyranopyridine **4a**; 4 h, 100 °C, CH_2Cl_2/MeOH 99:1, 55%; mp 230.0−232.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.05−1.12 (2H, m, CH_2, cyclopropyl), 1.20−1.50 (5H, m, CH_2 cyclopropyl, CH_2CH_3), 2.70 (2H, t, J = 5.4 Hz, CH_2, C-8), 2.75−2.85 and 3.15−3.25 (each 2H, m, CH_2, C-3 and C-2), 3.38−3.58 (3H, m, CH, cyclopropyl, CH_2,

C-7), 4.06 (2H, s, CH_2, C-5), 4.38 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.32 (1H, d, J = 7.2 Hz, H-8), 8.06 (1H, d, J = 13.4 Hz, H-5), 8.52 (1H, s, H-2). Anal. ($C_{23}H_{23}FN_2O_4S$) C, H, N.

Ethyl 1-Cyclopropyl-6-fluoro-7-(3-methyl-4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (8b). Compound **8b** was obtained from **5**[30] and thiopyranopyridine **4b**; 16 h, 100 °C, CH_2Cl_2/MeOH 99:1, 71%; mp 188.0−190.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.08−1.22 (2H, m, CH_2, cyclopropyl), 1.24−1.36 (5H, m, CH_2, cyclopropyl, CH_3), 1.40 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.50−2.80 (3H, m, CH, CH_2, C-2), 3.00−3.23 (2H, m, CH_2, C-8), 3.35−3.55 (1H, m, CH, cyclopropyl), 3.55−3.80 (2H, m, CH_2, C-7), 4.05 and 4,20 (each 1H, d, J = 13.4 Hz, CH_2, C-5), 4.42 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.45 (1H, d, J = 7.2 Hz, H-8), 8.00 (1H, d, J = 13.4 Hz, H-5), 8.62 (1H, s, H-2). Anal. ($C_{24}H_{25}FN_2O_4S$) C, H, N.

Ethyl 1-Cyclopropyl-6-fluoro-7-[4-(hydroxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate (8c). Compound **8c** was obtained from **5**[30] and thiopyranopyridine **4c**; 12 h, 100 °C, 48%; mp 199.0−202.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.10−1.35 (4H, m, CH_2, cyclopropyl), 1.45 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.48−2.65 (2H, m, CH_2, C-3), 2.90−3.15 (4H, m, CH_2, C-2 and C-8), 3.30−3.70 (3H, m, CH cyclopropyl, CH_2, C-7), 4.12 (2H, s, CH_2, C-5), 4.37 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.30 (1H, d, J = 7.2 Hz, H-8), 8.08 (1H, d, J = 13.4 Hz, H-5), 8.45 (1H, bs, NOH), 8.55 (1H, s, H-2). Anal. ($C_{23}H_{24}FN_3O_4S$) C, H, N.

Ethyl 1-Cyclopropyl-6-fluoro-7-[4-(hydroxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate (8d). Compound **8d** was obtained from **5**[30] and thiopyranopyridine **4d**; 12 h, 100 °C, 49%; mp 188.0−190.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.00−1.35 (10H, m, CH_2 cyclopropyl, CH_2CH_3, CH_3), 2.18−2.55 (3H, m, CH, CH_2, C-8), 2.70 and 3.10 (each 1H, d, J = 12.6 Hz, CH_2, C-2), 3.25−3.45 (1H, m, CH, cyclopropyl), 3.55−3.75 (2H, m, CH_2, C-7), 3.80−3.95 (1H, m, CH_2, C-5), 4.05−4.30 (3H, m, CH_2CH_3, CH_2, C-5), 7.30 (1H, d, J = 7.7 Hz, H-8), 7.70 (1H, d, J = 13.3 Hz, H-5), 8.45 (1H, s, H-2), 11.05 (1H, s, NOH,). Anal. ($C_{24}H_{26}FN_3O_4S$) C, H, N.

Ethyl 1-Cyclopropyl-6-fluoro-7-[4-(methoxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate (8e). Compound **8e** was obtained from **5**[30] and thiopyranopyridine **4e**; 12 h, 95 °C, 89%; mp 216.0−220.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.08−1.35 (4H, m, CH_2, cyclopropyl), 1.40 (3H, t, J = 6.9 Hz, CH_2CH_3), 2.45−2.60 (2H, m, CH_2, C-3), 2.80−3.05 (4H, m, CH_2, C-2 and C-8), 3.45−3.50 (1H, m, CH, cyclopropyl), 3.55−3.70 (2H, m, CH_2, C-7), 3.95 (3H, s, OCH_3), 4.20 (2H, s, CH_2 C-5), 4.45 (2H, q, J = 6.9 Hz, CH_2CH_3), 7.35 (1H, d, J = 8.1 Hz, H-8), 8.05 (1H, d, J = 13.0 Hz, H-5), 8.55 (1H, s, H-2). Anal. ($C_{24}H_{26}FN_3O_4S$) C, H, N.

Ethyl 1-Cyclopropyl-6-fluoro-7-[4-(methoxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate (8f). Compound **8f** was obtained from **5**[30] and thiopyranopyridine **4f**; 24 h, 100 °C, 14%; mp 232.0−233.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.00−1.30 (10H, m, CH_2, cyclopropyl, CH_2CH_3 and CH_3), 2.20−2.40 (2H, m, CH_2, C-8), 2.50−2.60 (1H, m, CH), 2.75 and 3.15 (each 1H, d, J = 12.9 Hz, CH_2, C-2), 3.25−3.45 (1H, m, CH, cyclopropyl), 3.55−3.90 (6H, m, CH_2, C-7, CH, C-5, and OCH_3), 4.10−4.30 (3H m, CH, C-5 and CH_2CH_3), 7.40 (1H, d, J = 7.8 Hz, H-8), 7.75 (1H, d, J = 13.7 Hz, H-5), 8.40 (1H, s, H-2). Anal. ($C_{25}H_{28}FN_3O_4S$) C, H, N.

