ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Identification of a Series of Compounds with Potent Antiviral Activity for the Treatment of Enterovirus Infections

Angus M. MacLeod,*[,†,∥] Dale R. Mitchell,[†,∥] Nicholas J. Palmer,[†] Hervé Van de Poël,[†] Katja Conrath,[§] Martin Andrews,[§] Pieter Leyssen,[‡] and Johan Neyts[‡]

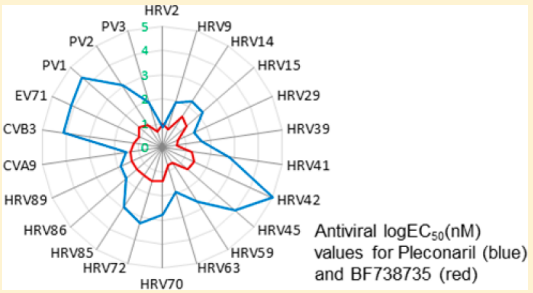
[†]Medicinal Chemistry Department, BioFocus, Chesterford Research Park, Cambridge, CB10 1XL, U.K.

[‡]Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium

[§]Galapagos NV, Generaal de Wittelaan, 2800 Mechelen, Belgium

Supporting Information

ABSTRACT: Rhinovirus (genus enterovirus) infections are responsible for many of the severe exacerbations of asthma and chronic obstructive pulmonary disease. Other members of the genus can cause life-threatening acute neurological infections. There is currently no antiviral drug approved for the treatment of such infections. We have identified a series of potent, broad-spectrum antiviral compounds that inhibit the replication of the human rhinovirus, Coxsackie virus, poliovirus, and enterovirus-71. The mechanism of action of the compounds has been established as inhibition of a lipid kinase, PI4KIIIβ. Inhibition of hepatitis C replication in a replicon assay correlated with enterovirus inhibition.



KEYWORDS: *antiviral, enterovirus, HCV, inhibitor, PI4KIIIβ*

Over the last 15 years, evidence has gathered that viral infections of the respiratory tract are a major cause of exacerbations in both chronic obstructive pulmonary disease (COPD)[1] and asthma.[2] Of these infections, half to two-thirds are caused by human rhinovirus (HRV), the most well-known etiologic agent of the common cold.[3] While these infections are merely inconvenient in otherwise healthy individuals, exacerbation of the symptoms of COPD and asthma are serious, leading to very large numbers of hospitalizations and significant mortality. In fact, the prevalence of COPD is increasing rapidly, and it is predicted to become the third leading cause of death globally by 2030.[4] Hence, the identification of compounds that can interfere with rhinovirus replication may lead to drugs that have important clinical value in the management of a growing medical need.

In the past, several drug candidates have been progressed into clinical trials for the treatment of HRV infections. These include Rupintrivir,[5] a viral 3C protease inhibitor, and a number of compounds that prevent virus entry into cells by binding to the viral capsid: Pirodavir,[6] Pleconaril,[7] and BTA798.[8] Only the latter of these is currently in clinical development, the others having been terminated due to lack of clinical efficacy or unacceptable side effects.

As part of an antiviral program directed toward hepatitis C (HCV), we identified a series of imidazo-pyrazines with modest (micromolar) activity in a genotype 1b replicon screen (Figure 1). These compounds were derived from a BioFocus SoftFocus library (SFK28) initially designed as potential kinase inhibitors.

Additional screening of the imidazo-pyrazines against a panel of other viruses revealed similar activity against the Enteroviruses, a genus of the *Picornaviridae* family, including HRV, polio virus (PV), and Coxsackie virus (CV). In most cases, the compounds were 3−5-fold more active against enteroviruses, which, in common with HCV, are positive-sense single-stranded RNA (+ssRNA) viruses. The compounds proved inactive against other RNA, DNA, and retroviruses and were not cytotoxic at antiviral concentrations, indicating a specific and selective antiviral effect.

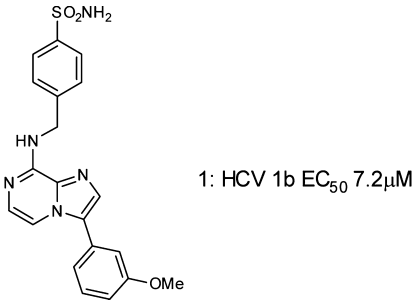


Figure 1. HCV 1b replicon screening hit.

