Journal of Medicinal Chemistry

Brief Article

pubs.acs.org/jmc

Pyrazolo[4,3-*c*][1,2]benzothiazines 5,5-Dioxide: A Promising New Class of *Staphylococcus aureus* NorA Efflux Pump Inhibitors

Stefano Sabatini,*[,†] Francesca Gosetto,[†] Serena Serritella,[†] Giuseppe Manfroni,[†] Oriana Tabarrini,[†] Nunzio Iraci,[†] Jean Pierre Brincat,[‡] Emanuele Carosati,[‡] Milena Villarini,[§] Glenn W. Kaatz,[∥] and Violetta Cecchetti[†]

[†]Dipartimento di Chimica e Tecnologia del Farmaco, [‡]Dipartimento di Chimica, and [§]Dipartimento di Specialità Medico-Chirurgiche e Sanità Pubblica, Università degli Studi di Perugia, 06123 Perugia, Italy

[∥]Department of Internal Medicine, Division of Infectious Diseases, School of Medicine, Wayne State University and the John D. Dingell Department of Veteran Affairs Medical Center, Detroit, Michigan 48201, United States

Supporting Information

ABSTRACT: The increasing resistance to antibacterials commonly employed in the clinic and the growth of multidrug resistant strains suggest that the development of new therapeutic approaches should be of primary concern. In this context, EPIs may restore life to old drugs. In the present work, the EPI activity of the COX-2 inhibitor celecoxib was confirmed and a new class of pyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide analogues acting as inhibitors of the *Staphylococcus aureus* NorA multidrug efflux pump was identified.

INTRODUCTION

Bacterial resistance to clinically important antibacterial agents represents a crucial and worldwide health care problem.[1] New strategies are needed to combat resistant pathogens and to avoid the increasing prevalence of multidrug resistance (MDR) bacteria.

The main mechanisms whereby the bacteria develop resistance to antimicrobial agents include enzymatic inactivation,[2] modification of the drug target(s),[3] and reduction of intracellular drug concentration by changes in membrane permeability[4] or by the overexpression of efflux pumps.[5] With respect to efflux pumps, these membrane-based proteins provide a self-defense mechanism by which antibiotics, made by other microbes 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.[6] Therefore, efflux pumps are viable antibacterial targets and identification and development of potent efflux pump inhibitors (EPIs) is a promising and valid strategy[7] which can restore the susceptibility of resistant strains to antibacterial agents that are substrates of efflux pumps.[8]

Among the multidrug resistant bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare-associated infections (HAIs) in the EU. In 2008, MRSA accounted for 44% (n = 171200) of HAIs and 22% (n = 5400) of attributable extra deaths.[9] In the U.S., infections caused by MRSA occur in approximately 94000 persons each year and are associated with about 19000 deaths.[10] This high death rate is due to the ability of MRSA to acquire resistance to many antibacterials such as tetracyclines, aminoglycosides, and fluoroquinolones. Moreover, since 2002, vancomycin resistant strains have also been identified.[11] These are just as deadly as MRSA but more challenging to treat. In a recent work, the increased expression of one or more MDR efflux pump genes was identified in 151/309 *S. aureus* clinical strains (49%) by quantitative reverse transcription-PCR. Among those over-expressing a single gene, *norA* was most common (43%), followed by *norB* (23.2%) and *mepA* (9.9%).[12]

The most studied efflux pump of *S. aureus* is NorA, a transporter belonging to the major facilitator superfamily (MFS).[13] MFS pumps such as NorA are capable of extruding multiple, structurally dissimilar substrates such as hydrophilic fluoroquinolones, various biocides, and dyes.[14] To date, the structural biology of NorA has not been determined. Nevertheless, the sequence homology and the sharing of different substrates with other MDR pumps have led to the hypothesis that NorA may have a large hydrophobic binding site. This structural peculiarity could explain the broad substrate specificity of MDR pumps.[15]

In recent years, many EPIs capable of potentiating the activity of antimicrobial substrates have been identified.[16] To date, there are only a few examples of rationally designed inhibitors and very little is known with respect to the structure−activity relationship (SAR) of NorA inhibitors.[16−18] 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 with secure economic benefit.[19]