Ethyl 1-Cyclopropyl-6-fluoro-7-(4-hydroxy-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (8g). Compound **8g** was obtained from **5**[30] and thiopyranopyridine **4g**; 12 h, 100 °C, $CHCl_3$/MeOH 90:10, 74%; mp 152.9−154.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.00−1.30 (7H, m, CH_2, cyclopropyl and CH_2CH_3), 1.80−2.40 (4H, m, CH_2, C-3 and C-8), 2.60−3.20 (4H, m, CH_2, C-2 and C-7), 3.50−3.80 (3H, m, CH cyclopropyl, CH_2, C-5), 3.90−4.30 (3H, m, CH, C-4 and CH_2CH_3), 7.32 (1H, d, J = 7.3 Hz, H-8), 8.06 (1H, d, J = 14.0 Hz, H-5), 8.52 (1H, s, H-2). Anal. ($C_{23}H_{25}FN_2O_4S$) C, H, N.

Ethyl 1-Cyclopropyl-6-nitro-4-oxo-7-(4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-1,4-dihydroquinoline-3-carboxylate (10a). Compound **10a** was obtained from **6**[26] and thiopyranopyridine **4a**; 7 h, 70 °C, 87%; mp 243.0−245.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.05−1.20 (2H, m, CH_2, cyclopropyl), 1.25−1.48 (5H, m, CH_2, cyclopropyl and CH_2CH_3), 2.63−2.75 (2H, m, CH_2, C-3), 2.78−2.87 (2H, m, CH_2, C-8), 3.10−3.30 (4H, m, CH_2, C-2 and C-7), 3.40−3.55 (1H, m, CH, cyclopropyl), 4.10 (2H, s, CH_2, C-5), 4.35 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.40 (1H, s, H-8), 8.55 (1H, s, H-2), 8.65 (1H, s, H-5).

Ethyl 1-Cyclopropyl-7-(3-methyl-4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (10b). Compound **10b** was obtained from **6**[26] and thiopyranopyridine **4b**; 30 h, 70 °C, 79%; mp 246.6−247.9 °C. ^{1}H NMR ($CDCl_3$): δ 1.10−1.20 (2H, m, CH_2, cyclopropyl), 1.30 (3H, d, J = 6.8 Hz, CH_3) 1.35−1.50 (5H, m, CH_2 cyclopropyl and CH_2CH_3), 2.50−2.60 (1H, m, CH, C-3), 2.65−2.95 (2H, m, CH_2, C-2), 3.05−3.20 (2H, m, CH_2, C-8), 3.22−3.35 (2H, m, CH_2, C-7),3.40−3.55 (1H, m, CH cyclopropyl), 4.05 and 4.15 (each 1H, d, J = 14.7 Hz, CH_2, C-5), 4.40 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.42 (1H, s, H-8), 8.55 (1H, s, H-2), 8.85 (1H, s, H-5).

Ethyl 1-Cyclopropyl-7-(4-hydroxy-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (10g). Compound **10g** was obtained from **6**[26] and thiopyranopyridine **4g**; 30 h, 70 °C, 86%; mp 205.1−206.8 °C. ^{1}H NMR ($CDCl_3$): δ 1.05−1.25 (2H, m, CH_2, cyclopropyl), 1.30−1.50 (5H, m, CH_2, cyclopropyl and CH_2CH_3), 1.90−2.20 (2H, m, CH_2, C-3), 2.30−2.60 (2H, m, CH_2, C-8), 2.70−2.85 (1H, m, CH, C-4), 3.00−3.35 (4H, m, CH_2, C-2 and C-7), 3.40−3.55 (1H, m, CH, cyclopropyl) 3.67 (1H, bs, OH), 4.00−4.25 (2H, m, CH_2, C-5), 4.40 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.30 (1H, s, H-8), 8.55 (1H, s, H-2), 8.75 (1H, s, H-5).

Ethyl 1-Cyclopropyl-8-methyl-6-nitro-4-oxo-7-(4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-1,4-dihydroquinoline-3-carboxylate (11a). Compound **11a** was obtained from **7**[23] and thiopyranopyridine **4a**; 10 days, 70 °C, CH_2Cl_2/Et_2O 70:30, 10%; mp 195.0−197.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.80−0.95 and 1.12−1.28 (each 2H, m, CH_2, cyclopropyl), 1.40 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.40−2.65 (5H, m, CH_2, C-8 and CH_3), 2.70−2.90 (2H, m, CH_2, C-2), 3.05−3.35 (4H, m, CH_2, C-3 and C-7), 3.85−4.10 (3H, m, CH, cyclopropyl and CH_2, C-5), 4.38 (2H, q, J = 7.1 Hz, CH_2CH_3), 8.60 (1H, s, H-5), 8.65 (1H, s, H-2).

Ethyl 1-Cyclopropyl-8-methyl-7-(3-methyl-4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (11b). Compound **11b** was obtained from **7**[23] and thiopyranopyridine **4b**; 10 days, 70 °C, $CHCl_3$/Et_2O 70:30, 14%; mp 187.0−188.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.15−1.22 and 1.25−1.35 (each 2H, m, CH_2, cyclopropyl), 1.40 (3H, d, J = 7.0 Hz CH_3), 1.41 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.42−2.60 (4H, m, CH, C-3 and CH_3), 2.65−2.85 (2H, m, CH_2, C-8), 3.00−3.22 (4H, m, CH_2, C-2 and C-7), 3.87−4.05 (3H, m, CH, cyclopropyl and CH_2, C-5), 4.38 (2H, q, J = 7.1 Hz, CH_2CH_3), 8.64 (1H, s, H-5), 8.66 (1H, s, H-2).