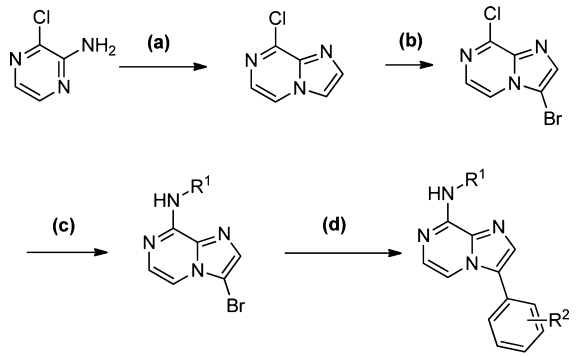
Enteroviruses present a range of threats to human health, from the common cold to life-threatening infections, including encephalitis and viral meningitis.[9] Polio is largely considered to be a disease of the past because of the success of the World Health Organization's Global Polio Eradication Initiative in

Received: March 8, 2013
Accepted: May 8, 2013
Published: May 8, 2013

reducing the incidence of polio over the past two decades. However, as the initiative moves toward its final goal, it has become apparent that polio antiviral drugs will be required for emergency use in a postvaccination era to deal with the threat of outbreaks caused by circulating vaccine-derived poliovirus (VDPV), including treatment of infections transmitted by immunocompromised patients who chronically shed vaccine-derived poliovirus.[10]

To improve on the antiviral potency of hit compound 1 and its congeners, we explored the effect of varying the substituents on the core scaffold, the aryl ring at the 3-position, and on the 8-position benzylamine. The synthesis of analogues (Scheme 1)

Scheme 1. Synthesis of Imidazopyrazines[a]



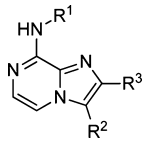
[a]Reagents and conditions: (a) $BrCH_2CHO$, ^iPrOH reflux; (b) NBS, DCM; (c) $R^1NH_2.HCl$, $^iPr_2EtN$, ^iBuOH, 108 °C; (d) $ArB(OH)_2$, Pd(0), DMF, microwave.

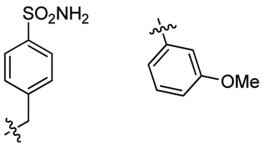
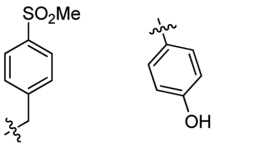
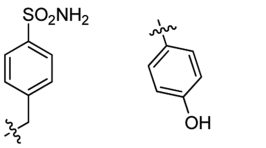
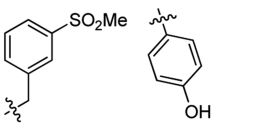
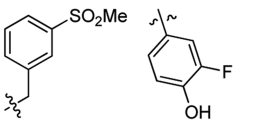
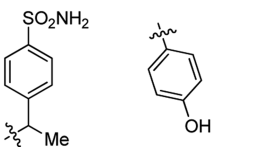
followed the methodology developed for the original library production and HRV14 in a Hela Rh cell line was used as the primary target for tracking potency optimization. The synthetic route was sufficiently flexible to readily allow variation of the substituents at both the 3- and 8-position of the core scaffold. Introduction of substituents at the 2-position of the core scaffold was achieved by the use of the appropriate haloacetate (replacing bromoacetaldehyde) under similar reaction conditions. Enhancement in antiviral activity was achieved by varying the position and nature of substituents on the terminal aryl rings, and modification at C2 of the imidazo-pyrazine ring. In particular, polar substituents at the meta position of the benzylamine and H-bond donor/acceptor groups para substituted on the C3 aryl ring were potency enhancing (Table 1). Methylation of the C2 position of the imidazopyrazine generally had no effect on activity or gave a small increase in potency. Methylation at the benzylic position of the benzylamine was also well tolerated with the (racemic) analogue **6** retaining good potency.

