Accepted Manuscript

Discovery and Characterization of Aryl Isonitriles as A New Class of Compounds versus Methicillin- and Vancomycin-resistant *Staphylococcus aureus*

Dexter C. Davis, Haroon Mohammad, Kwaku Kyei-Baffour, Waleed Younis, Cassidy Noel Creemer, Mohamed N. Seleem, Mingji Dai

PII: S0223-5234(15)30107-0

DOI: 10.1016/j.ejmech.2015.06.031

Reference: EJMECH 7958

To appear in: *European Journal of Medicinal Chemistry*

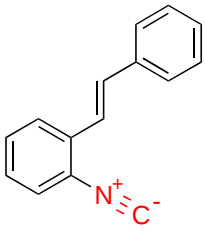
Received Date: 27 March 2015

Revised Date: 9 June 2015

Accepted Date: 13 June 2015

Please cite this article as: D.C. Davis, H. Mohammad, K. Kyei-Baffour, W. Younis, C.N. Creemer, M.N. Seleem, M. Dai, Discovery and Characterization of Aryl Isonitriles as A New Class of Compounds versus Methicillin- and Vancomycin-resistant *Staphylococcus aureus*, *European Journal of Medicinal Chemistry* (2015), doi: 10.1016/j.ejmech.2015.06.031.

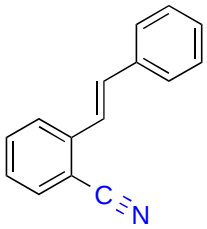
Insert Table of Contents Graphic and Synopsis Here.



13

MIC as low as 2 μM agaist MRSA and VRSA
No toxicity at 64 μM against mammalian cells

versus



43 (MIC > 128 μM)

A novel class of aryl isonitrile compounds has been discovered as potent antimicrobial compounds against several clinically relevant MRSA and VRSA isolates without any toxicity against mammalian cells up to a concentration of 64 μM.

Discovery and Characterization of Aryl Isonitriles as A New Class of Compounds versus Methicillin- and Vancomycin-resistant *Staphylococcus aureus*

Dexter C. Davis,[†,§] Haroon Mohammad,[†,‡] Kwaku Kyei-Baffour,[§] Waleed Younis,[‡] Cassidy Noel Creemer,[§] Mohamed N. Seleem,[,‡] and Mingji Dai*[,§]*

[§]Department of Chemistry and Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907, United States

[‡]Department of Comparative Pathobiology, Purdue University College of Veterinary Medicine, West Lafayette, Indiana 47907, United States

[†]These two authors contributed equally.

*Corresponding authors' E-mail addresses: mseleem@purdue.edu; mjdai@purdue.edu

KEYWORDS: Antibiotic, Drug Resistance, MRSA, VRSA, Isonitrile

ABSTRACT: Methicillin- and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA) have emerged as a global health concern. A new class of compounds featuring an aryl isonitrile moiety has been discovered that exhibits potent inhibitory activity against several clinically-relevant MRSA and VRSA isolates. Structure-activity relationship studies have been

conducted to identify the aryl isonitrile group as the key functional group responsible for the observed antibacterial activity. The most potent antibacterial aryl isonitrile analogs (MIC 2 μM) did not show any toxicity against mammalian cells up to a concentration of 64 μM.

INTRODUCTION

Multidrug-resistant bacterial infections pose a significant global health challenge afflicting more than 2 million people each year in the United States alone, resulting in over 23,000 fatalities.[1] Nearly half of these casualties are due to infections caused by a single pathogen, methicillin-resistant *Staphylococcus aureus* (MRSA). Currently prevalent in the community setting, MRSA is responsible for a wide spectrum of illnesses from superficial skin infections to invasive diseases including pneumonia, osteomyelitis, and bloodstream infections.[2-5] While a robust arsenal of antibiotics was once capable of treating MRSA infections, strains of this pathogen have emerged that exhibit resistance to nearly every class of antibiotics, including agents of last resort such as vancomycin and linezolid.[6-11] This underscores the urgent need for the identification and development of novel therapeutic options capable of treating infections due to MRSA.[12]

Recently, we have conducted a whole-cell screening of a small number of in house generated small molecules (about 250 molecules) against MRSA USA300 with the aim to identify compounds with novel skeletons to target antibiotic drug resistance. To our delights, among several hit molecules revealed by this screening effort, compound **1** with an isonitrile group attached to a stilbene system was shown to be capable of inhibiting bacterial growth at a concentration of 32 μM (Figure 1). Further analysis revealed this compound is bacteriostatic (the minimum bactericidal concentration exceeded 128 μM). The presence of an isonitrile moiety in

this compound is quite unique given that few antimicrobial compounds possessing the isonitrile moiety in their core structure have been described in literature and all of them are complex natural products and are difficult to access.[13-19] Natural terpene isonitrile-containing molecules and simplified analogs have been reported to show antimalarial activity as well.[20-22] The novel structural skeleton of compound **1** as an antibacterial compound against drug resistant strains prompted us to further study of this type of isonitrile compounds. Herein, we report our chemical synthesis, structure-activity relationship study, and evaluation of the antibacterial performance of compound **1** and closely related analogs against several clinically-relevant MRSA and VRSA strains. These efforts have led to the identification of more potent compounds with MIC as low as 2 μM but do not show any cytotoxicity against mammalian cells up to a concentration of 64 μM. Physiochemical analysis of this potent lead compound has been described to guide the next stage of developing these promising compounds into the antibiotic drug pipeline.

FIGURE 1 here.

CHEMICAL SYNTHESIS

In general, the stilbene isonitrile analogs were prepared from benzylic bromide **2**, which was converted to phosphonate **3** by Michaelis-Arbuzov reaction.[23] The nitro group of **3** was then converted to an isonitrile group upon a sequence of hydrogenation and Hofmann isonitrile synthesis using dichlorocarbene.[24] Compound **4** then served as a divergent point to synthesize a collection of analogs with a Horner-Wadsworth-Emmons reaction.[25] By treating various ketones and aldehydes with stabilized phosphonate carbanions derived from phosphonates **4**, we obtained thirty-three stilbene isonitrile analogs (**1**, **5**-**25**, and **27**-**37**) and one styrene isonitrile analog (**26**). This collection also includes compounds with the isonitrile group at different positions on the

aromatic ring as well as pyridine containing analogs. In order to investigate the importance of the isonitrile group for the observed biological activity, compound containing a hydrogen atom (**42**) or a nitrile group (**43**) at the isonitrile-substitution position was prepared as well using the Horner-Wadsworth-Emmons reaction. Additionally, four biary isonitrile analogs (**46** and **49**-**51**) were prepared. [26] Compound **45** was prepared from commercially available amine **33** via formamide formation followed by dehydration. Compounds **49**-**51** were synthesized from 2-bromoaniline derivatives (**47**) and arylboronic acids. Suzuki cross-coupling converted **47** to biaryl amines **48** smoothly. The latter was then converted to **49**-**51** via the aforementioned formamide formation and dehydration sequence. Lastly, we prepared compound **53** with a saturated two-carbon chain to investigate the importance of the double bond linker between the two aromatic moieties. All the newly synthesized compounds were purified using flash chromatography before entering biological evaluations.

SCHEME 1 here.

BIOLOGICAL RESULTS AND DISCUSSION

Antimicrobial susceptibility analysis of the isonitrile compounds against clinically-relevant isolates of MRSA and VRSA. The bacterial growth inhibiting activity of these synthetic analogs of hit compound **1** were subsequently evaluated (Table 2). When these derivatives were screened against MRSA, via the broth microdilution assay, the results revealed several interesting structural elements that appear to play an important role in the antimicrobial activity of these compounds. Initial inspection of the structural moieties of **1** revealed that the presence of an isonitrile group is essential for its antimicrobial activity. When the isonitrile group of **1** (MIC

against MRSA ranging from 8-64 μM) was removed (as in compound **42**), a complete loss in the anti-MRSA activity of **42** is observed (MIC > 128 μM). A similar pattern is observed when reviewing the MIC results for compounds **13** and **43**. Compound **13**, one of the most potent derivatives constructed (with MIC values against MRSA as low as 2 μM), contains the isonitrile group; when the isonitrile group of **13** is replaced with an isosteric nitrile group (resulting in compound **43**), complete loss of antimicrobial activity was observed. Similarly, compound **53** with an isonitrile group is active against several strains evaluated particularly MRSA USA100, MRSA USA300, MRSA NRS119, and VISA NRS1, while compound **52** without the isonitrile group lacks antimicrobial activity. These results confirm that the isonitrile group appears necessary for these compounds to possess activity against MRSA and may play an important role in binding to the compound's molecular target.

The presence of a second aromatic substituent (connected to the isonitrile-phenyl group) also appears critical to the biological activity observed; replacement of this moiety in **1** with a diethyl phosphonate (as in analog **4a** with an *ortho*-isonitrile group) results in complete loss of activity against MRSA (MIC > 128 μM). Likewise, substitution of this second aromatic substituent with a cycloalkane (cf. **26**) renders this compound inactive against several MRSA isolates (including MRSA USA300, MRSA USA500, and MRSA NRS119). The presence of an alkene bridge between the two aromatic substituents in **1** also appears to be important. When the alkene bridge between the two aromatic substituents is removed, as in compound **46**, this compound lacks activity against three strains of MRSA (USA300, USA500, and NRS119). A similar loss in antimicrobial activity is observed with compounds **49** and **50** indicating that the stilbene isonitrile core of **1** plays an important role in its antimicrobial activity. This notion was further supported by a direct comparison of compounds **13** and **53**. Compound **53** is a saturated

analog of compound **13** and contains a flexible two-carbon linker between the two aromatic moieties. In general, compound **53** is less potent than compound **13** against all the strains texted except for VISA NRS1.

We then evaluated how substituents on the double bond would affect the antimicrobial activity. Interestingly, removal of the ethyl group of **1** (cf. **13**) resulted in a dramatic improvement in antimicrobial activity (a two-to-eight fold reduction in the MIC against MRSA was observed). When the ethyl group was replaced by methyl (**5**), *n*-propyl (**6**), *n*-butyl (**7**) and phenyl (**8**) groups, a noticeable change in the MIC value for these compounds is observed.

We next assessed how substituents on the non-isonitrile-containing aromatic ring would affect the potency against MRSA. Analogs constructed include substitution of methoxy group (**14-16**), fluoride (**17-19**), trifluoromethyl group (**20-22**), methyl group (**23**), *n*-butyl group (**24**), and nitro group (**25**). Interestingly most of these modifications do not produce a major improvement in the MIC observed against MRSA, when compared to the activity of **13**. Additionally the positioning of these groups around the benzene ring do not appear to have an impact on the antimicrobial activity of the compound. While most of these modifications have little effect on improving the antimicrobial activity of these compounds, one substitution had an observed deleterious effect. Compound **24**, containing a *n*-butyl group, lacked activity against most MRSA strains tested (MIC >128 μM); interestingly, **23**, with a methyl group is active against all MRSA strains tested albeit at a higher concentration than **13** (MIC of **23** ranges from 4 to 64 μM against MRSA). This would appear to indicate that the presence of an alkyl group (in particular one of increased length) is undesirable and can have a negative effect on the activity of these compounds against MRSA. Analogs containing a pyridine ring were synthesized and tested as well (**27**, **30**, **33**) and reduced antimicrobial activities were observed.

