NHPyOx as a Chem-Bio Model for the Synergistic Discovery of Novel Ligands Facilitating Pharmaceutical Science

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Supporting Information Placeholder

Amino-pyridine-oxazoline (NH-PyOx) was conceived as a proof-of-concept of a chem-bio model (CBM) for function-oriented synergistic optimization. It culminated in the discovery of novel ligands showing much better performance in Hayashi-Miyaura coupling or antifungal performance than that of PyOx and boscalid, respectively.

There is a fascinating consensus that “ligands” play a pivotal role in both biology and chemistry ever since undergraduate study. In biology, a ligand is a special substance that can bind to a biomolecule for a biological purpose; in chemistry, it can coordinate with a central metal for a chemical transformation. Both empirical and rational discovery significantly affect the advances in synthetic methods and biological leads, which ultimately can accelerate the pharmaceutical discovery science. Subtle steric or electronic differences in the scaffold as well as minute conformational changes can drastically affect reaction or biological outcomes with regard to either activity or selectivity or both. Despite impressive progress, the discovery of suitable ligands is an eternal issue while remaining a formidable task.

Inspired by the profound importance of ligands in both chemistry and biology, we interrogate whether one can intentionally design a scaffold combining the pharmacophore and catalytic factors simultaneously, which we termed the “Chem-Bio Model” (CBM). One can easily get suitable ligands through function-oriented multidimensional optimization in view of either biological purpose or catalysis. Actually, N-containing pharmaceutically important small molecules have infiltrated the ligand discovery for transition-metal catalysis. This was also well exemplified by the odyssey of Cinchona alkaloids, which began with the antimalarial potentials followed by successful applications in asymmetric synthesis. As a proof-of-concept, we envisioned that our continuing efforts in the function-oriented exploration of the arylloxazoline model will have great potential to serve as a privileged “Chem-Bio Model” for accessing diverse molecular architectures to facilitate synthetic and medicinal chemistry.

Figure 1. Design and application of NHPyOx Ligands

Aryloxazolines are one of the most successful and versatile ligands or directing groups that have significant contributions to catalysis (e.g. PyOx). The previous works showcased that the amino arylloxazoline (NHPhOx) can serve as a pharmacophore for the discovery of novel antiviral, insecticidal or acaricidal, and antifungal leads (e.g. LE001). Interestingly, the core NHPhOx was utilized as an inherent directing group for versatile C-H functionalizations and the efficient synthesis and discovery of pharmaceutically important chemotypes possessing antifungal profiles. These progress intrigued our recent discovery of the natural β-carboline inspired CarOx as a kind of new ligands for
asymmetric transformations and pharmaceutically interesting molecules.¹⁹ The inconvenience in expeditious and modular diversification of this kind of ligands (via infra) impeded the efficient exploration of structure-function relationship aiming for the discovery of pharmaceutical leads or asymmetric catalysis.

Accordingly, the novel model comprised both the key factors (or coordination models) from PyOx and NHPPhox (Figure 1) was conceived, in which the electronic nature and steric hindrance can be finely and easily tuned by employing different amine substitutions. Importantly, this scaffold is poorly sampled by either synthetic chemists or medicinal researchers, which is yet to achieve the same level of refinement compared with its 6-substituted congeners (see Supporting Information for the Scifinder retrieval). We envision that the resultant amine-pyridine-oxazoline (NHPPhox) ligands can replenish the repertoire of the PyOx toolbox and facilitate multidimensional optimization. Taking advantage of these structural features, one will be able to discover suitable ligands with matched electronic and steric nature for either chemical or biological purposes. With this fascinating hypothesis, we herein document an engineered molecular design enumerated by the model NHPPhox shown in Figure 1. Fine-tuning of this simplified model from β-CarOx provides ligands showing better performance in Pd catalyzed Hayashi-Miyaura coupling of arylboronic acids to 4-quinolones, and also leads to novel pharmaceutically important chemotypes.