Ethyl 1-Cyclopropyl-7-(4-hydroxy-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-8-methyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (11g). Compound **11g** was obtained from **7**[23] and thiopyranopyridine **4g**; 7 days, 80 °C, 30%; mp 219.5−221.2 °C. ^{1}H NMR ($CDCl_3$): δ 0.80−1.00 and 1.10−1.30 (each 2H, m, CH_2, cyclopropyl), 1.41 (3H, t, J = 7.1 Hz, CH_2CH_3), 1.90−2.15 (2H, m, CH_2, C-8), 2.20−2.55 (2H, m, CH_2, C-3), 2.60−2.75 (5H, m, CH_2, C-2 and CH_3), 3.02−3.18 (2H, m, CH_2, C-7), 3.20−3.30 (1H, m, CH, cyclopropyl), 3.70 (1H, bs, OH), 3.80−3.95 (2H, m, CH_2, C-5), 4.05 (1H, s, CH, C-4), 4.40 (2H, q, J = 7.1 Hz, CH_2CH_3), 8.55 (H, s, H-5), 8.62 (H, s, H2).

Ethyl 6-Amino-1-cyclopropyl-4-oxo-7-(4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-1,4-dihydroquinoline-3-carboxylate (12a). Iron powder in a catalytic amount was

added to a solution of nitro derivative **10a** (0.20 g, 0.43 mmol) in acetic acid (15 mL) and warmed to 70 °C for 3 h. The reaction mixture was basified to pH 8−9 with NaOH 10% and extracted with EtOAc. The organic layers were combined, dried over Na_2SO_4, and concentrated to obtain a residue that was purified by flash column chromatography ($CHCl_3$/MeOH 90:10) to obtain 0.11 g (58.2%) of compound **12a** as a yellow solid; mp 246.0−247.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.05−1.28 (4H, m, CH_2, cyclopropyl), 1.37 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.60−2.70 (2H, m, CH_2, C-3), 2.75−2.90 (2H, m, CH_2, C-8), 3.15−3.30 (3H, m, CH cyclopropyl and CH_2, C-2), 3.40−3.55 (2H, m, CH_2, C-7), 3.80−3.95 (2H, m, CH_2, C-5), 4.35 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.45 (1H, s, H-8), 7.75 (1H, s, H-5), 8.45 (1H, s, H-2). Anal. ($C_{23}H_{25}N_3O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-(3-methyl-4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (12b). Compound **12b** was prepared according to the procedure for **12a** above, except that intermediate **10b** was used instead of **10a**, to give the title compound **12b** after 30 h; 53%; mp 228.0−230.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.10−1.50 (10H, m, CH_2, cyclopropyl, CH_3 and CH_2CH_3), 2.50−2.80 (3H, m, CH_2, C-2 and CH, C-3), 3.05−3.20 (2H, m, CH_2, C-8), 3.25−3.55 (3H, m, CH, cyclopropyl and CH_2, C-7), 3.80 and 3.95 (each 1H, d, J = 17.8 Hz, CH_2, C-5), 4.40 (2 H, q, J = 7.1 Hz, CH_2CH_3), 4.70 (2H, bs, NH_2), 7.48 (1H, s, H-8), 7.75 (1H, s, H-5), 8.52 (1H, s, H-2). Anal. ($C_{24}H_{27}N_3O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-(4-hydroxy-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (12g). A catalytic amount of Raney-Ni was added to a solution of nitro derivative **10g** (0.72 g, 1.53 mmol) in a mixture of EtOH (10 mL) and DMF (25 mL), and H_2 was bubbled for 20 min. The suspension was filtered over Celite to remove Raney-Ni, and the filtrate was evaporated to dryness under reduced pressure to obtain a residue that was purified by flash column chromatography ($CHCl_3$/MeOH 95:5) to give 0.35 g (52%) of compound **12g** as a yellow solid; mp 88.0−91.0 °C. ^{1}H NMR (DMSO-d_6): δ 0.92−1.03 (2H, m, CH_2, cyclopropyl), 1.12−1.30 (5H, m, CH_2, cyclopropyl and CH_2CH_3), 1.40−1.55 (2H, m, CH_2, C-3), 2.10−2.30 (2H, m, CH_2, C-8), 2.35−2.45 (2H, m, CH_2, C-2), 2.90−3.20 (2H, m, CH_2, C-7), 3.50−3.85 (3H, m, CH, cyclopropyl and CH_2, C-5), 4.15 (2H, q, J = 7.0 Hz, CH_2CH_3), 4.75 (1H, d, J = 4.4 Hz, OH), 5.05 (2H, s, NH_2), 5.75 (1H, bs, CH, C-4), 7.45 (1H, s, H-8), 7.50 (1H, s, H-5), 8.25 (1H, s, H-2). Anal. ($C_{23}H_{27}N_3O_4S$) C, H, N.

Following this procedure, starting from nitro derivatives **11a**, **11b**, and **11g**, amino derivatives **13a**, **13b**, and **13g** were obtained in reported yields.

