De novo design and optimization of Aurora A kinase inhibitors†‡

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Drug discovery programs urgently seek new chemical entities that meet the needs of a demanding pharmaceutical industry. Consequently, de novo ligand design is currently re-emerging as a novelty-generating approach. Using ligand-based de novo design software, we computationally generated, chemically synthesized and biochemically tested a new arylsulfonamide against Aurora A kinase, a validated drug target in several types of cancer. The designed compound exhibited desired direct inhibitory activity against Aurora A kinase. By chemical optimization we obtained a lead structure exhibiting sustained activity in phenotypic assays. These results emphasize the potential of ligand-based de novo design to consistently deliver functional new chemotypes within short timeframes and limited effort.

Introduction

Future success of drug discovery programs critically relies on innovation, in particular designing new chemical entities (NCEs) capable of modulating drug target functions.1,2 Computer-assisted molecular design offers tools for exploring uncharted chemical space in an expeditious way, and meets the needs of an increased pressure in the pharmaceutical industry to deliver NCEs.3 De novo design methods were conceived approximately 25 years ago, but have been scarcely exploited for their scaffold-hopping potential.4–6 Here we present the application of ligand-based de novo design to swiftly and successfully discover a new class of compounds efficiently blocking Aurora A kinase (AurA) activity.

AurA is a serine/threonine kinase that plays a pivotal role in mitosis, and is up-regulated in several human tumors.7–9 Despite AurA being an attractive target for inhibitor design, approved drugs have been elusive as therapeutic agents. VX-680 (Fig. 1, 1) is a pan-Aurora inhibitor with high potency against AurA (Ki = 0.6 nM), but its development was abandoned in clinical trials due to QT interval prolongation.10 Having 1 as a template, we employed the software DOGS (Design Of Genuine Structures)11 to create new chemotypes in an unbiased manner, which would mimic pharmacophoric features of the template, and thus jump to new activity islands in chemical space. We intended to explore the performance of this design approach in a research area, which has been extensively studied, and might thus leave little space for finding new chemotypes exhibiting a desired activity. Most notably, DOGS was successfully applied to ligand design when the decisive pharmacophore features were unknown.12 The algorithm generates molecules by starting with a user-defined number of fragments, from a 25 000 set of commercially available building blocks, and applying up to 58 virtual organic reactions. DOGS grows molecules sequentially, guided by a two-dimensional graph kernel method for similarity evaluation of the intermediates to the template.13

Results and discussion

The software generated a total of 210 drug-like candidates (172 unique), which featured a set of 88 diverse unique scaffolds...
Among the top solutions we found a high prevalence of arylsulfonamides. Furthermore, these compounds could be easily accessible through synthesis. As such, two compounds, 2a and 2b, were selected for synthesis and in vitro evaluation.

Compounds 2a and 2b were obtained in good yields following the synthesis pathway suggested by the software. Both inhibitor candidates were acquired from amide bond formation, reacting sulfamethizole with the required carboxylic acid in CH₂Cl₂ and using EDCI–HOBt as coupling agents (ESI†). With these two compounds in hand, we performed preliminary activity screenings against human AurA. To our surprise, compound 2a inhibited AurA by only 5% at 100 μM, while compound 2b presented an IC₅₀ value of 10 μM (Fig. 2A). Dynamic light scattering analysis of 2b showed no detectable aggregation at 50 μM. Both compounds differ solely by their terminal substituents, which were proven critical for activity according to our data. In a crystal structure complex of reference compound 1 in the ATP binding site of AurA (PDB ID: 3E5A), the pyrazole moiety in 1 interacts via hydrogen bonds with the hinge region of the kinase, while the cyclopropylcarboxamide group stabilizes the protein–ligand complex deep in the binding site.

To confirm that a substantial scaffold-hop had been achieved with our hit compound 2b, we tested the corresponding carboxylic acid starting material for AurA inhibitory activity. The building block alone showed only 6% inhibition at 100 μM, confirming that the arylsulfonamide scaffold significantly contributes to potency, either by interacting with key residues in the binding site or acting as a spacer. In order to rationalize this finding, we docked 2b into the ATP binding site of AurA (PDB ID: 3E5A) using the software GOLD 5.1. Hydrogen bonds between the pyrazole moiety and Ala₂¹³/Glu₂¹¹ from the hinge region, as well as between nitrogen atoms from the amide region and Thr₂¹⁷ from the phosphate binding site were present in the predicted complex (Fig. 2B). Albeit several classes of molecules have been described to inhibit AurA, only one structure-based designed compound features the arylsulfonamide scaffold, and none combines a sulfonamide moiety with a pyrazole ring. Most notably, using our computational approach we were able to obtain a new lead-like molecule by feeding the software solely with a two-dimensional template structure.