All the analogs discussed above contain an *ortho*-substituted isonitrile group. We wondered how the relative position of the isonitrile group would affect the antimicrobial activity and prepared eight analogs with the isonitrile group in *para*- and *meta*-relationship to the double bond (cf. **27**, **28**, **31**, **32**, **34**-**37**). Different antimicrobial activity patterns are observed. For the group of **13**, **36**, and **37**, the *ortho*-substituted compound **13** is still the most potent one against most of the strains tested and slight improvement was observed for the *para*-substituted compound **37** against MSSA (NRS72) and MRSA USA500. Interestingly, for the group of **33**, **34**, and **35**, the *para*-substituted compound **35** is much more active against all the strains tested than the *ortho*- and *meta*-substituted ones. The groups of **27**-**29** and **30**-**31** are less potent than the aforementioned two groups, which indicate that the position of the nitrogen atom in the pyridine ring is important for the observed antimicrobial activity as well.

After completing a preliminary examination of the structure-activity relationship of these compounds, we next moved to assess whether these compounds would retain their activity against several of the most challenging strains of MRSA (Table 1 and Table 2). When tested against an array of clinically-relevant MRSA isolates, the most potent compounds (**6**, **8**-**18**, **20**-**21**, **25**, and **37**) did retain their antimicrobial activity. Indeed these compounds possess potent activity against MRSA isolates prevalent in the healthcare-setting such as MRSA USA100 (responsible for invasive diseases in infected hospitalized patients),[27] and MRSA USA200 (associated with more severe morbidity in affected patients due to the production of toxins that can lead to toxic shock syndrome).[28] In addition to this, these compounds exhibit potent activity against MRSA USA300, a strain that has been linked to the majority of MRSA skin and soft tissue infections present in the community setting.[10,29] Furthermore, these compounds demonstrate strong antimicrobial activity against MRSA strains exhibiting resistance to

numerous antibiotic classes including penicillins, aminoglycosides (NRS1, USA200, and USA500), macrolides (USA100, USA200, USA300, USA500, and USA700), lincosamides (USA100, USA200, USA500), tetracyclines (NRS1, USA300, and USA500), and fluoroquinolones (USA100 and USA500). Additionally, compounds **10**, **11**, **12**, **21**, **25**, **32**, and **35** exhibit potent antimicrobial activity (MIC between 4 and 16 μM) against clinical isolates of *S. aureus* exhibiting resistance to antibiotics deemed agents of last resort, namely vancomycin (VRS2). These results indicate cross-resistance between these antibiotics and the aryl isonitrile compounds is unlikely; this lends further credence to the notion that the aryl isonitrile compounds have potential to be developed as future alternatives to these antibiotics.

Table 1 and Table 2 here.

Toxicity analysis of most potent aryl isonitrile compounds against mammalian cells. Identification of compounds exhibiting potent antimicrobial activity is the first step in a lengthy process for drug development. Many compounds with promising antimicrobial activity fail to advance further in this process due to concerns about toxicity to mammalian tissues. Selective toxicity is a critical feature novel antimicrobial compounds must possess. The ability for antimicrobial agents to exhibit their activity on the target microorganism while not causing harm to host (mammalian) tissues is important to ascertain early in the drug discovery process. To determine if compound **1** and its most potent derivatives against MRSA exhibited toxicity to mammalian tissues, these compounds were screened against a murine macrophage (J774) cell line utilizing the MTS assay (Figure 2). Initial inspection of the structure-activity relationship revealed that the isonitrile moiety appeared to be a vital component in the antimicrobial activity

of these compounds. This was a point of concern given the isonitrile group has been associated with a high degree of toxicity in certain compounds present in nature.[30] However, when the most potent compound, **13** (containing the isonitrile moiety), and its analog **42** (lacking the isonitrile moiety) were tested against J774 cells, they produced identical results (neither compound was toxic up to a concentration of 64 μM). This would indicate that the isonitrile group in these compounds does not contribute to undesirable toxicity to mammalian cells. This result is similar to a study conducted at Bayer AG that found compounds, in their discovery pipeline, containing the isonitrile moiety were not toxic to mice when administered orally or subcutaneously (even at concentrations in excess of 500 mg/kg).[31] In addition to this, at a concentration of 32 μM, all of the compounds tested, with the exception of **25** with a nitro group, were not toxic. When the compounds were tested at a concentration of 64 μM, nineteen out of twenty-three compounds were found to not be toxic to J774 cells (Figure 2). Compounds **11**, **12**, **19**, and **25** were found to be toxic at 64 μM. When the compounds were tested at 128 μM, all compounds were found to be toxic with the exception of compounds **15**, **30**, and **37** (data not presented). For the most active compounds (such as **13**), a 16-to-32 fold difference exists between the concentration at which the compounds exhibit anti-MRSA activity (MIC) compared to the concentration where toxicity is observed.

Figure 2 here.

Preliminary study of physicochemical properties of the isonitrole compounds using kinetic solubility analysis and Caco-2 permeability assay. After confirming that most of the isonitrile compounds exhibited strong antimicrobial activity against MRSA were not toxic to

mammalian cells up to a concentration of 64 µM, we next moved to analyze the physicochemical properties of the most promising compound **13**. These properties play an important role in determining the appropriate route of administration (i.e. systemic vs. local) by which compounds with biological activity can be delivered to the host.[32] Additionally, the physicochemical properties of a compound will have a direct impact on its pharmacokinetic profile (in particular absorption and metabolism), and ability to be translated into a viable drug candidate. Indeed, one study found that 40% of new drug candidates were withdrawn due to issues pertaining to significant pharmacokinetic problems.[33] Compounds possessing a limited physicochemical profile can have issues pertaining to solubility and permeability which can hinder a compound's ability to cross biological membranes, reach the bloodstream, and arrive at the target site of an infection (thus limiting their use systemically).[34]

A kinetic solubility screen (using phosphate-buffered saline) and Caco-2 permeability analysis was performed with compound **13**. The solubility screen determined the highest concentration **13** and three control drugs were capable of being fully dissolved in an aqueous solvent (PBS). As presented in Table 3, this experiment revealed that compound **13** possessed partial aqueous solubility (soluble up to 15.6 µM), identical to the control drugs reserpine and tamoxifen.

Table 3 here.

The Caco-2 permeability assay revealed that compound **13** was not able to permeate across the Caco-2 bilayer. As presented in Table 4, this compound was unable to cross from the apical (A) to basolateral (B) surface of the membrane (apparent permeability, P_{app} = 0.0 cm/sec).

A similar pattern is observed in the basolateral to apical direction with P_{app} = 0.0 cm/sec (indicating this compound is unlikely a substrate for an efflux transporter, like talinolol, which would be one plausible explanation for the inability of this compound to traverse the membrane). This is in stark contrast to the control drug warfarin, which is able to effectively permeate across the membrane from the basolateral to apical surface (P_{app} = 27.0 x 10^{-6} cm/sec). This result is a bit surprising given the size, structure, and calculated partition coefficient (clog P = 4.107) for **13**. Thus, in addition to possessing only partial aqueous solubility, **13** also possesses a poor permeability profile, indicating that, in its present state, this compound would not be suitable for use systemically.

Table 4 here.

The result from the Caco-2 permeability analysis is in agreement with the overall result obtained from the kinetic solubility screen indicating that **13**, though a promising antimicrobial candidate, needs to undergo further structural modifications to enhance its physicochemical profile (in order for it to be used systemically). In addition to modifying the structure of this compound, formulation technology can be utilized to overcome this compound's current limitations. This technology has been used to improve the drug-like properties of promising compounds with similar kinetic profiles to **13** in order to propel these compounds into further stages of drug development. By using a spray drying dispersion technique,[35] the antisolvent crystallization method,[36] or combining the active compound with an excipient (to create an amorphous solid dispersion),[37] the aqueous solubility, permeability and bioavailability profile of this compound can be significantly improved. Identifying that **13** has a problematic

physicochemical profile early in the drug discovery process will permit medicinal chemists and formulation scientists to invest time and effort to enhancing both the physiochemical and pharmacokinetic profiles of this promising new antimicrobial compound.

*Metabolic stability analysis of **13** via microsomal stability analysis.* In addition to studying the solubility and permeability profile of compound **13**, the stability of this compound to metabolic processes present in the liver was investigated using human liver microsomes (Table 5). Drugs administered systemically often are subject to various metabolic processes that can convert the active compound to inactive metabolites. Pharmaceutical compounds that are slow to be metabolized have multiple advantages including an improved pharmacokinetic profile, reduced frequency of doses that need to be given to patients (leading to better patient compliance), while also ensuring the active drug circulates within the patient's system to assist with treating and clearing an infection. As the liver is the primary organ for metabolism of drugs administered systemically in the body, incubating compounds with liver microsomes can shed valuable insight into the stability of these compounds to metabolic processes.[32]

When **13** was incubated with human liver microsomes, it was found to be rapidly metabolized (only 24% of the parent compound remained after one hour) similar to the highly metabolized control drug, verapamil (13% remained after one hour incubation with liver microsomes) (Table 5). While verapamil appeared to be metabolized via a NADPH-mediated process (as 94% of the drug remained after one hour when the co-factor NADPH was removed from the reaction mixture), **13** does not appear to mimic this result as only 51% of the parent compound remained after one hour when NADPH was not present. This would appear to suggest that **13** is metabolized by more than one enzyme system/reaction (one dependent on the co-factor

NADPH (most likely the cytochrome P450 system), and one independent of NADPH). The metabolic stability analysis performed lends further credence to the argument that in their present state, **13**, would not be suitable for use in systemic applications to treat MRSA infections.

Table 5 here.

CONCLUSION

In summary, we have discovered a novel class of aryl isonitrile compounds as promising and potent antimicrobial compounds without apparent toxicity against mammalian cells up to a concentration of 64 μM. Physicochemical profiling, including solubility, membrane permeability, and metabolic stability of one of the most potent compounds, **13**, has been conducted as well. These results indicates that modification of the physical structure of compound **13** is needed to enhance its physicochemical and pharmacokinetic profile so that it can be developed for systemic use against MRSA infections. In addition, identifying other routes of administration (such as topical/local administration) is another avenue to pursue to further develop this promising compound as a novel antimicrobial candidate. Given that *S. aureus* and its resistant strains (including MRSA) are a leading cause of uncomplicated skin infections (such as abscesses, impetigo, and cellulitis),[2,10] it is logical to assess if compound **13**, and its analogs, can be used as topical antimicrobial agents for treatment of MRSA skin infections. Topical agents avoid many concerns relating to solubility, permeability, and systemic toxicity associated with drugs administered orally or intravenously. Future work with these compounds will look to pursue two avenues – determining if these compounds can be used as topical antimicrobial agents for MRSA skin infections and improving the physicochemical profile of these compounds

(via structural modifications or using formulation technology) so they can potentially be used for treatment of more invasive MRSA infections.