This work was initially driven by our continuous effort in applying chiral heterocycle-oxazolines in the asymmetric addition of arylboronic acids to Michael acceptors (Hayashi-Miyaura reaction). Attracted by the promising pharmaceutical profiles of 2-aryl-2,3-dihydro-4-quinolones,²⁰-²⁴ the underdeveloped palladium-catalyzed asymmetric addition of phenylboronic acid 1 to N-Chz-4-quinolone 2 was chosen as a model. Guided by the pioneering Pd catalysis from the Stoltz group,²² the PyOx ligands (L₁-L₄) and the indole fused congeners β-CarOx ligands (L₅-L₇) were tested comparatively (Table 1A). β-CarOx L₅ provided better enantioselectivity and was selected for an extensive screening campaign by optimizing the continuous variables (see Supporting Information for more details). It was found that improving the ligand loading to 12 mol% and variation of the reaction media to PhCF₃ gave more promising results in view of both productivity and enantioselectivity, from which the desired dihydroquinolone 3 was prepared in 67% yield and 92% ee (Table 1B, entry 4).

The modular modification of β-CarOx is necessary but challenging for the further improvement of this transformation. The key to the success is the conceptual design of the easily available and programmable congeners possessing the coordination potentials of both PyOx and NHPPhox ligands. The simplification and versatile optimization of the β-CarOx framework commenced with the formal ring-opening of indole leading to 3-amino-PyOx ligands (Scheme 1). Alongside the steric and electronic modifications, the performance resides either the classic PyOx or NHPPhox competitively, which was finely adjusted by each other through the proximity to the oxazoline core. These ligands represent the unprecedented combination of PyOx and NHPPhox fragments for function-oriented optimization.

As shown in Scheme 1, two types of hybridized ligands including 3-arylamido-PyOx ligands (CL₁-CL₁₃) and 3-arylamino-PyOx ligands (NL₁-NL₂₄) can be easily prepared from the readily available 3-bromo-PyOx as an advanced intermediate through one-step C-N coupling. Copper catalyzed Goldberg coupling of different arylamides and 3-bromo-PyOx were smoothly recruited for expeditious construction of 3-arylamido PyOx type ligands CL₁-CL₁₃ in 22-50% yield. Meanwhile, the 3-arylamino PyOx type ligands NL₁-NL₂₄ (45-75% yield) featuring various electronic properties and steric factors are accessible via Pd(OAc)₂/Xantphos catalyzed Buchwald-Hartwig coupling with various amines.

### Table 1. Optimization of reaction parameters

<table>
<thead>
<tr>
<th>Entry</th>
<th>Parameters variation</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>64%</td>
<td>91%</td>
</tr>
<tr>
<td>2</td>
<td>MeOH instead of DCE</td>
<td>7%</td>
<td>91%</td>
</tr>
<tr>
<td>3</td>
<td>PPh₃ instead of DCE</td>
<td>67%</td>
<td>92%</td>
</tr>
<tr>
<td>5</td>
<td>L₁ instead of β-CarOx, PhCF₃</td>
<td>67%</td>
<td>92%</td>
</tr>
</tbody>
</table>

**Table 1 continued...**
Having an optimal procedure in hand, the scope of aryloboronic acids was first explored. Both monosubstituted and disubstituted aryloboronic acids are shown to be suitable substrates (Scheme 2), delivering the desired dihydroquinolones 4-22 in moderate to good yields with good to excellent enantioselectivities. To our delight, various functional groups, such as halogens (9, 10, 14, and 18), trifluoromethyl (11), ether (5, 7, 16, and 17), ester (12), and fused rings (20 and 21) are readily accommodated. These products can be used directly or after protection for the divergent establishment of pharmacologically important chiral dihydroquinolone collections. It is noteworthy that the introduction of thiophene-3-yl boronic acid is also tolerant to produce hydroyquinolone 22 with 90% ee. The lower productivity could be explained by the competitive coordination of sulfur to deactivate the catalyst. Among the monomethylated aryloboronic acids, the para- and the meta-substituted ones gave much better yields and selectivity (4 and 13), which can be rationalized by the sterically demanding nature of the aryloboronic acids. A similar unfavorable effect on the newly established chirality was also detected in the synthesis of naphthyl dihydroquinolones (20 versus 21). The limitation considering aryloboronic acids was observed in the synthesis of compound 23.