We compared the computationally designed molecules with 178 commercially available kinase inhibitors. All compounds were represented by a topological pharmacophore descriptor (CATS²²), and nearest neighbors to the design series among the kinase reference inhibitors were determined. As a result, EGFR and RET kinase targets showed the highest frequencies, while AurA and B were not observed (Fig. 2C). Then we projected the kinase reference inhibitors and the design series to the plane by t-distributed stochastic neighbor...
embedding\cite{20} (Fig. 2D). In the resulting map, the template VX-680 appears to be at the interface of the design series and kinase references. Compound 2b as well as a multitude of other homogeneously grouped designs reveal no kinase reference in their immediate neighborhood. Therefore they may be considered as new chemotypes. Furthermore, we performed \(k\)-means clustering\cite{21} of the designed molecules (Fig. S1\textsuperscript{\ref{S1}}), so that \(k = 9\) groups of structurally related designs were obtained. The cluster means were subject to creating a neighbor-joining\cite{22} tree, in which nodes are labeled with all kinases showing less than 20\% remaining activity in presence of the cluster-representative reference inhibitor (Fig. 2E). The tree fully concurs with the histogram presented in Fig. 2C, by exhibiting frequent appearances of EGFR and no Aurora kinase label. Having used a highly potent AurA/B inhibitor as a template for de novo design, the latter might be astonishing. However, this observation stresses the capability of \( \text{de novo} \) design to access new chemical space, and a profound distance of our designed compounds to known Aurora inhibitors. It also points to limitations of similarity-based approaches for target profile prediction.\cite{23}

We screened compound 2b against a panel of 48 kinases, in which moderate selectivity was observed (Gini\cite{24} coefficient = 0.6). At 10 \( \mu \text{M} \) the compound was inactive against most enzymes, but inhibited a few serine/threonine kinases (CaMK2a, CHK2, MAP4K4; 41\%, 65\%, 69\% inhibition; cf. ESI\textsuperscript{\ref{ESI}}). Having noticed that the predicted docking pose of 2b insufficiently occupies the most buried part of the ATP-binding site, we reasoned that an aromatic moiety connected to the pyrazole ring could enhance by increased molecular size and log\( \text{P} \), which is linked to high attrition rates in drug development.\cite{25,26}

Several indices have been suggested to guide hit prioritization.\cite{27} Accordingly, our compounds fully qualify as lead candidates (Table 1). Size-independent ligand efficiency (SILE)\cite{28} shows that 4a is a better lead than 2b, and both are in the desirable range of log\( \text{P} \) per ligand efficiency unit (LELP),\cite{29} and present fit-quality (FQ) values\cite{30} close to 1.

Compared to 2b, the kinase panel screen showed a marked selectivity drop for 4a (ESI\textsuperscript{\ref{ESI}}). For example, it presented 87\%, 95\%, 75\% and 77\% inhibition at 10 \( \mu \text{M} \) against Abl, MAP4k4, KDR and TRKA, respectively. This activity inflation could be associated with newly found hinge region interactions by the introduction of a pyridyl moiety to the compound’s framework. With these preliminary biochemical data in hand, and having in mind the relevance of AurA in breast cancer, we tested compounds 2b, 4a and 4b against the ductal breast epithelial tumor cell line T47D \textit{in vitro}. Previously, expression of AurA and B had been shown in the T47D cell line.\cite{31} Treatment of T47D cells with 2b, 4a and 4b resulted in concentration-dependent inhibition of cell proliferation, with a minimal effective concentration of 20 \( \mu \text{M} \) for 2b (−21\%; \( p < 0.01 \); Dunnett test), 10 \( \mu \text{M} \) for 4a (−21\%; \( p < 0.01 \)) and 20 \( \mu \text{M} \) for 4b (−31\%, \( p < 0.01 \)) (Fig. 3). Among the three tested compounds, 4a exhibited the highest cellular activity.

### Table 1 Optimization metrics for compounds 2b, 4a and 4b

<table>
<thead>
<tr>
<th>Metric</th>
<th>2b</th>
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</table>

\( ^{a} \text{Ligand efficiency.} ^{b} \text{Lipophilic ligand efficiency.} ^{c} \text{clogP per unit of LE.} ^{d} \text{Size-independent ligand efficiency.} ^{f} \text{Fit quality.} ^{j} \text{Binding efficiency index.} \)

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![Scheme 1 Synthesis of compounds 4a, b from intermediates 3a, b. Reagents and conditions: (i) (a) MeOH, Pd/C 10\%, \text{H}_2, \text{N}_2, \text{reflux.} (b) MeOH, \text{Br}_2, \text{aldehyde, rt.}

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Conclusions

Our study exemplifies the robustness of ligand-based de novo design for generating ideas for lead discovery. With VX-680 as a design template the software identified a structurally different chemotype that shows promising activity in biochemical and phenotypic assays. Our findings hold promise to inhibit the growth of breast cancers in vivo by de novo designed AurA kinase inhibitors. Overall, this study confirms reaction-based computational de novo design as a valuable source of synthetically feasible compounds that are readily amenable to medicinal chemistry.

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Notes and references