ACKNOWLEDGMENT

The authors would like to thank the Network of Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program supported under NIAID/NIH Contract # HHSN272200700055C for providing the MRSA, VISA, and VRSA strains used in this study and the NIH P30CA023168 for supporting shared NMR resources to Purdue Center for Cancer Research. M.D. thanks Purdue University for startup support.

Appendix A. Supplementary data

Supplementary data contain the detailed synthetic procedures, compound characterization data, and biological test procedures.

REFERENCES

(1) Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2013. http://www.cdc.gov/drugresistance/threat-report-2013/.

(2) Giordano, P.; Weber, K.; Gesin, G.; Kubert, J. Skin and skin structure infections: treatment with newer generation fluoroquinolones. *Ther. Clin. Risk Manag.* **2007**, 3, 309-317.

(3) Gillet, Y.; Issartel, B.; Vanhems, P.; Fournet, J. C.; Lina, G.; Bes, M.; Vandenesch, F.; Piémont, Y.; Brousse, N.; Floret, D.; Etienne, J. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet.* **2002**, *359*, 753-759.

(4) Bocchini, C. E.; Hulten, K. G.; Mason, E. O.; Gonzalez, B. E.; Hammerman, W. A.; Kaplan, S. L. Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children. *Pediatrics* **2006**, *117*, 433-440.

(5) David, M. Z.; Daum, R. S. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin. Microbiol. Rev.* **2010**, *23*, 616-687.

(6) Chambers, H. F. Community-associated MRSA-resistance and virulence converge. *N. Engl. J. Med.* **2005**, *352*, 1485-1487.

(7) Frazee, B. W.; Lynn, J.; Charlebois, E. D.; Lambert, L.; Lowery, D.; Perdreau-Remington, F. High prevalence of methicillin-resistant *Staphylococcus aureus* in emergency department skin and soft tissue infections. *Ann. Emerg. Med.* **2005**, *45*, 311-320.

(8) Han, L. L.; McDougal, L. K.; Gorwitz, R. J.; Mayer, K. H.; Patel, J. B.; Sennott, J. M.; Fontana, J. L. High frequencies of clindamycin and tetracycline resistance in methicillin-resistant *Staphylococcus aureus* pulsed-field type USA300 isolates collected at a Boston ambulatory health center. *J. Clin. microbiol.* **2007**, *45*, 1350-1352.

(9) Hiramatsu, K. Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance. *Lancet Infect. Dis.* **2001**, *1*, 147-155.

(10) Moran, G. J.; Krishnadasan, A.; Gorwitz, R. J.; Fosheim, G. E.; McDougal, L. K.; Carey, R. B.; Talan, D. A. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N. Engl. J. Med.* **2006**, *355*, 666-674.

(11) Wilson, P.; Andrews, J. A.; Charlesworth, R.; Walesby, R.; Singer, M.; Farrell, D. J.; Robbins, M. Linezolid resistance in clinical isolates of *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **2003**, *51*, 186-187.

(12) Podoll, J. D.; Liu, Y.; Chang, L.; Walls, S.; Wang, W.; Wang, X. Bio-inspired synthesis yields a tricyclic indoline that selectively resensitizes methicillin-resistant *Staphylococcus aureus* (MRSA) to β-lactam antibiotics. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 15573-15578.

(13) Marconi, G. G.; Molloy, B. B.; Nagarajan, R.; Martin, J. W.; Deeter, J. B.; Occolowitz, J. L. A32390A, a new biologically active metabolite. II. Isolation and structure. *J. Antibiot.* **1978**, *31*, 27-32.

(14) Mo, S.; Krunic, A.; Chlipala, G.; Orjala, J. Antimicrobial ambiguine isonitriles from the cyanobacterium *Fischerella ambigua*. *J. Nat. Prod.* **2009**, *72*, 894-899.

(15) Raveh, A.; Carmeli, S. Antimicrobial ambiguines from the cyanobacterium *Fischerella sp.* collected in Israel. *J. Nat. Prod.* **2007**, *70*, 196-201.

(16) Sugawara, T.; Tanaka, A.; Imai, H.; Nagai, K.; Suzuki, K. YM-47515, a novel isonitrile antibiotic from *Micromonospora echinospora* subsp. *echinospora*. *J. Antibiot.* **1997**, *50*, 944-948.

(17) Fujiwara, A.; Okuda, T.; Masuda, S.; Shiomi, Y.; Miyamoto, C.; Sekine, Y.; Tazoe, M.; Fujiwara, M. Fermentation, isolation and characterization of isonitrile antibiotics. *Agric. Biol. Chem.* **1982**, *46*, 1803-1809.

(18) Marquez, J. A.; Horan, A. C.; Kalyanpur, M.; Lee, B. K.; Loebenberg, D.; Miller, G. H.; Patel, M.; Waitz, J. A. The hazimicins, a new class of antibiotics taxonomy, fermentation, isolation, characterization, and biological properties. *J. Antibiot.* **1983**, *36*, 1101-1108.

(19) Bettley, F. R. Xanthocillin cream for local treatment. *Br. Med. J.* **1959**, *1*, 1226-1227.

(20) Wright, A. D.; Wang, H.; Gurrath, G.; König, G. M.; Kocak, G.; Neumann, G.; Loria, P.; Foley, M.; Tilley, L. Inhibition of heme detoxification processes underlies the antimalarial activity of terpene isonitrile compounds from marine sponges. *J. Med. Chem.* **2001**, *44*, 873-885.

(21) Schwarz, O.; Brun, R.; Bats, J. W.; Schmalz, H. Synthesis and biological evaluation of new antimalarial isonitriles related to marine diterpenoids. *Tetrahedron Lett.* **2002**, *43*, 1009-1013.

(22) Singh, C.; Srivastav, N. C.; Puri, S. K. In vivo active antimalarial isonitriles. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2277-2279.

(23) Arbuzov, B. A. Michaelis–Arbusow- und Perkow-Reaktionen. *Pure. Appl. Chem.* **1964**, *9*, 307-353.

(24) Weber, W. P.; Gokel, G. W. An improved procedure for the Hofmann carbylamine synthesis of isonitriles. Tetrahedron Lett. **1972**, 1637-1640.

(25) Zhang, B.; Studer, A. 2-Trifluoromethylated indoles via radical trifluoromethylation of isonitriles. *Org. Lett.* **2014**, *16*, 1216-1219.

(26) Zhang, B.; Mück-Lichtenfeld, C.; Daniliuc, C. G.; Studer, A. 6-Trifluoromethyl-phenanthridines through radical trifluoromethylation of isonitriles. *Angew. Chem. Int. Ed.* **2013**, *52*, 10792-10795.

(27) 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*, **2007**, *298*, 1763-1771.

(28) Lin, Y. C.; Anderson, M. J.; Kohler, P. L.; Strandberg, K. L.; Olson, M. E.; Horswill, A. R.; Schlievert, P. M.; Peterson, M. L. Proinflammatory exoprotein characterization of toxic shock syndrome *Staphylococcus aureus*. *Biochemistry*, **2011**, *50*, 7157-7167.

(29) McDougal, L. K.; Steward, C. D.; Killgore, G. E.; Chaitram, J. M.; McAllister, S. K.; Tenover, F. C. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: Establishing a national database. *J. Clin. Microbiol.* **2003**, *41*, 5113-5120.

(30) Goda, M.; Hashimoto, Y.; Shimizu, S.; Kobayashi, M. Discovery of a novel enzyme, isonitrile hydratase, involved in nitrogen-carbon triple bond cleavage. *J. Biol. Chem.* **2001**, *276*, 23480-23485.

(31) Ugi, I.; Fetzer, U.; Eholzer, U.; Knupfer, H.; Offerman, K. Isonitrile Syntheses. *Angew. Chem. Int. Ed.* **1965**, *4*, 472-484.

(32) Kerns, E. H.; Di, L. Drug-like properties: concepts, structure design and methods- from ADME to toxicity optimization. Amsterdam; Boston: Academic Press; 2008.

(33) Prentis, R. A.; Lis, Y.; Walker, S. R. Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964-1985). *Br. J. Clin. Pharmacol.* **1988**, *25*, 387-396.

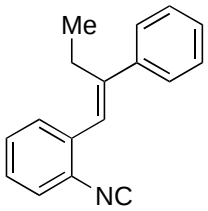
(34) Lin, J. H.; Lu, A. Y. Role of pharmacokinetics and metabolism in drug discovery and development. *Pharmacol. Rev.* **1997**, *49*, 403-449.

(35) Kwong, A. D.; Kauffman, R. S.; Hurter, P.; Mueller, P. Discovery and development of telaprevir: an NS3-4A protease inhibitor for treating genotype 1 chronic hepatitis C virus. *Nat. Biotechnol.* **2011**, *29*, 993-1003.

(36) Lonare, A. A.; Patel, S. R. Antisolvent Crystallization of Poorly Water Soluble Drugs. *Int. J. Chem. Eng. Appl.* **2013**, *4*, 337-341.

(37) Van den Mooter, G. The use of amorphous solid dispersions: A formulation strategy to overcome poor solubility and dissolution rate. *Drug Discov. Today. Technol.* **2012**, 9, e71-e174.