Cytotoxicity of the compounds in the Hela Rh cells was assessed in parallel with antiviral efficacy. The compounds were found to have a high selectivity index for antiviral activity relative to cellular toxicity with CC_{50} values generally >1000-fold higher than the EC_{50} values.

Comparison of the HRV14 activity of **1**–**6** against other members of the enteroviruses (Table 2) showed the rank order of potencies is essentially the same regardless of the species. This includes enterovirus-71, an emerging virus that has been the cause of several outbreaks in Asia, resulting in a number of deaths through neurological inflammation.[11] The consistency in activity across the enteroviruses suggested that the molecular target of the compounds is either a viral target with very high

Table 1. Antiviral Activities against HRV-14



	R^1	R^2	R^3	EC_{50}[a]	CC_{50}[a]
1			H	3900	>100,000
2			H	150	>100,000
3			Me	90	>100,000
4			Me	39	>100,000
5			Me	31	61,000
6			H	15	21,000

[a]Activity in nM.

Table 2. Cross-Species Antienteroviral Activity[a]

compd	HRV14	CVB3	PV1	EV71
1	3900	3000	3000	470
2	150	550	140	80
3	90	490	150	34
4	39	68	38	15
5	31	15	19	11
6	15	66		

[a]EC_{50} values in nM

conservation of sequence within the family or a single common target, most likely a host cell protein essential for the replication of the viruses.

Compound **5** (BF738735) was selected for more extensive antienterovirus profiling and revealed a remarkable consistency in activity across a wide range of species (Table 3). Pleconaril (Figure 2), an anti-HRV agent that acts by preventing viral entry into cells by binding to the viral capsid, had a range of

dx.doi.org/10.1021/ml400095m | ACS Med. Chem. Lett. 2013, 4, 585–589

Table 3. Comparison of the Antienteroviral Activity[a] of BF738735 with Pleconaril

virus	BF738735	pleconaril
HRV2	9	7
HRV9	6	83
HRV14	31	177
HRV15	21	166
HRV29	5	26
HRV39	4	44
HRV41	17	655
HRV42	27	100000
HRV45	25	8900
HRV59	6	471
HRV63	6	85
HRV70	24	578
HRV72	28	1800
HRV85	24	883
HRV86	24	89
HRV89	24	75
CVA9	21	33
CVB3	15	13000
EV71	11	13400
PV1	19	24200
PV2	13	1084
PV3	5	84

[a]EC_{50} values in nM

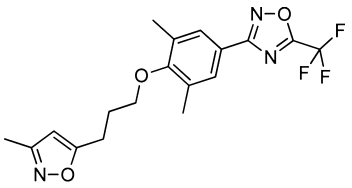


Figure 2. Pleconaril.

more than 3 orders of magnitude in EC_{50} values and was essentially inactive against some serotypes.

Pleconaril reached phase III clinical trials but did not progress to registration due to drug–drug interactions with oral contraceptives and insufficient efficacy in treating infections.[12] The relatively weak activity of Pleconaril against many of the HRV serotypes may have contributed to its poor clinical efficacy in a community setting where patients presenting with similar symptoms are infected with a spectrum of HRV serotypes. The broad, uniformly potent activity of BF738735 observed in the test panel offers a clear advantage over Pleconaril.

Although the potency of the compounds against HCV was generally slightly lower than that against HRV14 (e.g., BF738735 HCV1b EC_{50} 56 nM), a strong correlation in activity (Figure 3) was observed during the optimization process, again suggesting a common mechanism of action. Because HCV belongs to a different virus family (*Flaviviridae*), this observation reinforced the likelihood that the target may be a host cell protein rather than a viral enzyme or structural protein.

