**Open Source Challenge- Diaryl Imidazoles to counter multidrug resistant bacteria**

**Background**

This challenge is to explore a new chemical space for antibacterial drug development. The parent compounds/leads are derived from screens of kinase inhibitors which led, in some cases, to clinical trials as inhibitors of mapKinase, B-Raf kinase, and TGF-kinase. Although numerous compounds were generated in those programs, none were ultimately approved for human use. Nevertheless, these trials indicate that the chemotype is host tolerant. A recent screen indicated that a subclass of the diaryl imidazoles (DAI) produced initially by SmithKline Beecham (SB), later GlaxoSmithKline (GSK), expressed antibacterial activity against MRSA. A preliminary study examined analogs of one of the compounds with the result that 6 analogs possessed activity at the MIC = 2-4 µg/mL level. The most active compound was evaluated for metabolic clearance (mouse liver microsomes Clint = 1170 mL/min/kg) and found to be too rapidly metabolized to achieve potential therapeutic levels in vivo. However, given the unusual structure of this class of compounds in the antibacterial armamentarium, we are proposing a challenge to develop new antibiotics, particularly against resistant organisms, based on this chemotype. The key issues to be addressed are to identify compounds that are more potent, i.e., MIC <0.5 µg/mL, have greater metabolic stability, and can demonstrate in vivo efficacy. We open this challenge to any investigator who will submit actual or virtual compounds that meet the parameters listed below.

**Parameters for the Diaryl imidazoles**

**Overall Target Pharmacodynamics-Phenotypic in vitro**

Activity- <0.5 µg/mL against target resistant organism

Activity > 64 µg/mL host cells

Selectivity vs other cell types (i.e., antifungal/antiviral)

Low resistance potential

**Target Properties- PD/Tox**

It is not known at this time what the specific mechanisms of action are for these compounds are at the bacterial level. The compounds prepared to date as therapeutic agents have been evaluated as kinase inhibitors (primarily) and in some cases as selective cyclooxygenase (COX) inibitors. Whether these compounds are active at bacterial homologs is not known presently and presumably there may be correlations between published inhibitory data against mammalian enzymes and the phenotypic MIC testing results. Such correlations should be interpreted conservatively. A number of diaryl imidazoles have undergone human clinical trials, indicating that there is limited toxicity to the host and providing support for transitioning their use against invasive bacteria present in the host organism.

For compounds provided from existing libraries, it would be helpful to know what assays have already been done, and if studies have been done on animals, which assays and which species.

Examples of screens include kinase panels, GPCRs, ligand gated ion channels, transporters, ion pumps, bacterial enzyme assays, toxicology, etc.

In looking for new compounds, it may not be important to look at the compound that is most active in the mammalian screen if that target poses potential side effects. Lower activity against host enzymes/receptors/transporters may present an advantage in generating selectivity as it will be less necessary to dial out host activity while dialing in antibacterial properties.

**Target properties -PK [Some of these can be calculated/estimated**

In targeting multidrug resistant bacteria, the route of administration may be either oral or i.v., and therefore concern for Lipinski’s Rules may be suspended, at least initially.

cLog P <3

Log D <2

Rat hepatocyte clearance Clint <27

Human liver microsomes clearance Clint <47

Drug solubility > 10µM

Plasma Protein Binding <95%

**Targeted Chemical Properties.**

We will be looking at the class of 4,5-diaryl imidazoles which will consist of essentially 3 domains. The first domain is the central imidazole core. N1- maybe either unsubstituted or have an sp3 carbon attached; C2-may have sp2 or sp3 carbon attached. [This site may constitute one of the greatest areas for diversity] N3-is unsubstituted ; C4- is substituted by an electron-deficient aromatic ring. Currently the most promising group is 2-pyridyl , however, 4-pyridyl (associated with high CytP-450 activity) has been examined as well as 4-pyrimidinyl. Other variations at this site are also possible.C5- is substituted by an electron rich aromatic ring. This site also offers potential for structural diversity.

Key considerations in providing or proposing structures in this series, in addition to phenotypic potency against the organism, are:

Avoid introduction of groups highly susceptible to metabolism

Avoid introduction of groups known to inhibit key metabolic enzymes

Avoid introduction of groups associated with teratogenicity or carcinogenicity

The compound should be synthetically accessible in <5 steps in an overall yield >25%.

Reagents for the synthesis should be commercially available or easily prepared.