DESIGN RATIONALE

It has been recently reported that celecoxib, a cyclooxygenase-2 (COX-2) specific inhibitor, not only helps the reversal of drug resistance in cancers by inhibiting the multidrug resistance protein 1 (MDR1 or P-gp1) efflux pump but also increases the sensitivity of *S. aureus* strains to different antibacterials

Received: October 26, 2011
Published: March 20, 2012

(ampicillin, kanamycin, chloramphenicol, and ciprofloxacin).[20] Taking this evidence into account, we decided to bring to light as possible EPIs a focused in-house library of more than 150 compounds, previously designed and synthesized as celecoxib analogues. All these molecules, endowed with low, if any, anti-inflammatory effect, bear the 1,4-dihydropyrazolo[4,3-*c*]-benzothiazine 5,5-dioxide nucleus, variably functionalized in different positions. We then used our previously reported NorA in silico model for ethidium bromide (EtBr) efflux inhibition,[17] which employs a virtual screening procedure based on the program Fingerprints for Ligands And Proteins (FLAP),[21] to select a focused subset of our library (see Supporting Information (SI) for the criteria). Given the peculiar chemical space covered by the model,[17] the greater part of the library molecules were lying out of the domain of the in silico model and were not considered further. However, 17 molecules (**3a**−**i**, **4a**−**g**, and **4i**) (Scheme 1) were considered to be within the chemical space of the model. An activity prediction for these molecules was obtained from the FLAP pharmacophoric model: three compounds (**3b**, **3c**, and **3g** (Figure 1)) were

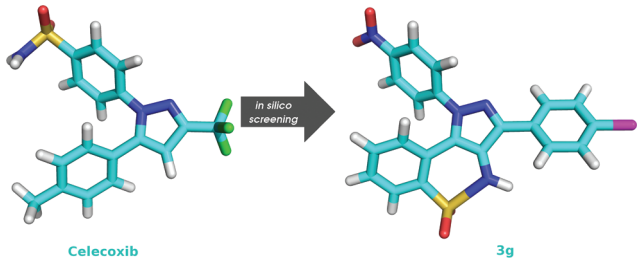
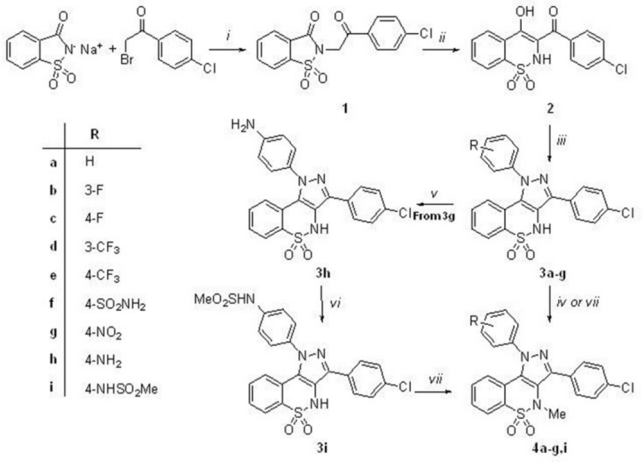


Figure 1. From celecoxib to the 3-(4-chlorophenyl)-1-(4-nitrophenyl)-1,4-dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide (**3g**).

predicted to be active NorA inhibitors, whereas 12 (**3d**−**f**, **3h**, **3i**, **4b**−**g**, and **4i**) were predicted as uncertain and the remaining two molecules (**3a**, and **4a**) were predicted to be not inhibitors (Scheme 1 and SI)

Scheme 1. Synthesis of the 1,4-Dihydropyrazolo[4,3-*c*]benzothiazine 5,5-Dioxide Derivatives[a]



[a]Reagents and conditions: (i) dry DMSO, 70 °C; (ii) EtONa, EtOH, 60 °C; (iii) phenylhydrazine hydrochloride, H_2SO_4, EtOH, reflux; (iv) MeI, NaH, dry DMF, rt; (v) Raney-Ni/hydrazine monohydrate, EtOH, 0 °C to rt; (vi) $MeSO_2Cl$, dry CH_2Cl_2, dry pyridine, 0 °C; (vii) MeI, K_2CO_3, dry DMF, rt.