Ethyl 6-Amino-1-cyclopropyl-8-methyl-4-oxo-7-(4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-1,4-dihydroquinoline-3-carboxylate (13a). One hour, 48%; mp 219.0−220.3 °C. ^{1}H NMR ($CDCl_3$): δ 0.75−0.95 and 1.00−1.20 (each 2H, m, CH_2, cyclopropyl), 1.37 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.25−2.55 (5H, m, CH_2, C-8 and CH_3), 2.70−2.82 (2H, m, CH_2, C-3), 3.00−3.50 (4H, m, CH_2, C-2 and C-7), 3.75−3.95 (3H, m, CH_2, C-5 and CH, cyclopropyl), 4.32 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.52 (1H, s, H-5), 8.52 (1H, s, H-2). Anal. ($C_{24}H_{27}N_3O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-8-methyl-7-(3-methyl-4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (13b). One hour, 42%; mp 233.8−235.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.75−0.95 (2H, m, CH_2, cyclopropyl), 1.00−1.33 (5H, m, CH_2, cyclopropyl, CH_3), 1.37 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.28−2.55 (4H, m, CH, C-3 and CH_3), 2.60−2.85 (2H, m, CH_2, C-8), 2.90−3.50 (4H, m, CH_2, C-2 and C-7), 3.70−4.05 (3H, m, CH_2, C-5 and CH, cyclopropyl), 4.35 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.54 (1H, s, H-5), 8.52 (1H, s, H-2). Anal. ($C_{25}H_{29}N_3O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-(4-hydroxy-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (13g). One hour, 41%; mp 289.4−290.8 °C. ^{1}H NMR (DMSO-d_6): δ 0.92−1.03 (2H, m, CH_2, cyclopropyl), 1.05−1.15 (5H, m, CH_2, cyclopropyl and CH_2CH_3), 1.85−2.15 (2H, m, CH_2, C-3), 2.50 (3H, s, CH_3), 2.60−2.75 (2H, m, CH_2, C-8), 2.80−3.05 (4H, m, CH_2, C-2 and C-7), 3.10−3.30 (3H, m, CH, cyclopropyl and CH_2, C-5), 4.15 (2H, q, J = 7.1 Hz, CH_2CH_3), 4.45−65 (1H, m, CH, C-4), 5.05−5.25 (1H, m, OH), 8.05 (1H, s, H-5), 8.55 (1H, s, H-2). Anal. ($C_{24}H_{29}N_3O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-[4-(hydroxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate (12c). A solution of derivative **12a** (0.18 g, 0.41 mmol) in MeOH (5 mL) was added to a mixture of NH_2OH hydrochloride (0.05 g, 0.66 mmol) and pyridine (0.07 g, 0.90 mmol) in MeOH (5 mL) and warmed to 60 °C for 6 h. Then, the reaction mixture was evaporated to dryness under reduced pressure, and the obtained residue was added to H_2O (10 mL) to obtain a precipitate that after filtration was purified by flash column chromatography (EtOAc/MeOH 95:5) to give 0.14 g (73%) of desired compound **12c** as a yellow solid; mp 267.5−269.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.05−1.40 (7H, m, CH_2, cyclopropyl and CH_2CH_3), 2.35−2.45 (2H, m, CH_2, C-3), 2.80−3.05 (4H, m, CH_2, C-2 and C-8), 3.15−3.45 (3H, m, CH_2, C-7 and CH, cyclopropyl), 3.85 (2H, s, C-5), 4.20 (2H, q, J = 7.2 Hz, CH_2CH_3), 5.30 (2H, bs, NH_2) 7.45 (2H, s, H-8 and H-5), 8.35 (1H, s, H-2), 11.00 (1H, bs, NOH). Anal. ($C_{23}H_{26}N_4O_4S$) C, H, N.

Following this procedure, starting from derivative **12b**, was obtained compound **12d**, and from derivatives **13a**,**b** were obtained compounds **13c**,**d**. Using NH_2OCH_3 hydrochloride instead NH_2OH hydrochloride, methoxyiminemethoxyimino derivatives **12e**,**f** and **13e**,**f** were obtained from compounds **12a**,**b** and **13a**,**b**, respectively.

Ethyl 6-Amino-1-cyclopropyl-7-[4-(hydroxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate (12d). Forty-eight hours, 70%, mp 259.3−261.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.02−1.35 (10H, m, CH_2, cyclopropyl, CH_3 and CH_2CH_3), 2.05−2.25 (2H, m, CH_2, C-8), 2.65−3.20 (3H, m, CH_2, C-2 and CH, C-3), 3.40−3.75 (5H, m, CH_2, C-5, C-7 and CH, cyclopropyl), 4.15 (2 H, q, J = 6.9 Hz, CH_2CH_3), 5.02 (2H, bs, NH_2) 7.40 (2H, s, H-8, H-5), 8.25 (1H, s, H-2), 10.95 (1H, bs, NOH). Anal. ($C_{24}H_{28}N_4O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-[4-(hydroxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (13c). Three hours, 41%; mp 215.0−217.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.75−1.30 (4H, m, CH_2, cyclopropyl), 1.40 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.15−2.70 (5H, m, CH_2, C-3 and CH_3), 2.80−3.20 (4H, m, CH_2, C-2 and C-8), 3.40−3.70 (2H, m, CH_2, C-7), 3.78−4.00 (3H, m, CH_2, C-5 and CH, cyclopropyl), 4.05−4.45 (4H, m, CH_2CH_3 and NH_2), 7.52 (1H, s, H-5), 8.52 (1H, s, H-2), 9.40 (1H, bs, NOH). Anal. ($C_{24}H_{28}N_4O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-[4-(hydroxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (13d). Twenty-four hours, 56%; mp 204.0−205.0 °C. ^{1}H NMR ($CDCl_3$): δ 0.75−1.30 (7H, m, CH_2, cyclopropyl and CH_3), 1.40 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.18−2.80 (6H, m, CH_2, C-8, CH, C-3 and CH_3), 3.12−3.35 (2H, m, CH_2, C-2), 3.40−3.60 (2H, m, CH_2, C-7), 3.72−4.28 (5H, m, CH_2, C-5, NH_2 and CH, cyclopropyl), 4.38 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.58 (1H, s, H-5), 7.70−7.90 (1H, bs, NOH), 8.56 (1H, s, H-2). Anal. ($C_{25}H_{30}N_4O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-[4-(methoxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate (12e). Twelve hours, 79%; mp > 300 °C. ^{1}H NMR ($CDCl_3$): δ 1.00−1.30 (4H, m, CH_2, cyclopropyl) 1.45 (3H, t, J = 6.9 Hz, CH_2CH_3), 2.90−3.05 (4H, m, CH_2, C-3 and C-8), 3.25 (2H, m, CH_2, C-2), 3.35−3.75 (3H, m, CH_2, C-7 and CH, cyclopropyl), 3.80−4.05 (5H, m, CH_2, C-5 and OCH_3), 4.40 (2H, q, J = 6.9 Hz, CH_2CH_3), 5. 95 (2H, bs, NH_2),7.45 (1H, s, H-8), 7.75 (H, s, H-5), 8.50 (H, s, H-2). Anal. ($C_{24}H_{28}N_4O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-[4-(methoxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate (12f). Forty-eight hours,