Total synthesis should require no more than one chromatographic step

**Submission of candidate compounds or structures**

**Actual compounds-synthetic or isolated from natural sources**

Source of compounds- lab book or registry numbers

Characterization- elemental analysis, LC-MS, other evidence of purity, spectral characterization-NMR, IR, etc

Prior biological characterization- if any

Number of steps in synthesis from initial precursor

**Virtual compounds generated computationally**

List program used to generate compound(s)

Provide calculated properties of the compound(s)

Provide a proposed synthesis for the compound(s) with references to support the synthetic methods

Reference for synthesis and biological activity of 4,5-diaryl-imidazoles

1. Bellina, F.; Cauteruccio, S.; Rossi, R. Tetrahedron (2007), 63: 4571-4624.

Relatively comprehensive review of the synthesis of all diaryl imidazole isomers and their biological activity. Most were active as kinase inhibitors, with some as COX-2 inhibitors. Only one paragraph reports antibacterial activity. The lead compound in this challenge project came from one of the kinase projects out of SmithKline Beecham (SB) which later became GlaxoSmithKline (GSK). GSK has been active in tropical and neglected disease research- providing screens and libraries for some investigators. Other companies with examples in the chapter are Merck and RWJ.

1. Rani, N.; Sharma, A.; Singh, R., Mini-Reviews in Medicinal Chemistry (2013), 13, 1812-1835

This review covers the field of substituted imidazoles as antibacterial agents, but it also includes the di(tri)aryl imidazoles as a subclass. In that regard, it intersects reference 1 and includes some of the compounds listed there, but also a number not covered by that review but which are still relevant. The review is limited sometimes by its references to levels of activity, i.e., not specific data. However, one example that is relevant was described in reference [89] which is listed below as reference 3. It contains a single 4,5-diaryl imidazole derivative possessing similar dipolar arrangement (one electron deficient ring and one electron rich ring) at the 4,5-positions, and a substituent at the 2-position of the imidazole that can be further modified.

1. Tanitame, A.; Oyamada, Y.; Ofuji, K.; Fujimoto, M.; Suzuki, K.; Ueda, T.; Terauchi, H.; Kawasaki, M.; Nagai, K.; Wachi, M.; Yamagishi, J. Synthesis and antibacterial activity of novel and potent DNA gyrase inhibitors with azole ring. Bioorg. Med. Chem. (2004), 12, 5515-5524.

This article describes the synthesis and evaluation of pyrazole, oxazole and imidazole derivatives as antibacterial agents. Of interest are the imidazole agents which are active at the 2-4µg/mL level against gram(-) and gram (+) bacteria as well as against MRSA and efflux pump mutants. A brief study suggests that these compounds may be inhibitors of DNA gyrase and/or Topoisomerase IV enzymes.

The question is why no further studies were done if these compounds were easy to make, and active.

1. Tanitame, A.; Oyamada, Y.; Ofuji, K.; Terauchi, H.; Kawasaki, M.; Wachi, M.; Yamaaguchi, J.-I., Bioorg. Med. Chem. Lett. (2005) 15; 4299-4303.

This is a follow-on to ref. 3 using similar scaffolding but a different pendant group. Introduction of a vinyl group between the core heterocycle and the aromatic group gives compounds with similar but not significantly improved biological potency. Compounds are still moderately active against gram (-) and gram(+) organisms including resistant strains. Mechanism of action may be against DNA gyrase and Topoisomerase IV enzymes. This suggests that in exploring structural modifications based on the ALM technology, one could consider Arylvinyl boronic acids(esters) as well.

1. Velaparthi, U.; Darne, C.P.; Warrier, J.; Liu, P.; Rahaman, H.; Augustine-Rauch, K.; Parrish, K.; Yang, Z.; Swanson, J.; Brown, J.; Dhar, G.; Anandam, A.; Holenarsipur, V.K.; Palanisamy, K.; Wautlet, B.S.; Fereshteh, M.P.; Lippy,J.; Tebben, A,J.; Sheriff,S .; Ruzanov, M.; Yan, C.; Gupta, A,; Gupta, A.K.; Vetrichelvan, M.; Mathur, A.; Gelman,M.; Singh, R.; Kinsella, T.; Murtaza, A,; Fargnoli, J.; Vite, G.; and. Borzilleri, R.M., ACS Med. Chem. Lett. 2020, 11, 172−178

This is a current publication on TGF-R agents based on the diaryl imidazole motif. Contains relevant synthetic strategies and chemistry directed toward orally active drugs. Although this is targeted for eukaryotic cells, it may be possible to repurpose the scaffold by modifying the pendant groups. Screening this library for antibacterial activity may generate lead.