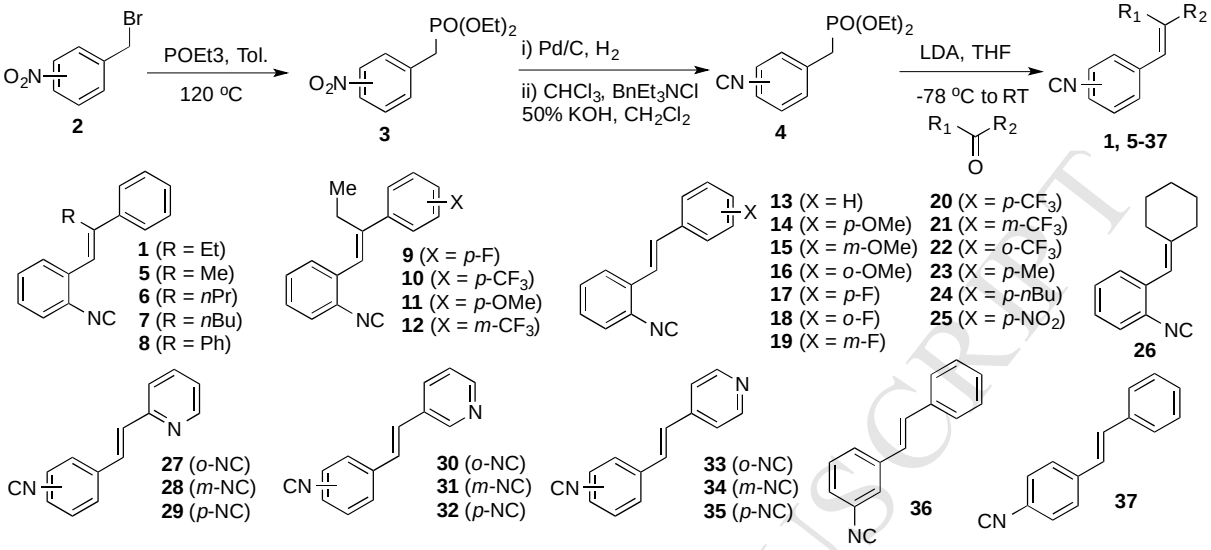
Schemes, Figures and Tables (see below)



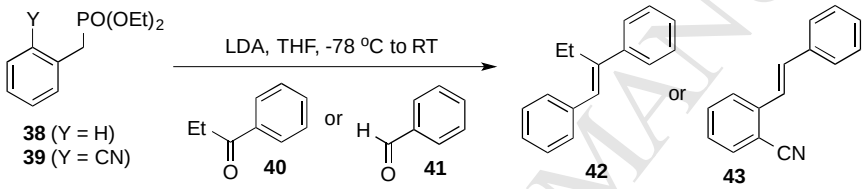
1

FIGURE 1. Structure of hit compound **1**.

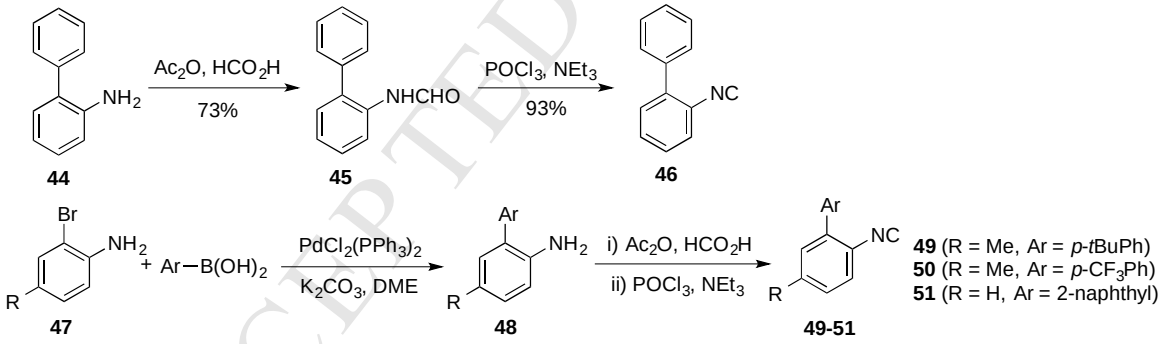
I. Synthesis of compounds **1**and **5-37**



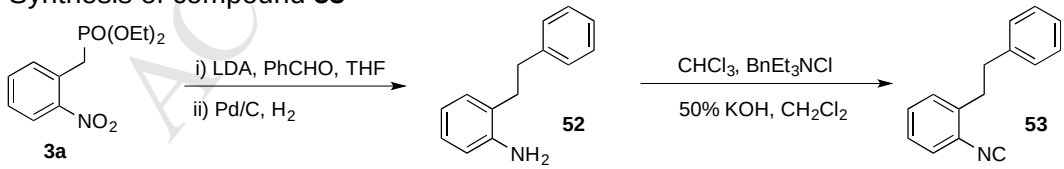
II. Synthesis of compounds **42** and **43**



III. Synthesis of compounds **46** and **38-40**



IV. Synthesis of compound **53**



SCHEME 1. Synthesis of Analogs of Lead Compound **1**.

Table 1: Strains of *Staphylococcus aureus* utilized in this study.

Strain Name		Isolation		Molecular Typing		Antimicrobial Resistance Phenotype
NARSA ID[1]	**Alternate Designation**	**Origin**	**Source**	**SCC*mec* type**	***spa* type**	
NRS1	ATCC700699 VISA	Japan	-	II	TJMBMDMGMK	Resistant to aminoglycosides and tetracycline (minocycline) Glycopeptide-intermediate *S. aureus*
NRS72[2]	MSSA 476	-	-	-	UKJFKBPE	None
NRS119	SA LinR #12	United States (Massachusetts)	Dialysis-associated peritonitis	IV	YHGCMBQBLO	Resistant to linezolid
NRS382	USA100	United States (Ohio)	Bloodstream	II	TJMBMDMGMK	Resistant to erythromycin, clindamycin and levofloxacin
NRS383	USA200	United States (North Carolina)	Bloodstream	II	WGKAKAOMQQQ	Resistant to erythromycin, clindamycin and gentamicin
NRS384	USA300-0114	United States (Mississippi)	Wound	IV	YHGFMBQBLO	Resistant to erythromycin, methicillin, and tetracycline
NRS385	USA500	United States (Connecticut)	Bloodstream	IV	YHGCMBQBLO	Resistant to erythromycin, clindamycin, trimethoprim/sulfamethoxazole, levofloxacin, gentamicin and tetracycline
NRS386	USA700	United States (Louisiana)	Bloodstream	IV	UJGFMGGM	Resistant to erythromycin and methicillin
VRS2	VRSA	United States (Pennsylvania)	Plantar ulcer	II	TJMBMDMGMK	Resistant to vancomycin

[1] NARSA = Network on Antimicrobial Resistance in *Staphylococcus aureus.*

[2] NRS72 = Methicillin-sensitive *Staphylococcus aureus* (MSSA).

Table 2: Minimum inhibitory concentration (MIC, in μM) of isonitrile compounds, linezolid, and vancomycin against methicillin-sensitive (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

Compound Name	MSSA[1] (NRS72)	MRSA USA100	MRSA USA200	MRSA USA300	MRSA USA500	MRSA USA700	MRSA NRS119	VISA[1] NRS1	VRSA[2] VRS2
1	8	8	16	32	32	8	64	8	32
4a	>128	>128	128	>128	>128	>128	>128	128	>128
5	8	8	64	>128	>128	16	>128	4	>128
6	16	16	16	32	16	16	32	8	32
7	32	32	32	>128	32	16	>128	16	64
8	8	8	8	16	8	4	16	4	16
9	8	16	16	32	16	8	64	8	32
10	8	16	16	32	16	16	64	8	16
11	8	8	8	16	8	4	>128	4	8
12	4	4	8	>128	8	2	8	2	8
13	16	2	4	4	32	4	4	2	32
14	4	8	2	16	>128	8	32	4	32
15	8	8	4	16	32	16	32	4	32
16	8	8	8	8	8	8	16	4	16
17	4	8	2	8	16	8	16	4	32
18	4	8	2	16	16	8	32	4	32
19	8	16	16	32	128	16	128	8	128
20	4	4	2	8	16	2	16	2	16
21	2	4	4	8	8	4	8	2	8
22	8	16	16	32	16	16	>128	8	32
23	16	4	4	16	32	8	64	4	32
24	>128	-	>128	>128	>128	-	>128	-	>128
25	2	4	4	8	8	8	16	4	8
26	8	16	64	>128	>128	16	>128	4	>128
27	64	64	64	16	64	32	128	64	32
28	64	32	64	32	64	64	128	128	128
29	64	128	128	32	64	128	128	64	64
30	64	32	32	16	32	32	64	64	32
31	64	32	64	32	64	64	64	64	64
32	16	16	32	8	32	16	64	32	8
33	64	32	32	32	32	32	64	64	64
34	64	32	64	32	64	64	32	32	64
35	2	8	8	4	4	8	4	8	4
36	16	32	32	16	>128	16	64	32	32
37	4	4	32	4	16	64	>128	>128	>128
42	>128	>128	>128	>128	>128	>128	>128	>128	>128
43	>128	>128	>128	>128	>128	>128	>128	>128	>128
46	>128	16	16	>128	>128	16	>128	8	>128
49	>128	16	16	>128	>128	16	64	4	>128
50	>128	>128	>128	>128	>128	>128	>128	>128	>128
51	16	16	64	64	32	16	32	4	64
52	>128	>128	32	>128	>64	>64	>128	8	>64
53	64	4	16	4	128	32	2	2	64
Linezolid	2	<1	2	2	<1	2	64	<1	<1
Vancomycin	<1	2	-	-	4	-	-	8	128

[1]VISA = Vancomycin-intermediate *Staphylococcus aureus*.
[2]VRSA = Vancomycin-resistant *Staphylococcus aureus*.

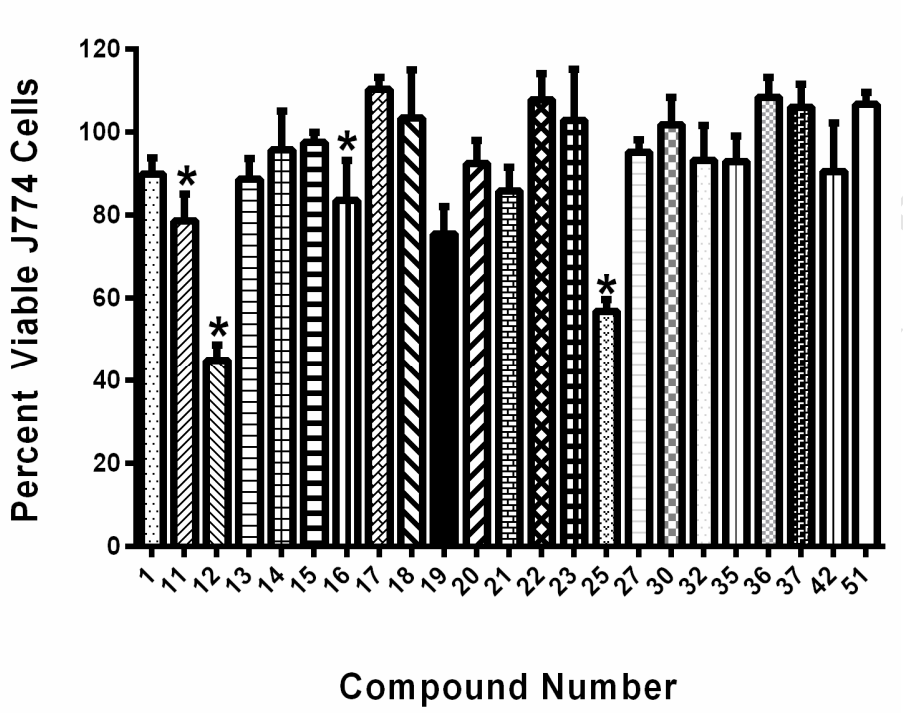


Figure 2: Percent viable mammalian cells (measured as average absorbance ratio (test agent relative to DMSO)) for cytotoxicity analysis of compounds **1**, **11**-**23**, **25**, **27**, **30**, **32**, **35**, **36**, **37**, **42**, and **51** at 64 µM. Compounds were tested against J774 cells using the MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) assay. DMSO was used as a negative control to determine a baseline measurement for the toxic impact of each compound. The values represent an average of three samples analyzed for each compound. Error bars represent standard deviation values for the absorbance values. Asterisks (*) indicate a statistical difference between the values obtained for the compound relative to the cells treated with DMSO ($P < 0.05$).