While medicinal chemistry efforts to optimize the potency of this series of compounds were carried out, parallel efforts were undertaken to determine the mechanism of action. Resistance selection experiments showed that, in contrast to Pleconaril, there is a high genetic barrier for HRV to become resistant to

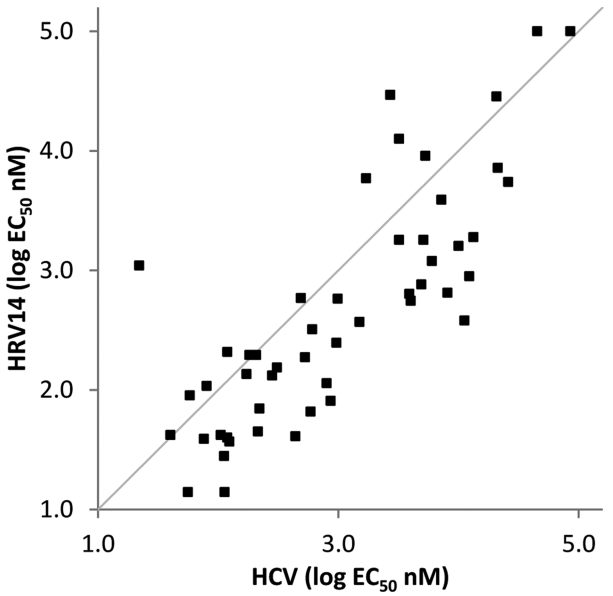


Figure 3. Correlation of activity against HRV and HCV. EC_{50} values were determined for inhibition of HRV replication in Hela Rh cells and against an HCV genotype 1b replicon in Huh 5.2. The correlation coefficient for the line of best fit is 0.81. The straight line drawn is for $y = x$.

the antiviral effect of the compounds. Time of drug-addition studies revealed that, whereas Pleconaril is only effective against HRV14 when added together with, or prior to, the addition of virus, BF738735 is active both when added before or after virus infection, indicating the mechanism is not at an early stage of the virus replication cycle. No significant activity of BF738735 was observed against isolated viral enzymes, which, taken together with the very narrow range of potencies seen within the genus enterovirus, pointed to a mechanism of action involving a host cell factor. The high correlation in activity against HCV suggested the mechanism is shared by this virus.

Representative compounds were selected for broad profiling against a range of enzymes, receptors, and ion channels and showed no significant activity at 1 μM. The highest activity observed in screening against a panel of >400 protein kinases was in the low micromolar range, considerably higher than the nanomolar antiviral activity that was observed in the virus-cell-based assays.

Concurrent with the work detailed here, several publications appeared detailing the results of target identification genomic screening to identify novel host targets involved in HCV replication. From these studies, the lipid kinases phosphatidylinositol-4 kinase III alpha and beta (PI4KIIIα and PI4KIIIβ) emerged as enzymes that appear to be important in the replication of the HCV virus. An siRNA study by Borawski et al.[13] showed that knockdown of either PI4KIIIα or PI4KIIIβ led to reduced production of viral RNA for HCV genotypes 1a and 1b. Studies by Reiss et al.[14] and Berger et al.[15] concluded that only enzymatic activity of PI4KIIIα is required for HCV replication, whereas van Kuppeveld et al.[16] demonstrated that PI4KIIIβ mediated enrichment of phosphatidyl inositol-4-phosphate in endoplasmic reticulum membranes is required for HCV replication. Arita et al.[17] recently disclosed the discovery of T-00127-HEV1 (Figure 4), which was demonstrated to be a selective PI4KIIIβ inhibitor (IC_{50} 60nM) with micromolar antiviral activity against PV1; T-00127-HEV1 was

dx.doi.org/10.1021/ml400095m | ACS Med. Chem. Lett. 2013, 4, 585–589

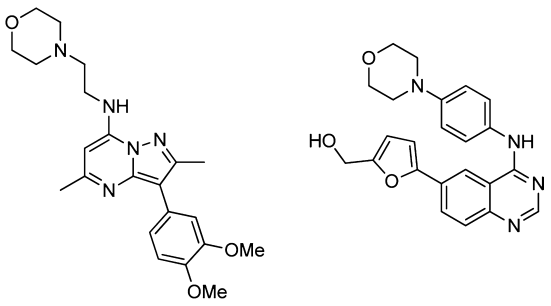


Figure 4.

not active against HCV. In contrast, the dual PI4KIIIα /PI4KIIIβ inhibitor AL-9,[18] which has a 5-fold preference for activity against PI4KIIIα (IC_{50} 0.57 vs 3.08 μM), does inhibit HCV replication (EC_{50} 0.29 μM for the 1b genotype) suggesting a greater dependence of this virus on the PI4KIIIα isoform.

