AI-driven drug discovery: identification and optimization of ALDH3A1 selective inhibitors with nanomolar activity

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Abstract:

In the field of medicinal chemistry, the discovery of novel compounds with therapeutic potential is of utmost importance. However, the conventional approach to drug discovery, relying on high-throughput screening (HTS), encounters limitations such as reagent availability and labor costs. Although a hit-finding campaign starts with a virtual screen of millions of compounds, the hit-to-lead and lead optimization stages often require designing, synthesizing, and profiling thousands of analogs before selecting clinical candidates. Recent advances offer an innovative solution - the virtual generation of billions of synthetically feasible compounds with an impressive 80% success rate for synthesis. This breakthrough has the potential to significantly expand the chemical space available for purchase and experimental validation, bringing about a revolution in the field. Our study presents a comprehensive approach to compound discovery and optimization, harnessing quantitative high-throughput screening (qHTS), chemical databases, and reaction-based enumeration. To further enhance the synthesis process and gain deeper insights into compound formation, we utilize the Reaction Cookbook from Biosolveit, which comprises reaction SMARTs for around 300 chemical reactions. By employing this wide range of reactions, we aim to uncover the full spectrum of a chemotype's potential and customize its structure to optimize desired properties. As an illustration of this approach, our work on the ALDH3A1 project resulted in the synthesis of 50 compounds, with 21 of them exhibiting activity (negative curve class values; hit-rate ~42%). Among these active compounds, 6 displayed IC_{50} values lower than 30 μM and efficacy values less than -50%, with the most potent compound achieving an impressive potency of 447 nM. This study demonstrates the successful synergy between in-silico reaction-based analogs enumeration, molecular modeling and AI/ML-based techniques in identifying compounds with improved biological activity, offering promising prospects for the development of

ALDH3A1-targeting agents as potential cancer therapeutics. Computational workflows developed in this study can be used for similar target-based drug discovery campaigns.

Introduction:

In the field of medicinal chemistry, discovering new therapeutic agents stands as a crucial and continuous challenge. While traditional drug discovery methods, notably high-throughput screening (HTS), have been helpful in identifying potential drug candidates, they are often hindered by factors such as limited availability of specialized reagents and the significant resource demands of conducting extensive experimental screens [1–4].

The advent of computational chemistry and advancements in synthetic methodologies have brought about a paradigm shift in compound discovery and optimization [5–7]. In recent years, *in silico* reaction-based enumeration has gained prominence, offering a novel solution to overcome the limitations inherent in traditional HTS methods [8–10]. This approach enables the virtual generation of a vast array of synthetically feasible compounds, thereby broadening the scope of chemical entities available for exploration and significantly improving the rate of successful compound synthesis [11, 12].

In our study, we complemented this modern approach by integrating it with quantitative structure-activity relationship (QSAR) modeling and quantitative high-throughput screening (qHTS). This combination allowed for a more systematic and data-informed identification of potential drug compounds [12, 13]. Our methodology was further enriched by incorporating extensive chemical databases [14–16]. These databases, replete with molecular building blocks and reaction templates offer a rich resource for data-driven drug design, facilitating the exploration of a wide range of chemical structures and properties [14]. QSAR modeling has been successfully used in the past for virtual screening, where it helps predict the biological activity of compounds based on their chemical structures [13, 17–21]. This prediction capability of QSAR models aids in narrowing down the vast pool of potential compounds to those most likely to exhibit desired biological activities [22].

To illustrate the effectiveness of this approach, we evaluated its potential using ALDH3A1 as a case study. ALDH3A1 serves a critical function in metabolizing a broad spectrum of aliphatic and aromatic aldehydes, safeguarding cells from oxidative damage, and maintaining cellular balance [23, 24]. Dysregulation of this enzyme is implicated in an array of diseases, such as cancers and neurodegenerative disorders, highlighting its value as a target for therapeutic intervention across these conditions [25, 26].

Applying this approach with the goal of discovering potent, novel, and selective inhibitors targeting ALDH3A1 through performing the comprehensive structure-activity relationships optimization resulted in the synthesis of 50 distinct compounds, demonstrating the method's ability to produce potent chemical compounds. Of these, 21 compounds showed activity, indicated by their negative curve class values. Notably, 6 of these active compounds had IC_{50} values below 30 μM and efficacy values were under -50%, suggesting their strong potency. The most potent compound identified had IC_{50} of 447 nM, showing the effectiveness of this approach in finding compounds with significant potential for drug discovery.