62%; mp 227.6−228.9 °C. ^{1}H NMR ($CDCl_3$): δ 0.95−1.05 (2H, m, CH_2, cyclopropyl), 1.10−1.25 (5H, m, CH_2, cyclopropyl and CH_3), 1.32 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.13−2.50 (2H, m, CH_2, C-8), 2.55−3.20 (3H, m, CH, C-3 and CH_2, C-2), 3.30−3.45 (2H, m, CH_2, C-7), 3.55−3.70 (3H, m, CH_2, C-5 and CH, cyclopropyl), 3.75 (3H, s, OCH_3), 4.30 (2 H, q, J = 7.1 Hz, CH_2CH_3), 6.17 (2H, bs, NH_2), 7.40 (1H, s, H-8), 7.68 (1H, s, H-5), 8.40 (1H, s, H-2). Anal. ($C_{25}H_{30}N_4O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-[4-(methoxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (13e). Twelve hours, 46%; mp > 300 °C. ^{1}H NMR ($CDCl_3$): δ 0.65−1.20 (4H, m, CH_2, cyclopropyl), 1.31 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.10−2.60 (5H, m, CH_2, C-3 and CH_3), 2.65−3.00 (2H, m, CH_2, C-8), 3.05−3.60 (4H, m, CH_2, C-2 and C-7), 3.65−3.95 (6H, m, CH_2, C-5, OCH_3 and CH, cyclopropyl) 4.30 (2 H, q, J = 7.1 Hz, CH_2CH_3), 7.50 (H, s, H-5), 8.50 (H, s, H-2). Anal. ($C_{25}H_{30}N_4O_4S$) C, H, N.

Ethyl 6-Amino-1-cyclopropyl-7-[4-(methoxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (13f). Twenty-four hours, 23%; mp 234.4−235.7 °C. ^{1}H NMR ($CDCl_3$): δ 0.75−1.30 (7H, m, CH_2, cyclopropyl and CH_3), 1.40 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.05−2.18 (1H, m, CH, C-3), 2.45−2.70 (5H, m, CH_2, C-8 and CH_3), 3.05−3.30 (2H, m, CH_2, C-2), 3.35−3.75 (2H, m, CH_2, C-7), 3.72−4.28 (6H, m, CH_2, C-5, OCH_3 and CH, cyclopropyl), 4.38 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.58 (1H, s, H-5), 7.70−7.90 (2H, bs, NH_2), 8.56 (1H, s, H-2). Anal. ($C_{26}H_{32}N_4O_4S$) C, H, N.

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-1,4-dihydroquinoline-3-carboxylic Acid (9a). A mixture of ester **8a** (0.10 g, 0.23 mmol) in 6 N HCl (2 mL) and EtOH (5 mL) was refluxed under stirring for 3 h. After the mixture was cooled, the yellow precipitate was separated by filtration and washed with Et_2O to obtain pure compound **9a** as a yellow solid 0.03 g (35%); mp 270.0−272.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.00−1.25 and 1.28−1.45 (each 2H, m, CH_2 cyclopropyl), 2.60−2.65 (2H, m, CH_2, C-8), 2.70−2.85 (2H, m, CH_2, C-3), 3.10−3.20 (2H, m, CH_2, C-2), 3.35−3.65 (3H, m, CH, cyclopropyl, CH_2, C-7), 4.05 (2H, s, CH_2, C-5), 7.32 (1H, d, J = 7.6 Hz, H-8), 7.95 (1H, d, J = 13.6 Hz, H-5), 8.70 (1H, s, H-2), 15.00 (1H, s, COOH). ($C_{21}H_{19}FN_2O_4S$) C, H, N.

1-Cyclopropyl-6-fluoro-7-(3-methyl-4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (9b). The suspension of ester **8b** (0.10 g, 0.22 mmol) in 4% NaOH (2.0 mL) was refluxed under stirring for 3 h. After it was cooled, the solution was acidified with 2 N HCl until pH ≅ 5 to obtain a precipitate that after filtration was crystallized from EtOH to give 0.03 g of compound **9b** (yield, 32%) as a pale yellow solid; mp 236.0−238.0 °C. ^{1}H NMR ($CDCl_3$): δ 1.08−1.24 (2H, m, CH_2, cyclopropyl), 1.27 (3H, d, J = 6.8 Hz, CH_3), 1.33−1.45 (2H, m, CH_2, cyclopropyl), 2.50−2.80 (3H, m, CH, C-3 and CH_2, C-2), 3.00−3.25 (2H, m, CH_2, C-8), 3.40−3.55 (2H, m, CH_2, C-7), 3.60−3.80 (1H, m, CH, cyclopropyl), 4.05 and 4.20 (each 1H, d, J = 16.0 Hz, CH_2, C-5), 7.45 (1H, d, J = 7.3 Hz, H-8), 8.07 (1H, d, J = 13.3 Hz, H-5), 8.62 (1H, s, H-2), 14.95 (1H, bs, COOH). Anal. ($C_{22}H_{21}FN_2O_4S$) C, H, N.

Following this procedure, starting from ethyl esters **8c−e**, acid derivatives **9c−e** were obtained in reported yields.

1-Cyclopropyl-6-fluoro-7-[4-(hydroxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (9c). Two hours, 41%; mp 274.0−276.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.15−1.30 (4H, m, CH_2, cyclopropyl), 2.40−2.50 (2H, m, CH_2, C-3), 2.75−3.00 (4H, m, CH_2, C-2 and C-8), 3.55−3.65 (2H, m, CH_2, C-7), 3.70−3.85 (1H, m, CH, cyclopropyl), 4.20 (2H, s, CH_2, C-5), 7.30 (1H, d, J = 10.0 Hz, H-8), 7.95 (1H, d, J = 13.4 Hz, H-5), 8.65 (1H, s, H-2), 11.10 (1H, s, NOH), 15.20 (1H, s, COOH). Anal. ($C_{21}H_{20}FN_3O_4S$) C, H, N.