Table 3: Kinetic solubility assessment of compound 13, reserpine, tamoxifen, and verapamil in phosphate-buffered saline (PBS).

Compound Tested	Solubility Limit (μM)[1]	Solubility Analysis
13	15.6	Low solubility
Reserpine	15.6	Low solubility
Tamoxifen	15.6	Low solubility
Verapamil	>500	High solubility

[1] Solubility limit corresponds to the highest concentration of test compound where no precipitate was detected.

Table 4: Permeability analysis of compound 13, ranitidine, warfarin, and talinolol via the Caco-2 permeability assay.

Compound/Drug Tested	Mean A → B[1] P_{app} (10^{-6} cm/sec)	Mean B → A[2] P_{app} (10^{-6} cm/sec)	Efflux Ratio[3]	Permeability Analysis
13	0.0[4]	0.0	N/A[5]	Not permeable
Ranitidine	0.23	3.1	13.5	Low permeability
Warfarin	27.0	7.2	0.3	High permeability
Talinolol	0.05	8.9	178	P-gp[6] efflux control

[1] Mean A → B P_{app} = mean apparent permeability of test compound from apical to basolateral surface

[2] Mean B → A P_{app} = mean apparent permeability of test compound from basolateral to apical surface

[3] Efflux ratio = $\frac{Papp(B \rightarrow A)}{Papp(A \rightarrow B)}$

[4] Compound not detected in receiver compartment

[5] N/A, not applicable

[6] P-gp, P--glycoprotein

Table 5: Evaluation of metabolic stability of compound 13, verapamil, and warfarin in human liver microsomes.

Compound/Drug Tested	Average remaining after 60 min (%), with NADPH	Average remaining after 60 min (%), without NADPH	Notes
13	24	51	-
Verapamil	13	94	High metabolism control
Warfarin	93	94	Low metabolism control

Highlights

1. Evaluated over forty aryl isonitrile compounds against MRSA and VRSA strains.
2. Identified compounds with MIC as low as 2 μM but no cytotoxicity at 64 μM.
3. Established SAR of these novel isonitrile compounds.
4. Profiled the most potent compound's physicochemical properties.

Supporting Information For:

Discovery and Characterization of Aryl Isonitriles as A New Class of Compounds versus Methicillin- and Vancomycin-resistant *Staphylococcus aureus*

Dexter C. Davis, Haroon Mohammad, Kwaku Kyei-Baffour, Waleed Younis, Cassidy Noel Creemer, Mohamed N. Seleem, and Mingji Dai

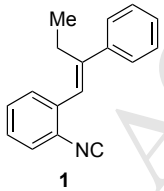
Table of Contents

Part I. Synthetic Procedures and Spectra Data

General Methods.

Reactions were performed using standard syringe techniques under argon unless stated otherwise. Starting materials and reagents were used as received from suppliers (Aldrich, Alfa Aeser, Acros). Anhydrous THF was distilled over sodium benzophenone under argon. Acetonitrile (CH_3CN), dichloromethane (CH_2Cl_2), methanol (MeOH), and toluene were purified by passing the previously degassed solvents through activated alumina columns. Flash chromatography was performed using silica gel (230-400 mesh). Thin layer chromatography (TLC) was performed using glass-backed silica plates (Silicycle). NMR spectra were recorded on a *Bruker ARX-300, Bruker ARX-400* spectrometer, *DRX-500* or *AV-500* spectrometer at room temperature. Chemical shifts (in ppm) are given in reference to the solvent signal [^{1}H NMR: $CDCl_3$ (7.26); ^{13}C NMR: $CDCl_3$ (77.2).]. ^{1}H NMR data are reported as follows: chemical shifts (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (Hz), and integration. ^{13}C NMR data are reported in terms of chemical shift and multiplicity. IR data were recorded on a Thermo Nicolet Nexus 470 FTIR.

A Representative Procedure for the Synthesis of Aryl Isonitriles:



1

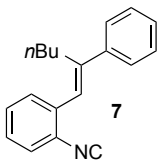
(E)-1-isocyano-2-(2-phenylbut-1-en-1-yl)benzene (1)[1]

To a stirred solution of diisopropyl amine (52 mg, 0.52 mmol) in THF (1.3 ml) was added a solution of *n*-BuLi (2.5 M in hexane, 0.174 ml, 0.43 mmol) dropwise at -78 ^{o}C. After stirring for 5 min, a solution of diethyl (2-isocyanobenzyl)phosphonate **4** (100 mg, 0.395 mmol) in THF (1 ml) was added dropwise

at -78 ^{o}C. The resulting solution was stirred for an additional 30 min and a solution of propiophenone (48 mg, 0.36 mmol) in THF (1 ml) was added dropwise. The reaction was stirred for an additional 30 min at -78 ^{o}C then warmed to room temperature and stirred for 1 h. A saturated aqueous ammonium chloride solution (4 ml) and Et_2O (4 ml) were added. The aqueous layer was extracted with Et_2O (3 x 5 ml) and the combined organic layers were washed with brine (10 ml), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (CH_2Cl_2/hexane = 1/4) to yield (E)-1-isocyano-2-(2-phenylbut-1-en-1-yl)benzene **1** (50 mg, 60% yield.)

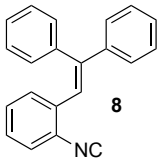
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.17 (m, 4H), 7.20-6.99 (m, 3H), 6.96 (td, *J* = 7.7, 1.4 Hz, 1H), 6.78 (dd, *J* = 7.9, 1.5 Hz, 1H), 6.63 (s, 1H), 2.62 (qd, *J* = 7.4, 1.5 Hz, 2H), 1.14 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 149.1, 140.3, 134.7, 130.2, 128.3 (3C), 128.2, 127.2, 126.7, 126.5, 119.7, 33.0, 12.8. The spectral data matches that reported by Studer and coworkers.[1]

Spectra Data of New Aryl Isonitriles:



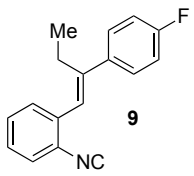
(E)-1-isocyano-2-(2-phenylhex-1-en-1-yl)benzene (7)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66-7.19 (m, 4H), 7.19-7.04 (m, 3H), 6.95 (td, *J* = 7.7, 1.3 Hz, 1H), 6.75 (d, *J* = 10 Hz, 1H), 6.60 (s, 1H), 2.59 (t, *J* = 7.5 Hz, 2H), 1.32-1.56 (m, 4H), 0.91 (t, *J* = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.9, 147.8, 140.3, 134.9, 130.3, 128.5 (2C), 128.4, 128.3, 127.3, 126.8, 126.6, 120.8, 39.8, 30.0, 22.2, 13.9; IR (neat): 2956, 2925, 2854, 2117, 1478, 1448 cm^{-1}; MS (ESI): *m/z* = 284.14 calc. for $C_{19}H_{19}N[M+Na]^+$, found 284.24.



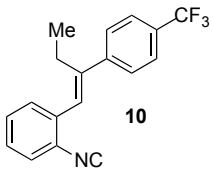
(2-(2-isocyanophenyl)ethene-1,1-diyl)dibenzene (8)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.28 (m, 8H), 7.16-7.11 (m, 3H), 7.10 (s, 1H), 7.00 (dt, *J* = 8.2, 1.1 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5, 146.7, 142.4, 139.5, 134.6, 130.4, 130.2, 128.5, 128.4, 128.3 (3C), 128.1, 127.9, 127.3, 126.9, 122.0; IR (neat) 3059, 3023, 2923, 2853, 2116, 1491, 1473, 1443, 1280, 1114, 1027, 942, 885 cm^{-1}; MS (ESI): *m/z* = 304.11 calc. for $C_{21}H_{15}N[M+Na]^+$, found 304.24.



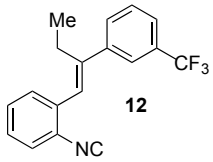
(E)-1-(2-(4-fluorophenyl)but-1-en-1-yl)-2-isocyanobenzene (9)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 7.07-7.04 (m, 2H), 7.00 (t, *J* = 7.8 Hz, 1H), 6.94 (m, 2H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.59 (s, 1H), 2.58 (qd, *J* = 7.4, 1.4 Hz, 2H), 1.11 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.0 162.0 (d, *J* = 244 Hz), 148.0, 136.1 (d, *J* = 4 Hz), 134.6, 130.3, 130.1 (d, *J* = 8 Hz), 128.4, 126.9, 126.6, 125.6, 120.2, 115.4 (d, *J* = 21 Hz), 32.9, 12.8; IR (neat) 2967, 2930, 2117, 1602, 1507, 1222, 1178, 879, 836 cm^{-1}; MS (ESI): *m/z* = 274.10 calc. for $C_{17}H_{14}FN[M+Na]^+$, found 274.16.



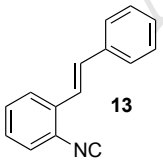
(E)-1-isocyano-2-(2-(4-(trifluoromethyl)phenyl)but-1-en-1-yl)benzene (10)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.0 Hz, 2H), 7.31 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.12 (td, *J* = 7.7, 1.4 Hz, 1H), 7.01 (td, *J* = 7.7, 1.3 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.67 (s, 1H), 2.61 (qd, *J* = 7.4, 1.5 Hz, 3H), 1.12 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 147.6, 144.1, 134.1, 130.1, 128.7 (2C), 128.5, 127.2, 126.6, 126.1 (q, *J* = 269 Hz), 125.30, 125.27, 121.1, 32.6, 12.7; IR (neat) 2968, 2931, 2118, 1322, 1164, 1109, 1066, 881, 843 cm^{-1}; MS (ESI): *m/z* = 324.10 calc. for $C_{18}H_{14}F_3N[M+Na]^+$, found 324.08.



(E)-1-isocyano-2-(2-(3-(trifluoromethyl)phenyl)but-1-en-1-yl)benzene (12)

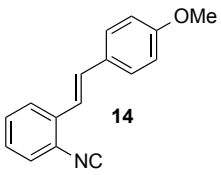
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.38-7.26 (m, 4H), 7.12 (td, *J* = 7.7, 1.4 Hz, 1H), 7.00 (td, *J* = 7.7, 1.4 Hz, 1H), 6.67-6.72 (m, 2H), 2.63 (qd, *J* = 7.4, 1.5 Hz, 2H), 1.14 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.6, 147.7, 141.3, 134.4, 132.2, 131.1, 130.9, 130.4, 129.1, 128.7, 127.5, 126.9, 126.2 (q, *J* = 267 Hz), 125.4, 124.3, 121.4, 32.6, 12.8; IR (neat) 2969, 2118, 1324, 1202, 1163, 1072, 903, 828 cm^{-1}; MS (ESI): *m/*z = 324.10 calc. for $C_{18}H_{14}F_3N[M+Na]^+$, found 324.08.