An assortment of 4-quinolones with neutral, electron-withdrawing, or electron-donating groups can be submitted and successfully attacked by aryloboronic acids with different electronic factors (24-31). It should be emphasized that this will open a new window for us to achieve promising analogs of our recently disclosed antifungal sakuranetin (Scheme 1) and revealed antifungal sakuranetin (Scheme 1) with the amino acid residues TYR128 and ALA77 via amide and amido PyOx scaffold that is distinct from the previous entities. Notably, the substitution on the oxazoline ring of 3-aryloxy PyOx ligands showed a crucial effect on the antifungal performance. The isomeric variation of sBu to tBu (CL15) resulted in a sharp decrement in the potential against Fusarium graminearum with the EC50 value of > 100 μM. Interestingly, the inversion of the configuration of oxazoline (CL1 vs CL7) underscored that the S enantiomer (EC50 = 13.35 μM) is more favorable for antifungal enhancement. Introducing fluorine to the aryloxime segment resulted in a further increase of potency, culminating in the acquirement of compound CL11 as a new antifungal lead against Fusarium graminearum with the EC50 value of 4.04 μM, which is > 20 fold more promising than boscalid. Additionally, a brief investigation of 3-aryloxy PyOx ligands demonstrated their potency against Rhizoctonia solani, in which the electron-deficient diylamines NL18 and NL23 were identified as equipotent antifungal leads with submicromolar potency (EC50 = 0.57 μM). Notable amongst this kind of ligands is that replacing the electron-deficient Cl group (NL18) with the electron-enriched MeO group (NL16) also afforded a good inhibitory effect against Rhizoctonia solani.

To investigate more insights into the biological difference, homology modeling and molecular docking were conducted for the 3-aryloxy PyOx CL11 and the SDH of Fusarium graminearum (FgSDH). The model of FgSDH was generated based on the reported crystal complex (PDB code: 3SFD). Molecular docking was carried out to simulate and compare the effective protein-ligand interactions via hydrogen bondings, which were graphically characterized in Figure 2. Though in a similar fashion to interact with the amino acid residue TRP205, the hydrogen bonding from the amide of CL11 (via carboxyl) is much stronger than that from boscalid, with the hydrogen-bonding distance of 1.60 Å and 2.50 Å, respectively. Additionally, ligand CL11 can effectively interact with the amino acid residues TYR128 and ALA77 via amide and oxazoline functional groups. Other interactions including π-π stacking, π-σ interaction, and halogen effect were also characterized in the Supporting Information.
Scheme 2. Substrate Scope for Asymmetric Addition of Arylboronic acids to Quinolones

Unless otherwise mentioned, the yields refer to the isolated yield. The ee values were determined by HPLC analysis on a chiral phase. *Data were reported in Chem. Eur. J. 2013, 19, 74-77. **Data in parentheses is yield brsm.

In summary, this work qualified NHPyOx as a valuable ChemBio Model (CBM) for function discovery, in which promising results have been achieved in both chemical catalysis and biological exploration. The function-oriented synergistic effort ultimately leads to the discovery of NL11 for high enantioselective addition of arylboronic acids to quinolones, and the achievement of CL4 and CL11 as novel antifungal leads against Fusarium graminearum. New promising chemotypes (NL18 and NL23) against Rhizoctonia solani were also acquired. The new leads are quite different from the commercially available fungicides either in antifungal phenotypical screening or molecular docking. We anticipate the current work will show a significant impact on the discovery of new ligands facilitating interdisciplinary innovation.

ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its online supplementary material.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Synthetic procedures, characteristic data, HPLC Traces, NMR spectra, and antifungal data (file type, i.e., PDF)

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Author Contributions

S. Li conceived and designed this work; J. Yang, J. Lai, and W. Li performed the experiments and provided results; S. Sun conducted molecular docking; all authors wrote the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was financially supported by the National Natural Science Foundation of China (21772094, 21977049), and the Research Foundation of Guizhou University (202016).

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