CHEMISTRY

The synthesis of the 3-(4-chlorophenyl)pyrazolbenzothiazines **3a**−**i** and their corresponding N-4 methyl derivatives **4a**−**g** and **4i** was performed by following the synthetic strategy depicted in Scheme 1. Starting from saccharin sodium salt and 2-bromo-1-(4-chlorophenyl)ethanone, using dry DMSO as solvent and heating at 70 °C, the 2-[2-(4-chlorophenyl)-2-oxoethyl]-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide **1** was obtained in good yield (84%). A subsequent ring expansion with a mixture of EtONa/EtOH at 60 °C gave the (4-chlorophenyl)(4-hydroxy-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)methanone **2** in a 80% yield. Condensation of the key intermediate **2** with the substituted phenylhydrazines hydrochloride in refluxing EtOH, adding H_2SO_4 conc as dehydrating agent, provided the target compounds **3a**−**g** in 51−68% yield.

Finally, alkylation of compounds **3a**−**e** and **3g** was performed in dry DMF using NaH and MeI to give the corresponding N-4 methyl derivatives **4a**−**e** and **4g**. A mild alkylation of compound **3f** using K_2CO_3 as a base instead of NaH to avoid the alkylation of the exocyclic sulphonamide group gave the N-4 monoalkylated derivative **4f**.

Catalytic reduction of the nitro derivative **3g** using Raney-Ni/hydrazine monohydrate in absolute EtOH gave the corresponding amino compound **3h** in 68% yield. Compound **3h**, reacting with $MeSO_2Cl$, in a mixture of dry CH_2Cl_2 and dry pyridine, gave the mesylated derivative **3i** which was then methylated with MeI and K_2CO_3 in dry DMF to obtain the corresponding N-4 monoalkylated derivative **4i**.

RESULTS AND DISCUSSION

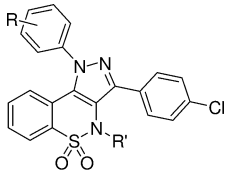
By analyzing the predictions obtained with our previously reported pharmacophoric model developed for NorA inhibitory activity,[17] a preference emerged for structures containing the NH versus the NMe in the benzothiazine scaffold. In fact, all the library structures containing the N-4 methyl group were predicted by the model to have either uncertain (**4b**−**g** and **4i**) or no (**4a**) EPI activity. Furthermore, all the compounds predicted to be inhibitors have a *para*-nitro group (**3g**) or a *meta*- or *para*-fluorine (**3b** and **3c**, respectively). To increase the diversity of the set of compounds to test, three additional compounds from among those predicted to have uncertain EPI activity were evaluated (**4d**, **3f**, and **3i**). Derivative **4d** was selected for in vitro screening among the four compounds carrying the *meta*- (**3d** and **4d**) or *para*-CF_3 (**3e** and **4e**) electron-withdrawing group. Because the effect of a *para*-bulky group was not clearly assigned in the model, the two compounds with an SO_2NH_2 (**3f**) or a $NHSO_2Me$ groups (**3i**) were also selected from among those having the unalkylated benzothiazine nitrogen. Moreover, compound **3f** bears, like celecoxib, a 4-phenylsuphonamide group linked to the pyrazole ring.

Thus, six compounds (**3b**, **3c**, **3f**, **3g**, **3i**, and **4d**) were screened for their ability to block the NorA efflux pump. This was performed by employing a 50 μM concentration of each putative EPI in a fluorescent EtBr efflux assay in which SA-1199B, a well-characterized *norA*-overexpressing strain, was used as the test strain.[22] Celecoxib was included to confirm its inhibitory activity against the NorA efflux pump. The well-known EPIs reserpine and paroxetine were also included for comparative purposes (Table 1).