Screening BF738735 against a panel of lipid kinases revealed potent and highly selective inhibitory activity on PI4KIIIβ, with an IC_{50} of 5.7 nM. Inhibition of the related isoform PI4KIIIα was substantially weaker with an IC_{50} of 1700 nM, and no activity was found against a range of other lipid kinases (IC_{50} > 10 000 nM).

In summary, we have discovered a novel series of antiviral compounds with broad applicability for the treatment of enterovirus infections and with a potential for use against HCV in combination with drugs working by complementary mechanisms. The clinical application of PI4KIIIβ inhibitors will depend on the therapeutic index that can be achieved, particularly in nonlife-threatening infections. A recent publication[19] has suggested that compounds acting by this mechanism cause reductions in T-cell count as a side effect that potentially will limit the use of this type of drug. Further studies will define the scope under which this may be managed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental procedures for the synthesis of compounds and antiviral assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(A.M.M.) E-mail: angus.macleod@glpg.com.

Author Contributions

‖These authors contributed equally to this work. The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to acknowledge Stijn Delmotte and Tom Bellon for their excellent assistance in generating the antiviral data.

■ ABBREVIATIONS

COPD, chronic obstructive pulmonary disease; HRV, human rhinovirus; PV, poliovirus; CV, Coxsackie virus

■ REFERENCES

(1) Papi, A.; Bellettato, C. M.; Braccioni, F.; Romagnoli, M.; Casolari, P.; Caramori, G.; Fabbri, L. M.; Johnston, S. L. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am. J. Respir. Crit. Care Med.* **2006**, *173*, 1114−1121.

(2) Corne, J. M.; Marshall, C.; Smith, S.; Schreiber, J.; Sanderson, G.; Holgate, S. T.; Johnston, S. L. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. *Lancet* **2002**, *359*, 831−834.

(3) Mallia, P.; Contoli, M.; Caramori, G.; Pandit, A.; Johnston, S. L.; Papi, A. Exacerbations of asthma and chronic obstructive pulmonary disease (COPD): focus on virus induced exacerbations. *Curr. Pharm. Des.* **2007**, *13* (1), 73−97.

(4) WHO. World health statistics 2008. http://www.who.int/whosis/whostat/EN_WHS08_Full.pdf.

(5) Hayden, F. G.; Turner, R. B.; Gwaltney, J. M.; Chi-Burris, K.; Gersten, M.; Hsyu, P.; Patick, A. K.; Smith, G. J.; Zalman, L. S. Phase II, randomized, double-blind, placebo-controlled studies of ruprintrivir nasal spray 2-percent suspension for prevention and treatment of experimentally induced rhinovirus colds in healthy volunteers. *Antimicrob. Agents Chemother.* **2003**, *47* (12), 3907−3916.

(6) Hayden, F. G.; Hipskind, G. J.; Woerner, D. H.; Eisen, G. F.; Janssens, M.; Janssen, P. A. J.; Andries, K. Intranasal Pirodavir (R77,975) treatment of rhinovirus colds. *Antimicrob. Agents Chemother.* **1995**, *39* (2), 290−294.

(7) Hayden, F. G.; Herrington, D. T.; Coats, T. L.; Kim, K.; Cooper, E. C.; Villano, S. A.; Liu, S.; Hudson, S.; Pevear, D. C.; Collett, M.; McKinlay, M. Efficacy and safety of oral pleconaril for treatment of colds due to picornaviruses in adults: results of 2 double-blind, randomized, placebo-controlled trials. *Clin. Infect. Dis.* **2003**, *36* (12), 1523−1532.

(8) Feil, S. C.; Hamilton, S.; Krippner, G. Y.; Lin, B.; Luttick, A.; McConnell, D. B.; Nearn, R.; Parker, M. W.; Ryan, J.; Stanislawski, P. C.; Tucker, S. P.; Watson, K. G.; Morton, C. J. An orally available 3-ethoxybenzisoxazole capsid binder with clinical activity against human rhinovirus. *ACS Med. Chem. Lett.* **2012**, *3* (4), 303−307.