(E)-1-isocyano-2-styrylbenzene (13)

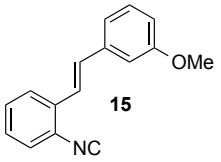
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.64-7.55 (m, 2H), 7.49-7.13 (m, 8H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 136.4, 133.7, 132.7, 129.4, 128.8, 128.6, 128.0, 127.3, 127.0, 125.4, 125.0, 122.2; IR (neat) 3044, 3022, 2119, 1632, 1598, 1480, 1446, 1288, 1263, 1221, 1198, 1092, 959, 875 cm^{-1}; MS (ESI): *m/z* = 228.08 calc. for $C_{15}H_{11}N[M+Na]^+$, found 228.00.



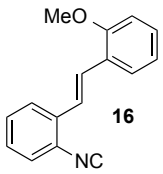
(E)-1-isocyano-2-(4-methoxystyryl)benzene (14)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.56-7.50 (m, 2H), 7.41-7.36 (m, 2H), 7.27-7.11 (m, 3H), 6.96-6.89 (m, 2H), 3.85 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.7, 160.0, 134.1, 132.2, 129.4, 129.2, 128.4, 127.5, 127.3, 127.0, 125.2, 112.0, 114.3, 55.4; IR (neat) 2924, 2843, 2122, 1633, 1604, 1511, 1481, 1270, 1253, 1172, 1091, 961, 868 cm^{-1}; MS (ESI): *m/z* = 258.09 calc. for $C_{16}H_{13}NO[M+Na]^+$, found 258.10.



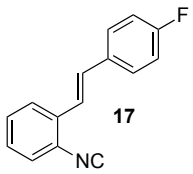
(E)-1-isocyano-2-(3-methoxystyryl)benzene (15)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 7.9 Hz, 1H), 7.42-7.36 (m, 3H), 7.32-7.26 (m, 2H), 7.19 (s, 1H), 7.16 (d, *J* = 7.8 Hz, 1H), 7.10 (m, 1H), 6.88 (dd, *J* = 8.2, 2.4 Hz, 1H), 3.86 (s 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0, 159.9, 137.8, 133.6, 132.6, 129.8, 129.4, 128.0, 127.3, 125.5, 124.9, 122.5, 119.6, 114.2, 112.2, 55.3; IR (neat) 3072, 3034, 2119, 1607, 1581, 1489, 1482, 1448, 1286, 1239, 1157, 1135, 1093, 941, 873 cm^{-1}; MS (ESI): *m/z* = 258.09 calc. for $C_{16}H_{13}NO[M+Na]^+$, found 258.00.



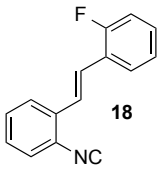
(E)-1-isocyano-2-(2-methoxystyryl)benzene (16)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.0 Hz, 1H); 7.66 (d, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 16.4 Hz, 1H), 7.46 (d, *J* = 16.5 Hz, 1H), 7.40-7.37 (m, 2H), 7.31 (t, *J* = 8.2 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.00 (t, *J* = 7.6 Hz, 1H), 6.93 (d, *J* = 8.2 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.6, 157.2, 134.3, 129.6, 129.2, 127.6, 127.5, 127.1 (2C), 125.4 (2C), 124.8, 122.5, 120.8, 110.9, 55.4; IR (neat) 3033, 2958, 2936, 2834, 2119, 1489, 1463, 1334, 1242, 1191, 1107, 1091, 1031, 992, 965, 879 cm^{-1}; MS (ESI): *m/z* = 258.09 calc. for $C_{16}H_{13}NO[M+Na]^+$, found 258.10.



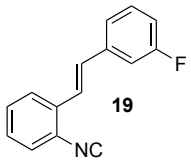
(E)-1-(4-fluorostyryl)-2-isocyanobenzene (17)

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.72 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.58-7.52 (m, 2H), 7.40-7.37 (m, 2H), 7.33 (d, *J* = 16.3 Hz, 1H), 7.28 (td, *J* = 7.6, 1.1Hz, 1H), 7.16 (d, *J* = 16.3 Hz, 1H), 7.10-7.06 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0, 162.9 (d, *J* = 287 Hz), 133.5, 132.6 (d, *J* = 3 Hz), 131.4, 129.4, 128.61, 128.55, 128.0, 127.3, 125.3, 121.9, 115.8 (d, *J* = 22 Hz); IR (neat) 3045, 2926, 2122, 1567, 1508, 1479, 1265, 1232, 1177, 1159, 960, 933, 817; MS (ESI): *m/z* = 224.09 calc. for $C_{15}H_{10}FN[M+H]$, found 224.08.



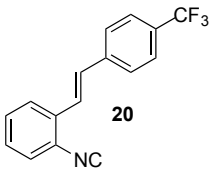
(E)-1-fluoro-2-(2-isocyanostyryl)benzene (18)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.70 (td, *J* = 7.7, 1.7 Hz, 1H), 7.48 (d, *J* = 16.5 Hz, 1H), 7.42-7.37 (m, 3H), 7.30-7.27 (m, 2H), 7.18 (td, *J* = 7.6, 1.2 Hz, 1H), 7.10 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2, 160.5 (d, *J* = 249 Hz), 133.6, 129.9 (d, *J* = 8 Hz), 129.4, 128.3, 127.2, 127.17 (d, *J* = 3 Hz), 125.5, 124.7 (d, *J* = 3 Hz), 125.0, 124.4, 124.3, 124.1 (d, *J* = 3 Hz), 115.8 (d, *J* = 22 Hz); IR (neat) 3057, 2924, 2122, 1635, 1487, 1476, 1452, 1336, 1283, 123, 1212, 1190, 1090, 960, 868 cm^{-1}; MS (ESI): *m/z* = 246.07 calc. for $C_{15}H_{10}FN[M+Na]^+$, found 246.00.



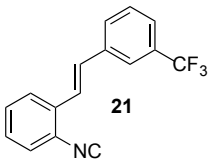
(E)-1-(3-fluorostyryl)-2-isocyanobenzene (19)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.42-7.25 (m, 7H), 7.16 (d, *J* = 16.3 Hz, 1H), 7.01-7.04 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.3, 163.2 (d, *J* = 244 Hz), 138.7 (d, *J* = 7.3 Hz), 133.2, 131.5, 130.3 (d, *J* = 8 Hz), 129.5, 128.4, 127.3, 125.6, 125.1, 123.5, 122.8, 115.4 (d, *J* = 21 Hz), 113.5 (d, *J* = 22 Hz); IR (neat) 3072, 3035, 2122, 1608, 1581, 1482, 1448, 1286, 1238, 1183, 1158, 941, 874, 864, 834; MS (ESI): *m/z* = 246.07 calc. for $C_{15}H_{10}FN$, found 246.00.



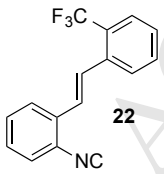
(E)-1-isocyano-2-(4-(trifluoromethyl)styryl)benzene (20)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.70-7.61 (m, 4H), 7.50 (d, *J* = 16.3 Hz, 1H), 7.42-7.41 (m, 2H), 7.32 (t, *J* = 6.8 Hz, 1H), 7.22 (d, *J* = 16.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.5, 139.8, 133.0, 131.0, 130.2 (q, *J* = 32 Hz), 129.5, 128.7, 127.4, 127.1, 125.8 (q, *J* = 4 Hz), 125.6, 125.1, 124.7, 124.1 (q, *J* = 270 Hz); IR (neat) 2924, 2123, 1614, 1483, 1321, 1190, 1155, 1104, 1065, 989, 964, 839 cm^{-1}; MS (ESI): *m/z* = 296.07 calc. for $C_{16}H_{10}FN[M+Na]^+$, found 295.84.



(E)-1-isocyano-2-(3-(trifluoromethyl)styryl)benzene (21)

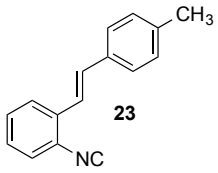
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82-7.77 (m, 3H), 7.61-7.45 (m, 5H), 7.36 (t, *J* = 7.5 Hz, 1H), 7.25 (d, *J* = 16.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.3, 137.2, 133.1, 131.3 (q, *J* = 31 Hz), 131.1, 129.6, 129.5, 129.3, 128.6, 127.4, 125.6, 125.02, 124.99, 124.0, 124.0 (q, *J* = 271 Hz), 123.9; IR (neat) 3049, 2118, 1489, 1341, 1324, 1287, 1224, 1163, 1194, 1114, 1093, 998, 983, 962 cm^{-1}; MS (ESI): *m/z* = 296.07 calc. for $C_{16}H_{10}FN[M+Na]^+$, found 296.08.



(E)-1-isocyano-2-(2-(trifluoromethyl)styryl)benzene (22)

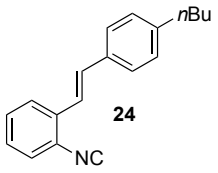
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, *J* = 7.8 Hz, 1H), 7.76 (d, *J* = 7.9 Hz, 1H), 7.70 (d, *J* = 7.9 Hz, 1H), 7.61-7.54 (m, 2H), 7.45-7.38 (m, 4H), 7.33 (td, *J* = 8.0, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$), δ 167.2, 135.4, 133.2, 132.1, 129.6, 128.7, 128.3, 128.1, 127.9 (q, *J* = 29 Hz), 127.5, 127.3, 126.1,

126.0 (q, *J* = 5.4 Hz), 125.9, 125.3, 124.3 (q, *J* = 269 Hz); IR (neat) 3065, 2926, 2125, 1490, 1311, 1291, 1227, 1202, 1060, 1033, 959 cm^{-1}; MS (ESI): *m/z* = 296.07 calc. for $C_{16}H_{10}F_3N[M+Na]^+$, found 296.16.



(E)-1-isocyano-2-(4-methylstyryl)benzene (23)

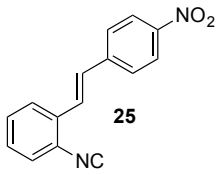
^1H NMR (500 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.48 (d, *J* = 8.1 Hz, 2H), 7.35-7.41 (m, 3H), 7.26 (td, *J* = 7.4, 1.2 Hz, 1H), 7.20 (d, *J* = 7.1 Hz, 2H), 7.18 (d, *J* = 16.0 Hz, 1H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.8, 138.7, 133.9, 133.6, 132.6, 129.5, 129.4, 127.7, 127.3, 126.9, 125.3, 124.8, 121.1, 21.3; IR (neat) 3023, 2919, 2115, 1630, 1510, 1478, 1445, 1290, 1110, 959, 839 cm^{-1}; MS (ESI): *m/z* = 242.09 calc. for $C_{16}H_{13}N[M+Na]^+$, found 242.00.