Data reported in Table 1 highlight that the derivative **3g**, belonging to the set of predicted active compounds, is the most active compound exhibiting 76.9% of EtBr efflux inhibition

dx.doi.org/10.1021/jm201446h | *J. Med. Chem.* 2012, 55, 3568−3572

Table 1. EtBr Efflux Inhibition (%) at 50 μM Concentration and MIC Values against SA-1199B of Selected Compounds



compd	R	R′	EtBr efflux inhib (%)	MIC[a] (μM)	MIC[a] (μg/mL)
3b	3-F	H	33.9		
3c	4-F	H	15.5		
3f	4-SO_2NH_2	H	29.8		
3g	4-NO_2	H	76.9	110	50
3i	4-$NHSO_2Me$	H	19.2		
4d	3-CF_3	Me	0.0		
celecoxib			64.5	>262	>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 60%.

while celecoxib appears to be slightly less active (64.5%). Although both compound **3g** and celecoxib were less active than the reference compounds reserpine and paroxetine, a dose–response curve was built to assess their activity at lower concentrations and to establish the respective IC_{50} values in the same test (Figure 2). Interestingly, Figure 2 shows that while

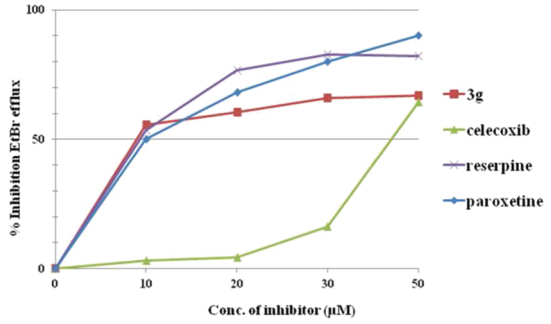


Figure 2. Effects of compound **3g**, celecoxib, reserpine, and paroxetine on EtBr efflux of SA-1199B.

both compound **3g** and celecoxib display similar EtBr efflux inhibition at 50 μM concentration, the trend of their dose–response curves appears to be very different. Compound **3g** was more active than celecoxib at every tested concentration less than 50 μM. Moreover, when comparing IC_{50} values, compound **3g** seems to be as active as the reference compounds ($IC_{50} \approx 10$ μM) and better than celecoxib, which has displayed an IC_{50} value higher than 40 μM.

To establish whether the NorA efflux inhibitory activity of compound **3g** and celecoxib results in a synergistic interaction with CPX, a fluoroquinolone antibacterial substrate of the NorA efflux pump, it was necessary to evaluate the intrinsic antibacterial activity of the new EPIs against the *S. aureus* strains included in the test (SA-1199, SA-1199B, SA-K1902, and SA-K2378) to avoid a misleading interpretation in assessing NorA inhibitory activity. Reserpine, paroxetine, and CPX were included for comparative purposes (Table 2).

Data presented in Table 2 reveal that compound **3g** shows weak (MICs 50–25 μg/mL) antibacterial activity in comparison

Table 2. Evaluation of Intrinsic Antibacterial Activity (MICs μg/mL) of Compounds 3g, Celecoxib, Reserpine, Paroxetine, and CPX against the Four *S. aureus* Strains Included in the Test of Synergism with CPX

	MIC (μg/mL)			
compd	SA-1199 (*norA* WT)	SA-1199B (*norA*++ +/ A116E GrlA)	SA-K1902 (*norA*−)	SA-K2378 (*norA*++)
3g	50	50	25	25
celecoxib	>100	>100	nd	nd
reserpine	>100	>100	>100	>100
paroxetine	>100	>100	>100	100
CPX	0.63	10	0.31	2.50

with the marketed antibacterial quinolone CPX. The effect of combining **3g**, celecoxib, reserpine, and paroxetine on CPX MICs was assessed by checkerboard assays,[23] which were performed using two pairs of *S. aureus* strains including SA-1199 (*norA* wild-type)/SA-1199B (*norA*++ and A116E GrlA) and SA-K1902 (*norA*−)/SA-K2378 (*norA*++) to confirm the efficacy of their NorA inhibitory activity (Figures 3 and 4, respectively).