1-Cyclopropyl-6-fluoro-7-[4-(hydroxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (9d). Four hours, 39%; mp 257.0−258.0 °C. ^{1}H NMR (DMSO-d_6): δ 0.95−1.10 (7H, m, cyclopropyl and CH_3), 2.15−2.35 (1H, m, CH, C-3), 2.55−2.80 (2H, m, CH_2, C-8), 3.20−3.40 (2H, m, CH_2, C-2), 3.55−3.80 (3H, m, CH, cyclopropyl and CH_2, C-7), 3.90 and 4.25 (each 1H, d, J = 16.7 Hz, CH_2, C-5), 7.40 (1H, d, J = 7.5 Hz, H-8), 7.8 (1H, d, J = 13.6 Hz, H-5), 8.65 (1H, s, H-2), 11.05 (1H, s, NOH), 15.15 (1H, s, COOH). Anal. ($C_{22}H_{22}FN_3O_4S$) C, H, N.

1-Cyclopropyl-6-fluoro-7-[4-(methoxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (9e). Thirty hours, 25%; mp 235.0−238.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.00−1.30 (4H, m, CH_2, cyclopropyl), 2.75−3.00 (4H, m, CH_2, C-3 and C-8), 3.10−3.25 (2H, m, CH_2, C-2), 3.45−3.70 (3H, m, CH, cyclopropyl and CH_2, C-7), 3.80 (3H, s, OCH_3), 4.10 (2H, s, CH_2, C-5), 7.45 (1H, d, J = 6.9 Hz, H-8), 7.80 (1H, d, J = 13.3 Hz, H-5), 8.55 (H, s, H-2). Anal. ($C_{22}H_{22}FN_3O_4S$) C, H, N.

1-Cyclopropyl-6-fluoro-7-[4-(methoxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (9f). Solid $LiOH \cdot H_2O$ (0.02 g, 0.41 mmol) was added to a solution of ester **8f** (0.10 g, 0.21 mmol) in a mixture of dioxane (6 mL) and H_2O (2 mL) and stirred at room temperature for 4 h. The mixture was evaporated to dryness under reduced pressure, and H_2O (5 mL) was added and acidified with AcOH until pH 4.5−5 to obtain a precipitate that was filtered, dried, and crystallized from EtOH to give 0.06 g (62.5%) of compound **9f** as a pale yellow solid; mp 177.0−178.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.00−1.30 (7H, m, CH_2, cyclopropyl and CH_3), 2.17−2.35 (1H, m, CH, C-3), 2.65 and 3.10 (each 1H, dd, J_1 = 13.5, J_2 = 2.9 Hz, CH_2, C-2), 3.15−3.40 (2H, m, CH_2, C-8), 3.45−3.55 (1H, m, CH cyclopropyl), 3.60−3.80 (5H, m, CH_2, C-7 and OCH_3), 3.85 and 4.20 (each 1H, d, J = 16.5, CH_2, C-5), 7.40 (H, d, J = 7.6 Hz, H-8), 7.80 (H, d, J = 13.4 Hz, H-5), 8.50 (H, s, H-2), 15.10 (H, s, COOH). Anal. ($C_{23}H_{24}FN_3O_4S$) C, H, N.

The acid **9g** was obtained following this procedure starting from the ethyl ester derivative **8g**; likewise, **14a−g** and **15a−g** were obtained from derivatives **12a−g** and **13a−g**.

1-Cyclopropyl-6-fluoro-7-(4-hydroxy-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic (9g). Two hours, 24%; mp 240.1−241.9 °C. ^{1}H NMR (DMSO-d_6): δ 0.85−1.05 (4H, m, CH_2, cyclopropyl), 1.55−160 (2H, m, CH_2, C-3), 2.09−2.25 (2H, m, CH_2, C-8), 2.45−2.52 (2H, m, CH_2, C-2), 3.45−3.55 (1H, m, CH, cyclopropyl), 3.80−3.92 (3H, m, CH, C-4 and CH_2, C-7), 5.55 and 5.65 (each 1H, s, CH_2, C-5), 7.35 (1H, d, J = 7.3 Hz, H-8), 7.60 (1H, d, J = 13.3 Hz, H-5), 8.40 (1H, s, H-2), 11.60 (1H, bs, OH) 14.95 (H, bs, COOH). Anal. ($C_{21}H_{21}FN_2O_4S$) C, H, N.

6-Amino-1-cyclopropyl-4-oxo-7-(4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-1,4-dihydroquinoline-3-carboxylic Acid (14a). Four hours, 31%; mp 308.0−310.0 °C. ^{1}H NMR (DMSO-d_6): δ 0.90−1.20 (4H, m, CH_2, cyclopropyl), 2.50−2.72 (4H, m, CH_2, C-3 and C-8), 3.05−3.35 (4H, m, CH_2, C-2 and C-7), 3.60−3.80 (3H, m, CH, cyclopropyl and CH_2, C-5), 5.40 (2H, bs, NH_2), 7.42 (1H, s, H-8), 7.50 (1H, s, H-5), 8.40 (1H, s, H-2), 15.78 (1H, bs, COOH). Anal. ($C_{21}H_{21}N_3O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-(3-methyl-4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (14b). Four hours, 46%; mp 243.0−244.0 °C. ^{1}H NMR (DMSO-d_6): δ 1.05−1.32 (7H, m, CH_2, cyclopropyl, CH_3), 2.60−2.90 (3H, m, CH, C-3 and CH_2, C-2), 3.05−3.35 (5H, m, CH, cyclopropyl and CH_2, C-7 and C-8), 3.70−3.95 (2H, m, CH_2, C-5), 5.45 (2H, bs, NH_2), 7.55 (1H, s, H-8), 7.60 (1H, s, H-5), 8.52 (1H, s, H-2), 15.88 (1H, bs, COOH). Anal. ($C_{22}H_{23}N_3O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-[4-(hydroxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (14c). Four hours, 51%; mp 270.8−272.4 °C. ^{1}H NMR (DMSO-d_6): δ 1.02−1.32 (4H, m, CH_2, cyclopropyl), 2.45−2.55 (2H, m, CH_2, C-3), 2.75−3.05 (4H, m, CH_2, C-2 and C-8), 3.22−3.40 (2H, m, CH_2, C-7), 3.62−3.77 (1H, m, CH,