(E)-1-(4-butylstyryl)-2-isocyanobenzene (24)

^1H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 7.9 Hz, 2H), 7.36-7.30 (m, 3H), 7.26 (t, *J* = 7.4 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 2H), 7.19 (d, *J* = 15.8 Hz, 1H), 2.64 (t, *J* = 7.8 Hz, 2H), 1.63 (m, 2H), 1.39 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.9, 143.8, 134.0, 133.9, 132.7, 129.4, 128.9, 127.8, 127.3, 127.0, 125.4, 124.8, 121.2, 35.5, 33.6, 22.4, 14.0; IR (neat) 2956, 2928, 2857, 2116, 1736, 1632, 1608, 1480, 1449, 1265, 1241, 1018, 962, 854 cm^{-1}; MS

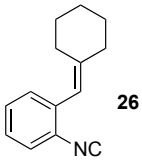
(ESI): *m/z* = 284.14 calc. for $C_{19}H_{19}N[M+Na]^+$, found 284.16.



25

(E)-1-isocyano-2-(4-nitrostyryl)benzene (25)

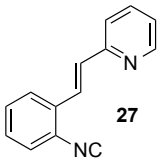
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, *J* = 11.7 Hz, 2H), 7.76 (d, *J* = 10.5 Hz, 1H), 7.70 (d, *J* = 11.7 Hz, 2H), 7.56 (d, *J* = 21.8 Hz, 1H), 7.30-7.46 (m, 3H), 7.24 (d, *J* = 21.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.8, 147.4, 142.7, 132.5, 130.2, 129.6, 129.2, 127.5 (2C), 126.6, 125.8, 124.2, 123.7; IR (neat) 2923, 2842, 2121, 1632, 1510, 1372, 1340, 1299, 1269, 1253, 1226, 1172, 1108, 1091, 1026, 959, 886, 870; MS (ESI): *m/z* = 273.06 calc. for $C_{15}H_{10}N_2O_2[M+Na]^+$, found 273.12.



26

1-(cyclohexylidenemethyl)-2-isocyanobenzene (26)

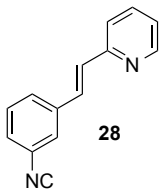
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.34 (m, 1H), 7.32 (d, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.5 Hz, 1H), 7.27-7.20 (m, 1H), 2.33 (t, *J* = 6.1 Hz, 2H), 2.25 (t, *J* = 5.6 Hz, 2H), 1.70-1.52 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 147.2, 135.3, 130.3, 128.6, 126.7, 126.6, 125.8, 116.9, 37.3, 29.8, 28.4, 27.7, 26.4; IR (neat) 2927, 2853, 2118, 1479, 1461, 1445, 1343, 1038, 838 cm^{-1}; MS (ESI): *m/z* = 220.11 calc. for $C_{14}H_{15}N[M+Na]^+$, found 220.08.



27

(*E*)-2-(2-isocyanostyryl)pyridine (27)

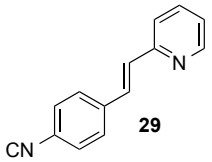
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65-8.63 (m, 1H), 7.88 (d, *J* = 16.2 Hz, 1H), 7.80-7.76 (m, 1H), 7.71 (td, *J* = 7.7, 1.8 Hz, 1H), 7.53 (dt, *J* = 7.9, 1.1 Hz, 1H), 7.45-7.38 (m, 2H), 7.35-7.28 (m, 2H), 7.21 (ddd, *J* = 7.6, 4.8, 1.1 Hz, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 167.6, 154.9, 149.9, 136.6, 133.1, 132.4, 129.5, 128.7, 127.5, 126.3, 126.1, 125.3, 122.8, 122.0; **IR** (neat) 3075, 3042, 2118, 1581, 1560, 1485, 1469, 1453, 1427, 1331, 1303, 1279, 1239, 1209, 1180, 1149, 1091, 1049, 992, 965 [1], 897, 889, 862 cm^{-1}; **MS** (GC-MS) *m/z* = 207.08 calc. for $C_{14}H_{10}N_2[M+H]^+$, found 207.1.



28

(*E*)-2-(3-isocyanostyryl)pyridine (28)

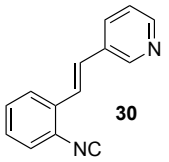
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 (ddd, *J* = 4.8, 1.8, 0.9 Hz, 1H), 7.69 (td, *J* = 7.7, 1.8 Hz, 1H), 7.64-7.55 (m, 3H), 7.43-7.35 (m, 2H), 7.32-7.28 (m, 1H), 7.20 (td, 1H, *J* = 4.8, 1.2 Hz), 7.17 (d, *J* = 16.1 Hz, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 164.2, 154.7, 149.9, 138.4, 136.7, 130.4, 130.1, 129.8, 128.0, 127.1, 125.8, 124.5, 122.7 (2 C); **IR** (neat) 3065, 3002, 2925, 2131, 1597, 1583, 1560, 1471, 1441, 1430, 1334, 1302, 1275, 1240, 1209, 1146, 1094, 1083, 978, 951, 892, 863 cm^{-1}; **MS** (GC-MS) *m/z* = 207.08 calc. for $C_{14}H_{10}N_2[M+H]^+$, found 207.1.



29

(*E*)-2-(4-isocyanostyryl)pyridine (29)

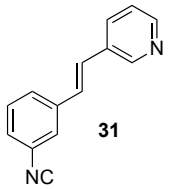
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 (ddd, *J* = 4.8, 1.8, 0.9 Hz, 1H), 7.69 (td, *J* = 7.7, 1.8 Hz, 1H), 7.63 (d, *J* = 16.1 Hz, 1H), 7.60-7.57 (m, 2H), 7.40-7.36 (m, 3H), 7.22-7.16 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 164.8, 154.8, 149.8, 137.9, 136.7, 130.8, 130.1, 127.8 (2 C), 126.8 (2 C), 122.7 (2 C), 122.7; **IR** (neat) 3056, 2922, 2851, 2130, 1583, 1562, 1505, 1468, 1430, 975; **MS** (GC-MS) *m/z* = 207.08 calc. for $C_{14}H_{10}N_2[M+H]^+$, found 207.1.



30

(*E*)-3-(2-isocyanostyryl)pyridine (30)

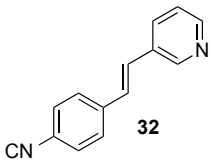
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (d, *J* = 2.3, 1H), 8.55 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.95-7.92 (m, 1H), 7.77-7.73 (m, 1H), 7.46 (d, 1H, *J* = 16.3 Hz), 7.44-7.38 (m, 2H), 7.36-7.29 (m, 2H), 7.18 (d, 1H, *J* = 16.3 Hz); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 167.5, 149.5, 149.2, 133.0, 132.9, 132.1, 129.6, 128.9, 128.7, 127.4, 125.6, 125.1, 124.4, 123.7; **IR** (neat) 3033, 2119, 1583, 1565, 1485, 1450, 1423, 1278, 1228, 1184, 1161, 1091, 1043, 1022, 962, 909 cm^{-1}; **MS** (GC-MS) *m/z* = 207.08 calc. for $C_{14}H_{10}N_2[M+H]^+$, found 207.1.



31

(*E*)-3-(3-isocyanostyryl)pyridine (31)

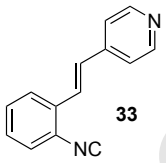
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (dd, *J* = 2.3, 0.9 Hz, 1H), 8.53 (dd, *J* = 4.7, 1.6 Hz, 1H), 7.84 (ddd, *J* = 8.0, 2.3, 1.6 Hz, 1H), 7.56-7.52 (m, 2H), 7.44-7.38 (m, 1H), 7.34-7.28 (m, 2H), 7.11 (s, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 164.4, 149.3, 148.7, 138.3, 132.9, 132.2, 129.9, 128.5, 127.5, 127.4, 127.2, 125.7, 124.2, 123.7; **IR** (neat) 3027, 2124, 1598, 1581, 1567, 1481, 1435, 1411, 1273, 1182, 1024, 966, 885 cm^{-1}; **MS** (GC-MS) *m/z* = 207.08 calc. for $C_{14}H_{10}N_2[M+H]^+$, found 207.1.



32

(*E*)-3-(4-isocyanostyryl)pyridine (32)

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (d, *J* = 2.2 Hz, 1H), 8.53 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.83 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.57-7.51 (m, 2H), 7.41-7.36 (m, 2H), 7.31 (ddd, *J* = 8.0, 4.8, 0.9 Hz, 1H), 7.11 (d, *J* = 4.3 Hz, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 164.9, 149.2, 148.7, 137.9, 132.9, 132.2, 128.9, 127.4 (2 C), 127.3, 126.9 (2 C), 123.6; **IR** (neat) 3028, 2920, 2850, 2127, 1644, 1600, 1574, 1567, 1503, 1483, 1421, 1409, 1329, 1304, 1252, 1166, 1130, 1100, 1022.75, 964, 942, 865 cm^{-1}; **MS** (GC-MS) *m/z* = 207.08 calc. for $C_{14}H_{10}N_2[M+H]^+$, found 207.1.

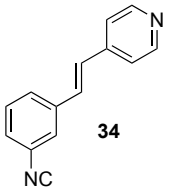


33

(*E*)-4-(2-isocyanostyryl)pyridine (33)

KKB-1-19: **^{1}H NMR** (500 MHz, Benzene-d_6) δ 8.54-8.46 (m, 2H), 7.40 (d, *J* = 16.3 Hz, 1H), 7.01 (dd, *J* = 8.0, 1.3 Hz, 1H), 6.82-6.71 (m, 4H), 6.60 (td, *J* = 7.7, 1.4 Hz, 1H), 6.49 (d, *J* = 16.3 Hz, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$**)** δ 167.8, 150.4 (2 C), 143.6, 132.5, 130.1, 129.6, 129.2, 127.5, 126.6, 125.9, 125.3, 121.1 (2 C); **IR** (neat) 3052, 2922, 2852, 2122, 1592, 1549, 1495, 1479, 1451, 1413, 1309, 1274,

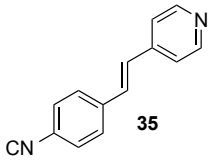
1243, 1213, 1090, 991, 966, 958, 880 cm^{-1}; **MS** (GC-MS) *m/z* = 207.08 calc. for $C_{14}H_{10}N_2[M+H]^+$, found 207.1.