Isobolograms reveal no appreciable synergistic activity between any of the tested compounds and CPX against the NorA wild-type strain SA-1199 (Figure 3a). These data are in agreement with a low level of expression of the NorA efflux pump in this strain. For SA-1199B (CPX MIC 10 μg/mL), which overexpresses *norA* and also has a A116E GrlA mutation, all tested compounds displayed a different level of synergism with CPX with the only exception of celecoxib. Compound **3g** displays good synergism with CPX, resulting in an 8-fold MIC reduction at concentrations above 6.25 μg/mL and an 16-fold MIC reduction (MIC of CPX from 10 to 0.63 μg/mL) at concentrations ≥12.5 μg/mL (Figure 3b).

These results were superior to that observed by the reference compounds reserpine and paroxetine. Compound **3g** completely restored the antibacterial activity of CPX against SA-1199B at concentrations less than $^1/_4$ of the MIC of the EPIs for this strain.

The high IC_{50} value displayed by celecoxib in the EtBr efflux inhibition assay does not permit appreciable synergistic activity with CPX against SA-1199B. Therefore we decided to test its synergistic activity with EtBr, a better substrate of NorA efflux pump, against the same strain (Figure 3c). Synergistic activity with EtBr was observed at celecoxib concentrations higher than 6.25 μg/mL (≥4-fold MIC of CPX reduction).

Further confirmation of the synergistic activity with CPX of **3g** was obtained when testing the compound, as well as the reference compounds, against strains SA-K1902 (*norA*−)/SA-K2378 (*norA*++) that differ only by the absence/presence of the NorA efflux pump (Figure 4).

Compound **3g** demonstrated an interesting synergic activity with CPX in this assay, being similar to that of the reference compound reserpine and better than that displayed by paroxetine.

Finally, compound **3g** was assayed for its cytotoxic activity with a lactate dehydrogenase (LDH) leakage assay against HepG2 cells and showed a $CC_{50} > 100$ μg/mL that was the same as that reported for the antibacterial quinolone CPX on that cell line.[24]

dx.doi.org/10.1021/jm201446h | J. Med. Chem. 2012, 55, 3568–3572

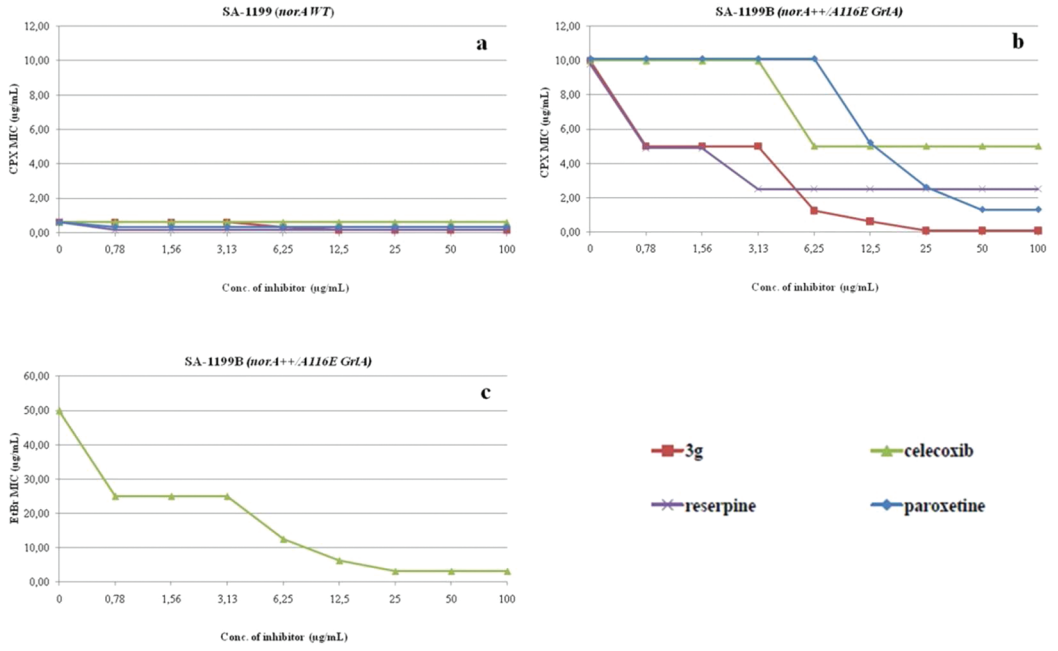


Figure 3. Effect of compound **3g**, celecoxib, reserpine, and paroxetine on the MIC of ciprofloxacin (a,b) and EtBr (c) against SA-1199 (a) and SA-1199B (b,c) strains.