cyclopropyl), 3.85−4.00 (2H, m, CH_2, C-5), 5.50 (2H, bs, NH_2), 7.48 (1H, s, H-8), 7.55 (1H, s, H-5), 8.48 (1H, s, H-2), 11.05 (1H, bs, NOH), 15.80 (1H, bs, COOH). Anal. ($C_{21}H_{22}N_4O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-[4-(hydroxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (14d). Four hours, 31%; mp 245.7−246.7 °C. ^{1}H NMR (DMSO-d_6): δ 0.95−1.25 (7H, m, CH_2, cyclopropyl and CH_3), 2.05−2.35 (2H, m, CH_2, C-8), 2.45−2.55 (1H, m, CH, C-3), 2.60−2.70 (2H, m, CH_2, C-2), 2.90−3.20 (2H, m, CH_2, C-7), 3.30−3.45 (1H, m, CH, cyclopropyl), 3.55−3.75 (2H, m, CH_2, C-5), 5.35 (2H, bs, NH_2), 7.40 (1H, bs, H-8), 7.50 (1H, bs, H-5), 8.40 (1H, s, H-2), 10.95 (1H, bs, NOH), 15.80 (1H, bs, COOH). Anal. ($C_{22}H_{24}N_4O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-[4-(methoxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (14e). Four hours, 82%; mp 224.1−225.9 °C. ^{1}H NMR (DMSO-d_6): δ 1.08−1.28 (4H, m, CH_2, cyclopropyl), 2.30−2.45 (2H, m, CH_2, C-3), 2.75−2.95 (4H, m, CH_2, C-2 and C-8), 3.20−3.30 (2H, m, CH_2, C-7), 3.68−3.88 (6H, m, CH_2, C-5, CH cyclopropyl, OCH_3), 5.48 (2H, bs, NH_2), 7.50 (1H, s, H-8), 7.60 (1H, s, H-5), 8.45 (1H, s, H-2), 15.82 (H, bs, COOH). Anal. ($C_{22}H_{24}N_4O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-[4-(methoxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (14f). Four hours, 46%; mp 152.0−154.0 °C. ^{1}H NMR (DMSO-d_6): δ 0.88−1.25 (7H, m, CH_2, cyclopropyl and CH_3), 2.10−2.78 (3H, m, CH, C-3 and CH_2, C-8), 2.90−3.20 (2H, m, CH_2, C-2), 3.20−3.45 (1H, m, CH, cyclopropyl), 3.50−3.65 (2H, m, CH_2, C-7), 3.70−4−05 (5H, m, CH_2, C-5 and OCH_3) 5.35 (2H, bs, NH_2), 7.42 (1H, s, H-8), 7.51 (1H, s, H-5), 8.42 (1H, s, H-2), 15.80 (1H, bs, COOH). Anal. ($C_{23}H_{26}N_4O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-(4-hydroxy-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (14g). Two hours, 45%; mp 173.7−175.0 °C. ^{1}H NMR (DMSO-d_6): δ 0.92−1.03 (4H, m, CH_2, cyclopropyl), 1.15−1.25 (2H, m, CH_2, C-3), 1.40−1.55 (2H, m, CH_2, C-8), 1.85−2.10 (2H, m, CH_2, C-2), 2.70−3.05 (2H, m, CH_2, C-7), 3.25 and 3.40 (each 1H, d, J = 14.7 Hz, CH_2, C-5), 3.45−3.55 (1H, m, CH, cyclopropyl), 4.45 (1H, bs, CH, C-4), 5.05 (2H, s, NH_2), 5.45 (1H, s, OH), 7.20 (1H, s, H-8), 7.30 (1H, s, H-5), 8.25 (1H, s, H-2), 15.70 (1H, bs, COOH). Anal. ($C_{21}H_{23}N_3O_4S$) C, H, N.

6-Amino-1-cyclopropyl-8-methyl-4-oxo-7-(4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-1,4-dihydroquinoline-3-carboxylic Acid (15a). Three hours, 29%; mp 198.3−199.5 °C. ^{1}H NMR (DMSO-d_6): δ 0.60−1.00 and 1.00−1.25 (each 2H, m, CH_2, cyclopropyl), 2.27−2.82 (7H, m, CH_2, C-3, C-8 and CH_3), 2.90−3.45 (4H, m, CH_2, C-2, C-7), 3.55−3.70 (1H, m, CH, cyclopropyl), 4.15−4.45 (2H, m, CH_2, C-5), 5.30 (2H, bs, NH_2) 7.40 (1H, s, H-5), 8.55 (1H, s, H-2), 15.60 (1H, bs, COOH). Anal. ($C_{22}H_{23}N_3O_4S$) C, H, N.