(9) De Palma, A. M.; Vliegen, I.; De Clercq, E.; Neyts, J. Selective inhibitors of picornavirus replication. *Med. Res. Rev.* **2008**, *28* (6), 823−884.

(10) Barrett, S. Polio eradication: strengthening the weakest links. *Health Aff.* **2009**, *28* (4), 1079−1090.

(11) McMinn, P. C. Recent advances in the molecular epidemiology and control of human enterovirus 71 infection. *Curr. Opin. Virol.* **2012**, *2* (2), 199−205.

(12) Fleischer, R.; Laessig, K. Safety and efficacy evaluation of pleconaril for treatment of the common cold. *Clin. Infect. Dis.* **2003**, *37* (12), 1722.

(13) Borawski, J.; Troke, P.; Puyang, X.; Gibaja, V.; Zhao, S.; Mickanin, C.; Leighton-Davies, J.; Wilson, C. J.; Myer, V.; Cornellataracido, I.; Baryza, J.; Tallarico, J.; Joberty, G.; Bantscheff, M.; Schirle, M.; Bouwmeester, T.; Mathy, J. E.; Lin, K.; Compton, T.; Labow, M.; Wiedmann, B.; Gaither, L. A. Class III phosphatidylinositol 4-kinase alpha and beta are novel host factor regulators of hepatitis C virus replication. *J. Virol.* **2009**, *83* (19), 10058−10074.

(14) Reiss, S.; Rebhan, I.; Backes, P.; Romero-Brey, I.; Erfle, H.; Matula, P.; Kaderali, L.; Poenisch, M.; Blankenburg, H.; Hiet, M. S.; Longerich, T.; Diehl, S.; Ramirez, F.; Balla, T.; Rohr, K.; Kaul, A.; Bühler, S.; Pepperkok, R.; Lengauer, T.; Albrecht, M.; Eils, R.; Schirmacher, P.; Lohmann, V.; Bartenschlager, R. Recruitment and activation of a lipid kinase by hepatitis C virus NS5A is essential for integrity of the membranous replication compartment. *Cell Host Microb.* **2011**, *9* (1), 32−45.

(15) Berger, K. L.; Cooper, J. D.; Heaton, N. S.; Yoon, R.; Oakland, T. E.; Jordan, T. X.; Mateu, G.; Grakoui, A.; Randall, G. Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106* (18), 7577−7582.

(16) Hsu, N. Y.; Ilnytska, O.; Belov, G.; Santiana, M.; Chen, Y. H.; Takvorian, P. M.; Pau, C.; van der Schaar, H.; Kaushik-Basu, N.; Balla,

T.; Cameron, C. E.; Ehrenfeld, E.; van Kuppeveld, F. J.; Altan-Bonnet, N. Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. *Cell* **2010**, *141* (5), 799−811.
(17) Arita, M.; Kojima, H.; Nagano, T.; Okabe, T.; Wakita, T.; Shimizu, H. Phosphatidylinositol 4-kinase III beta is a target of enviroxime-like compounds for antipoliovirus activity. *J. Virol.* **2011**, *85* (5), 2364−2372.
(18) Bianco, A.; Reghellin, V.; Donnici, L.; Fenu, S.; Alvarez, R.; Baruffa, C.; Peri, F.; Pagani, M.; Abrignani, S.; Neddermann, P.; De Francesco, R. *PLoS Pathog.* **2012**, *8* (3), e1002576.
(19) Lamarche, M. J.; Borawski, J.; Bose, A.; Capacci-Daniel, C.; Colvin, R.; Dennehy, M.; Ding, J.; Dobler, M.; Drumm, J.; Gaither, L. A.; Gao, J.; Jiang, X.; Lin, K.; McKeever, U.; Puyang, X.; Raman, P.; Thohan, S.; Tommasi, R.; Wagner, K.; Xiong, X.; Zabawa, T.; Zhu, S.; Wiedmann, B. Anti-hepatitis C virus activity and toxicity of type III phosphatidylinositol-4-kinase beta inhibitors. *Antimicrob. Agents Chemother.* **2012**, *56* (10), 5149−5156.