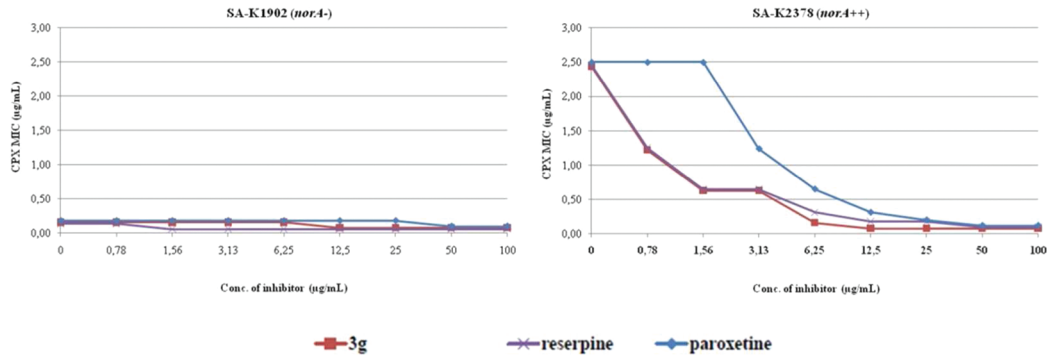


Figure 4. Effect of compounds **3g**, reserpine, and paroxetine on the MIC of ciprofloxacin against SA-K1902 and SA-K2378 strains.

CONCLUSION

In conclusion, in this work we have identified the 3-(4-chlorophenyl)-1-(4-nitrophenyl)-1,4-dihydropyrazolo[4,3-*c*]-[1,2]benzothiazine 5,5-dioxide **3g**, which shows modest intrinsic antistaphylococcal activity and is able to completely restore, in a concentration-dependent manner, the antibacterial activity of CPX against *norA*-overexpressing *S. aureus* strains. Compound **3g** was identified by means of a focused in vitro screen of only six compounds selected with the help of our recently presented model.[17] EtBr inhibition efflux experiments confirm the predictions obtained from the in silico screening by the NorA inhibitory activity model.[17] From the six compounds tested, it appears that the presence of an N-4 unalkylated in the benzothiazine ring is preferred to the NMe, whereas in the phenyl ring linked at the pyrazole a potent electron-withdrawing group (such as the nitro group) is preferred to bulkier groups and to the *meta*- or *para*-fluorine. However, these simple observations need to be substantiated with a proper SAR analysis before being taken as guidelines for EPI synthesis.

Although the EPI activity of derivative **3g** in the EtBr efflux inhibition assay was lower than that of reserpine, its synergistic activity with CPX was similar or slightly better than that shown by the reference compound against the *norA*-overexpressing strain SA-1199B. Moreover, EPI activity of celecoxib was confirmed against the NorA efflux pump even though synergistic activity with CPX against resistant strains was not demonstrable due to its high IC_{50} value. Finally, a new chemotype with potent NorA EPI activity, a limited intrinsic antibacterial activity, and a reduced cytotoxicity was identified. Compound **3g** could serve as a lead compound for the development of a second generation of NorA pyrazolo[4,3-*c*][1,2]benzothiazine inhibitors having an even higher potency.

EXPERIMENTAL SECTION

Synthesis. All reactions were routinely checked by thin-layer chromatography (TLC) on silica gel 60_{F254} (Merck) and visualized using UV illumination. Flash column chromatography was performed on Merck silica gel 60 (mesh 230−400) using the indicated 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 (≥95% purity). ^{1}H NMR spectra were recorded at 400 MHz 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

dx.doi.org/10.1021/jm201446h | J. Med. Chem. 2012, 55, 3568−3572

given in Hz. Reagents and solvents were purchased from common commercial suppliers and were used as received. All starting materials were commercially available unless otherwise indicated.