34

(*E*)-4-(3-isocyanostyryl)pyridine (34)

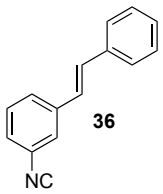
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64-8.58 (m, 2H), 7.58-7.52 (m, 2H), 7.42 (t, *J* = 8.1 Hz, 1H), 7.38-7.35 (m, 2H), 7.34-7.31 (m, 1H) 7.23 (d, *J* = 16.3 Hz, 1H), 7.05 (d, *J* = 16.3 Hz, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 164.7, 150.4 (2 C), 143.7, 137.8, 130.8, 129.9, 128.4, 127.8, 127.3, 126.2, 124.5, 121.0 (2 C); **IR** (neat) 3056, 3029, 2922, 2128, 1591, 1549, 1494, 1478, 1450, 1415, 1242, 1217, 1174, 991, 971, 922, 893, 874, 859 cm^{-1}; **MS** (GC-MS) *m/z* = 207.08 calc. for $C_{14}H_{10}N_2[M+H]^+$, found 207.1.



35

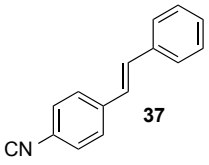
(*E*)-4-(4-isocyanostyryl)pyridine (35)

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64-8.59 (m, 2H), 7.60-7.53 (m, 2H), 7.42-7.35 (m, 4H), 7.26 (d, 1H, *J* = 16.3 Hz), 7.04 (d, *J* = 16.3 Hz, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 165.3, 150.4 (2 C), 143.8, 137.3, 131.1, 128.4, 127.8 (2 C), 126.9 (2 C), 126.2, 121.0 (2 C); **IR** (neat) 3031, 2923, 2118, 1582, 1559, 1504, 1486, 1469, 1453, 1427, 1331, 1302, 1279, 1239, 1209, 1180, 1148, 1091, 992, 962, 942, 898, 858 cm^{-1}; **MS** (GC-MS) *m/z* = 207.08 calc. for $C_{14}H_{10}N_2[M+H]^+$, found 207.1.



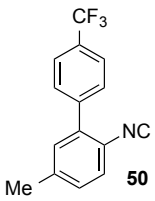
(*E*)-1-isocyano-3-styrylbenzene (36)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54-7.50 (m, 4H), 7.41-7.35 (m, 3H), 7.34-7.28 (m, 1H), 7.26-7.23 (m, 1H), 7.14 (d, *J* = 16.3 Hz, 1H), 7.04 (d, *J* = 16.3 Hz, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 164.1, 139.0, 136.4, 131.1, 129.7, 128.8 (2 C), 128.4, 127.4, 127.1, 126.8 (2 C), 126.4, 125.1, 124.0; **IR** (neat) 3024, 2922, 2126, 1597, 1578, 1496, 1485, 1472, 1449, 1267, 1226, 1168, 1145, 1081, 1074, 965, 911, 884 cm^{-1}; **MS** (GC-MS) *m/z* = 206.09 calc. for $C_{15}H_{11}N[M+H]^+$, found 206.1.



(*E*)-1-isocyano-4-styrylbenzene (37)

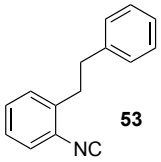
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54-7.49 (m, 4H), 7.41-7.35 (m, 4H), 7.33-7.28 (m, 1H), 7.16 (d, *J* = 16.3 Hz, 1H), 7.08 (d, *J* = 16.3 Hz, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 164.5, 138.6, 136.5, 131.1 (2 C), 128.8 (2 C), 128.4 (2 C), 127.2 (2 C), 126.8 (3 C), 125.2; **IR** (neat) 3023, 2920, 2850, 2123, 1633, 1576, 1503, 1448, 1417, 1335, 1305, 1220, 1198, 1160, 1107, 1073, 967, 950, 918, 866 cm^{-1}; **MS** (GC-MS) *m/z* = 206.09 calc. for $C_{15}H_{11}N[M+H]^+$, found 206.1.



2-isocyano-5-methyl-4'-(trifluoromethyl)-1,1'-biphenyl (50)

Prepared according to a procedure reported by Studer[2] from 2-bromo-4-methylanaline and 4-(trifluoromethyl)phenylboronic acid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.1 Hz, 2H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.44 (d, *J* = 8.6 Hz, 1H), 7.26-7.27 (m, 2H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 166.8, 140.9, 140.4, 137.3, 131.1, 130.4 (q, *J* = 32 Hz), 129.8, 129.6, 127.9, 125.7, 124.2 (q, *J* = 270 Hz), 122.3, 21.3; IR (cm^{-1}): 2922, 2125, 1620, 1571, 1493, 1396, 1328, 1198, 1179, 1155, 1110, 965, 952, 897, 880, 845; MS (ESI): *m/z* = 262.09 calc. for $C_{15}H_{10}F_3N[M+H]^+$, found 262.2.



53

1-isocyano-2-phenethylbenzene (53)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.33-7.28 (m, 3H), 7.27-7.19 (m, 5H), 3.07 (dd, *J* = 8.9, 7.6 Hz, 2H), 2.95 (dd, *J* = 8.9, 5.6 Hz, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 166.1, 140.8, 138.2, 130.0, 129.4, 128.6 (2 C), 128.5 (2 C), 127.1, 126.9, 126.2, 126.1, 36.0, 34.7; **IR** (neat) 3063, 3028, 2926, 2855, 2119, 1603, 1496, 1487, 1453 cm^{-1}; **MS** (GC-MS) *m/z* = 208.11 calc. for $C_{15}H_{13}N[M+H]^+$, found 208.1.

Compounds **46**[2], **49**[2] and **51**[2] were prepared according to the literature procedure and their spectral data match with the reported ones. Compounds **5**[1], **6**[1], **11**[1], **42**[3], and **43**[4] were prepared according to the representative procedure aforementioned and their spectral data match with the reported ones.

Part II. Biological Materials and Evaluation Methods

Bacterial strains and reagents. Clinical isolates of MRSA, vancomycin-intermediate *S. aureus* (VISA), and vancomycin-resistant *S. aureus* (VRSA) were obtained through the Network of Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program (Table 1). Vancomycin hydrochloride (Gold Biotechnology, St. Louis, MO, USA) and linezolid (Chem-Impex International, Inc., Wood Dale, IL, USA) powders were purchased commercially and dissolved in DMSO to prepare a stock 10 mM solution.

Assessment of antimicrobial activity of the isonitrile compounds against multidrug-resistant S. aureus strains. The minimum inhibitory concentration (MIC) of each compound and linezolid was determined against eight different strains of MRSA, VISA, and VRSA using a modified version of the broth microdilution method, outlined by the CLSI.[5] The same analysis was performed with vancomycin against the VISA and VRSA strains tested. A bacterial suspension (~1×10^5 CFU/mL) was prepared in Tryptic soy broth (TSB) and then transferred to a microtiter plate. Each agent tested was added (in triplicate) to wells in the first row of the plate and then serially diluted downward. Plates were incubated at 37 ºC for 18-20 h before the MIC was determined as the lowest concentration of each test agent where bacterial growth was not visible.

Toxicity analysis of selected isonitrile compounds tested against mammalian cells. Selected isonitrile compounds were assayed at concentrations of 16 μM, 32 μM, 64 μM, and 128 μM against a murine macrophage (J774) cell line to asses if the compounds exhibited toxicity to mammalian cells *in vitro*. Cells were cultured in Dulbeco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA) with

10% fetal bovine serum (USA Scientific, Inc.) at 37 ºC with 5% CO_2. Controls received DMSO alone at a concentration equal to that in drug-treated cell samples. The cells were incubated with each compound (in triplicate) in a 96-well tissue-culture plate at 37 ºC and 5% CO_2 for 2 h prior to addition of the assay reagent MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl) -2-(4-sulfophenyl)-2H-tetrazolium) (Promega, Madison, WI, USA). Absorbance readings (at OD_{490}) were taken using a kinetic microplate reader (Molecular Devices, Sunnyvale, CA, USA). The quantity of viable cells after treatment with each compound was expressed as a percentage of the viability of DMSO-treated control cells (average of triplicate wells ± standard deviation). Statistical analysis was performed (comparing cells treated with compound versus cells treated with DMSO) using the paired t-test ($P < 0.05$) utilizing Microsoft EXCEL software.

*Caco-2 permeability analysis of compound **13***. The ability of compound **13**, ranitidine (low permeability control), warfarin (high permeability control), and talinolol (P-glycoprotein efflux substrate), to effectively permeate across a biological membrane was assessed using a Caco-2 cell monolayer, as described elsewhere.[6] The amount of permeation was determined both from the apical (A) to basolateral (B) direction and the basolateral (B) to apical (A) direction. Data for apparent permeability (P*app*) and the efflux ratio (R_E) were determined as explained elsewhere.[6] An $R_E > 2$ indicates the test agent may be a potential substrate for P-glycoprotein or other active efflux transporters.

Kinetic solubility screen. A kinetic solubility analysis of compound **13**, reserpine, tamoxifen, and verapamil was performed as has been described elsewhere.[6] The solubility limit (in μM) reported is the

maximum concentration of each test agent where turbidity was not observed. Values below 1 μM indicate compound is insoluble, values between 1 to 100 μM indicate partial aqueous solubility, and values above 100 μM indicate test agent is fully soluble.

Metabolic stability analysis using pooled human liver microsomes. To analyze the stability of compound **13** to metabolic processes in the liver, this compound was incubated in duplicate with pooled human liver microsomes at 37 ºC (for 60 min), using a similar protocol described elsewhere, with two modifications.[7,8] First, the reaction mixture utilized 0.3 mg/mL microsomal protein. Additionally, samples were collected after 0 and 60 min and analyzed accordingly. Data are reported as % remaining by dividing by the time zero concentration value.

References:

1. Zhang, B.; Studer, A., *Org. Lett.* **2014**, *16*, 1216.
2. Zhang, B.; Mueck-Lichtenfeld, C.; Daniliuc, C. G.; Studer, A., *Angew. Chem., Int. Ed.* **2013**, *52*, 10792.
3. Tanaka, R.; Sanjiki, H.; Urabe, H., *J. Am. Chem. Soc.* **2008**, *130*, 2904.
4. Hahn, B. T.; Tewes, F.; Fröhlich, R.; Glorius, F., *Angew. Chem., Int. Ed.* **2010**, *49*, 1143.
5. Institute. CaLS. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Seventh Edition: Approved Standard M7-A7.* 7 ed. Wayne, PA2011.
6. Mohammad, H.; Mayhoub, A. S.; Cushman, M.; Seleem, M. N. *J. Antibiot.* **2014**, doi: 10.1038/ja.2014.142.
7. Zhang, W.; Benmohamed, R.; Arvanites, A. C.; Morimoto, R. I.; Ferrante, R. J.; Kirsch, D. R.; Silverman, R. B. *Bioorgan. Med. Chem.* **2012**, *20*, 1029.
8. Papadopoulou, M. V.; Bloomer, W. D.; Rosenzweig, H. S.; Ashworth, R.; Wilkinson, S. R.; Kaiser, M.; Andriani, G.; Rodriguez, A. *Future Med. Chem.* **2013**, *5*, 1763.