6-Amino-1-cyclopropyl-8-methyl-7-(3-methyl-4-oxo-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (15b). Three hours, 27%; mp 239.0−240.7 °C. ^{1}H NMR (DMSO-d_6): δ 0.62−1.20 (7H, m, CH_2, cyclopropyl and CH_3), 2.20−2.75 (6H, m, CH, C-3, CH_2, C-2 and CH_3), 2.90−3.40 (4H, m, CH_2, C-7, C-8), 3.50−3.65 (1H, m, CH, cyclopropyl) 3.70−4.20 (2H, m, CH_2, C-5), 5.20 (2H, bs, NH_2) 7.28 (1H, s, H-5), 8.52 (1H, s, H-2), 15.50 (1H, bs, COOH). Anal. ($C_{23}H_{25}N_3O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-[4-(hydroxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (15c). Three hours, 40%; mp 247.2−248.6 °C. ^{1}H NMR (DMSO-d_6): δ 0.77−0.94 and 1.08−1.24 (each 2H, m, CH_2, cyclopropyl), 2.10−2.30 (2H, m, CH_2, C-2), 2.55 (3H, s, CH_3), 2.82−3.27 (4H, m, CH_2, C-3 and C-8) 3.30−3.75 (3H, m, CH_2, C-7 and CH, cyclopropyl), 4.05−4.30 (2H, m, CH_2, C-5), 5.30 (2H, bs, NH_2), 7.40 (1H, s, H-5), 8.60 (1H, s, H-2), 11.00 (1H, bs, NOH), 15.60 (1H, bs, COOH). Anal. ($C_{22}H_{24}N_4O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-[4-(hydroxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (15d). Three hours, 58%; mp 251.4−252.5 °C. ^{1}H NMR (DMSO-d_6): δ 0.75−1.00 (2H, m, CH_2, cyclopropyl), 1.08−1.23 (5H, m, CH_2, cyclopropyl and CH_3), 2.10−2.20 (1H, m, CH, C-3), 2.55−2.80 (5H, m, CH_2, C-8 and CH_3), 3.17−3.35 (2H, m, CH_2, C-2), 3.40−3.80 (4H, m, CH_2, C-5 and C-7), 4.25−4.32 (1H, m, CH, cyclopropyl), 5.28 (2H, bs, NH_2) 7.40 (1H, s, H-5), 8.61 (1H, s, H-2), 10.82 (1H, bs, NOH), 15.60 (1H, bs, COOH). Anal. ($C_{23}H_{26}N_4O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-[4-(methoxyimino)-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (15e). Three hours, 28%; mp 280.2−282.0 °C. ^{1}H NMR (DMSO-d_6): δ 0.60−1.22 (4H, m, CH_2, cyclopropyl), 2.00−2.72 (7H, m, CH_2, C-3, C-8 and CH_3), 3.05−3.60 (7H, m, CH_2, C-2, C-7 and OCH_3), 3.70−3.85 (2H, m, CH_2, C-5), 4.00−4.15 (1H, m, CH, cyclopropyl), 5.20 (2H, bs, NH_2), 7.28 (1H, s, H-5), 8.53 (1H, s, H-2), 15.50 (1H, bs, COOH). Anal. ($C_{23}H_{26}N_4O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-[4-(methoxyimino)-3-methyl-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (15f). Three hours, 40%; mp 224.0−225.3 °C. ^{1}H NMR (DMSO-d_6): δ 0.60−1.22 (7H, m, CH_2, cyclopropyl and CH_3), 2.00−2.72 (6H, m, CH, C-3, CH_2, C-8 and CH_3), 3.05−3.25 (2H, m, CH_2, C-2), 3.30−3.90 (7H, m, CH_2, C-5, C-7 and OCH_3), 4.12−4.28 (1H, m, CH, cyclopropyl), 5.20 (2H, bs, NH_2) 7.28 (1H, s, H-5), 8.53 (1H, s, H-2), 15.50 (1H, bs, COOH). Anal. ($C_{24}H_{28}N_4O_4S$) C, H, N.

6-Amino-1-cyclopropyl-7-(4-hydroxy-3,4,7,8-tetrahydro-2*H*-thiopyrano[3,2-*c*]pyridin-6(5*H*)-yl)-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (15g). Eight hours, 18%; mp 165.2−166.9 °C. ^{1}H NMR (DMSO-d_6): δ 0.92−1.03 (4H, m, CH_2, cyclopropyl), 1.15−1.25 (2H, m, CH_2, C-3), 1.40−1.55 (2H, m, CH_2, C-8), 1.85−2.10 (2H, m, CH_2, C-2), 2.70−3.05 (5H, m, CH_2, C-7e CH_3), 3.25 and 3.40 (each 1H, d, J = 14.7 Hz, CH_2, C-5), 3.45−3.55 (1H, m, CH, cyclopropyl), 4.45 (1H, s, OH), 5.05 (2H, s, NH_2), 5.40−5.50 (1H, m, CH, C-4), 7.30 (1H, s, H-5), 8.25 (1H, s, H-2), 15.70 (1H, bs, COOH). Anal. ($C_{22}H_{25}N_3O_4S$) C, H, N.

Ethyl 1-Cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,4-dihydroquinoline-3-carboxylate (CPXE).[31] A mixture of compound **5**[30] (0.30 g, 1.02 mmol), piperazine (0.29 g, 3.36 mmol), and Et_3N (0.17 mL, 1.23 mmol) in dry *N*-methylpyrrolidinone (6.0 mL) and *t*-butanol (0.8 mL) was warmed to 60 °C for 12 h. Then, the reaction mixture was evaporated to dryness under reduced pressure to obtain a residue that was purified by flash column chromatography (CH_2Cl_2/MeOH 90:10) to give 0.26 g (70.2%) of the desired compound as a white solid; mp 211.2−212.7 °C. ^{1}H NMR (DMSO-d_6): δ 1.05−1.15 (2H, m, CH_2, cyclopropyl), 1.22−1.35 (5H, m, CH_2, cyclopropyl and CH_2CH_3), 2.85−2.98 (4H, m, CH_2, piperazine), 3.13−3.22 (4H, m, CH_2, piperazine), 3.62−3.73 (1H, m, CH, cyclopropyl), 4.23 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.44 (1H, d, J = 7.4 Hz, H-8), 7.76 (1H, d, J = 13.7 Hz, H-5), 8.45 (1H, s, H-2). Anal. ($C_{19}H_{22}FN_3O_3$) C, H, N.

Supporting Information Available: Elemental analysis data for intermediate esters and final compounds. ^{1}H−^{1}H 2D NOESY NMR spectral data for methoxyimines **4e**,**f**. Properties of the PLS model for the 16 quinolone esters. This material is available free of charge via the Internet at http://pubs.acs.org.

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