3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1,4-dihydropyrazolo-[4,3-*c*][1,2]benzothiazine 5,5-Dioxide (3g). To a suspension of **2** (4.00 g, 11.92 mmol) in 35 mL of absolute EtOH, a solution of (4-nitrophenyl)hydrazine hydrochloride (4.52 g, 23.84 mmol) in 35 mL of absolute EtOH was added dropwise. Then 5 mL of concentrated H_2SO_4 was added to the mixture and refluxed for 48 h. The solvent was evaporated and the solid crushed with 30 mL of a solution of EtOH:H_2O (50:50). After column chromatography eluting with CH_2Cl_2:MeOH (99:1), compound **3g** (3.20 g) was obtained as an orange solid (yield 59%, mp 283.7−284.9 °C). ^{1}H NMR (DMSO-d_6): δ 7.25 (d, J = 6.68 Hz, 1H), 7.64−7.73 (m, 4H), 7.92−7.97 (m, 2H), 8.04−8.11 (m, 3H), 8.48 (d, J = 9.07 Hz, 2H). Anal. ($C_{21}H_{13}ClN_4O_4S$) C, H, N.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full experimental procedures, analytical data for compounds **1**, **2**, **3a**−**f**, **3h**, **3i** and **4a**−**g**, **4i**, computational methods, NorA predicted activity and biological data for the molecules screened in silico, bacterial strains, microbiologic procedures and cytotoxicity test method. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +39 075 5855130. Fax: +39 075 5855115. E-mail: stefano.sabatini@unipg.it.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. F. Modarelli for helpful discussions and R. Bianconi and C. Moriconi for their technical support.

■ ABBREVIATIONS USED

EPI, efflux pump inhibitor; COX-2, cyclooxygenase-2; MDR, multidrug resistance; MRSA, methicillin-resistant *S. aureus*; HAI, healthcare-associated infection; MFS, major facilitator superfamily; SAR, structure−activity relationship; MDR1 or P-gp1, multidrug resistance protein 1; EtBr, ethidium bromide; CPX, ciprofloxacin; FLAP, fingerprints for ligands and proteins

■ REFERENCES

(1) Prabhavathi, F. Antibacterial discovery and development—the failure of success? *Nature Biotechnol.* **2006**, *24*, 1497−1503.

(2) Bush, K.; Miller, G. H. Bacterial enzymatic resistance: beta-lactamases and aminoglycoside-modifying enzymes. *Curr. Opin. Microbiol.* **1998**, *1*, 509−515.

(3) Ruiz, J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J. Antimicrob. Chemother.* **2003**, *51*, 1109−1117.

(4) Nikaido, H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 593−656.

(5) Li, X. Z.; Nikaido, H. Efflux-mediated drug resistance in bacteria: an update. *Drugs* **2009**, *69*, 1555−1623.

(6) Kohler, T.; Pechere, J. C.; Plesiat, P. Bacterial antibiotic efflux systems of medical importance. *Cell. Mol. Life Sci.* **1999**, *56*, 771−778.

(7) Mahamoud, A.; Chevalier, J.; Alibert-Franco, S.; Kern, W. V.; Pages, J. M. Antibiotic efflux pumps in Gram-negative bacteria: the inhibitor response strategy. *J. Antimicrob. Chemother.* **2007**, *59*, 1223−1229.

(8) Poole, K.; Lomovskaya, O. Can efflux inhibitors really counter resistance? *Drug Discovery Today: Ther. Strategies* **2006**, *3*, 145−152.

(9) ECDC/EMEA Joint Working Group. *The Bacterial Challenge: Time to React*; ECDC: Stockholm, September 2009; http://www.ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf (Accessed Oct 20, 2011).

(10) Klevens, R. M.; Morrison, M. A.; Nadle, J.; Petit, S.; Gershman, K.; Ray, S.; Harrison, L. H.; Lynfield, R.; Dumyati, G.; Townes, J. M.; Craig, A. S.; Zell, E. R.; Fosheim, G. E.; McDougal, L. K.; Carey, R. B.; Fridkin, S. K. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA, J. Am. Med. Assoc.* **2007**, *298*, 1763−1771.

(11) Centers for Disease Control and Prevention. Public Health Dispatch: Vancomycin-Resistant *Staphylococcus aureus*—Pennsylvania, 2002. *Morbidity Mortality Wkly. Rep.*, **2002**, *51*, 902.

(12) Patel, D.; Kosmidis, C.; Seo, S. M.; Kaatz, G. W. Ethidium bromide MIC screening for enhanced efflux pump gene expression or efflux activity in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2010**, *54*, 5070−5073.

(13) Pao, S. S.; Paulsen, I. T.; Saier, M. H. Jr. Major facilitator superfamily. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 1−34.

(14) Yoshida, H.; Bogaki, M.; Nakamura, S.; Ubukata, K.; Konno, M. Nucleotide sequence and characterization of the *Staphylococcus aureus norA* gene, which confers resistance to quinolones. *J. Bacteriol.* **1990**, *172*, 6942−6949.

(15) Neyfakh, A. A. Mystery of multidrug transporters: the answer can be simple. *Mol. Microbiol.* **2002**, *44*, 1123−1130.

(16) Zhang, L.; Ma, S. Efflux pump inhibitors: a strategy to combat P-glycoprotein and the NorA multidrug resistance pump. *ChemMedChem* **2010**, *5*, 811−822.

(17) Brincat, J. P.; Carosati, E.; Sabatini, S.; Manfroni, G.; Fravolini, A.; Raygada, J. L.; Patel, D.; Kaatz, G. W.; Cruciani, G. Discovery of Novel Inhibitors of the NorA Multidrug Transporter of *Staphylococcus aureus*. *J. Med. Chem.* **2011**, *54*, 354−365.

(18) Sabatini, S.; Gosetto, F.; Manfroni, G.; Tabarrini, O.; Kaatz, G.; Patel, D.; Cecchetti, V. Evolution from a natural flavones nucleus to obtain 2-(4-propoxyphenyl)quinoline derivatives as potent inhibitors of the *S. aureus* NorA efflux pump. *J. Med. Chem.* **2011**, *54*, 5722−5736.

(19) Wright, G. D. Resisting resistance: new chemical strategies for battling superbugs. *Chem. Biol.* **2000**, *7*, R127−R132.

(20) Kalle, A. M.; Rizvi, A. Inhibition of bacterial multidrug resistance by celecoxib, a cyclooxygenase-2 inhibitor. *Antimicrob. Agents Chemother.* **2011**, *55*, 439−442.

(21) Baroni, M.; Cruciani, G.; Sciabola, S.; Perruccio, F.; Mason, J. S. A common reference framework for analyzing/comparing proteins and ligands. Fingerprints for Ligands and Proteins (FLAP): theory and application. *J. Chem. Inf. Model* **2007**, *47*, 279−294.

(22) Kaatz, G. W.; Seo, S. M. Mechanisms of fluoroquinolone resistance in genetically related strains of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **1997**, *41*, 2733−2737.

(23) Eliopoulos, G. M.; Moellering, R. C. J. Antimicrobial combinations. In *Antibiotics in Laboratory Medicine*; Lorian, V., Ed.; Williams and Wilkins: Baltimore, MD, 1991; pp 432−492.

(24) Wang, Q.; Lucien, E.; Hashimoto, A.; Pais, G. C.; Nelson, D. M.; Song, Y.; Thanassi, J. A.; Marlor, C. W.; Thoma, C. L.; Cheng, J.; Podos, S. D.; Ou, Y.; Deshpande, M.; Pucci, M. J.; Buechter, D. D.; Bradbury, B. J.; Wiles, J. A. Isothiazoloquinolones with enhanced antistaphylococcal activities against multidrug-resistant strains: effects of structural modifications at the 6-, 7-, and 8-positions. *J. Med. Chem.* **2007**, *50*, 199−210.