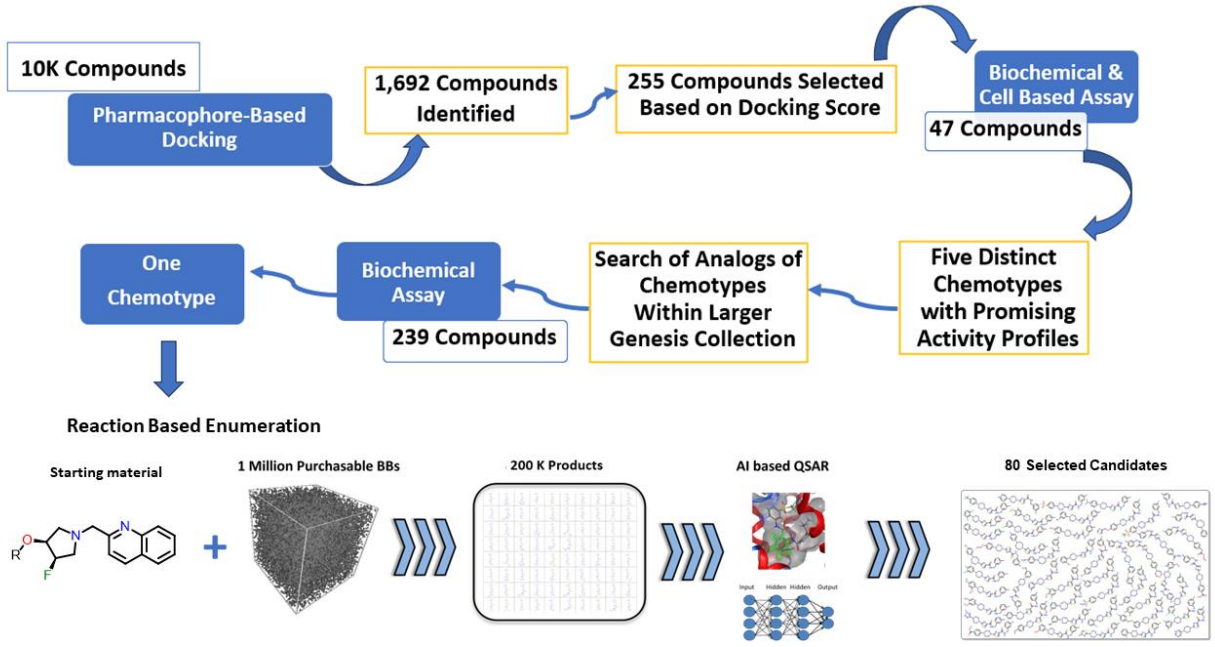


Figure 1: Flowchart of Study Approach: A flowchart that outlines the steps taken in the study: pharmacophore-based docking, reaction-based enumeration, QSAR modeling, and experimental validation.

In summary, our method, which synergistically combines data-driven compound selection, reaction-based synthesis, and comprehensive computational analysis, marks a significant advancement in the field of medicinal chemistry. It paves the way for the expedited development of novel compounds with enhanced therapeutic potential, potentially bringing substantial benefits to patient care and advancing public health initiatives.

Methods:

Pharmacophore-Based Docking and Selection

The pharmacophore-based molecular docking was performed using the Molecular Operating Environment (MOE) software [27–29]. The compound library for docking comprised a diverse set of molecular compounds (approximately 9,000 compounds), each of which was prepared using MOE's ligand preparation tools. This involves energy minimization and the generation of 3D conformations. The crystal structure of human ALDH3A1, PDB ID: 3SZA, was selected as the starting point for our pharmacophore-based molecular docking studies. Initial steps involved cleaning the protein structure, which included the removal of water molecules, irrelevant heteroatoms, and the addition of hydrogens. Protonation states were assigned, followed by energy minimization to optimize the protein's conformation [30, 31].

Active Site Identification

The active site of ALDH3A1 was identified based on the ligand-binding region observed in the crystal structure. This region was defined as the docking site for subsequent simulations.

Pharmacophore Model Development

A pharmacophore model was developed (in MOE) to represent the essential features required for interaction with the crystal ligand in its protein binding site (PDB ID: 3SZA). The model included key features such as hydrogen bond donors and acceptors, hydrophobic regions, and aromatic rings, which are critical for molecular interactions.

Docking Protocol

The docking protocol involved aligning the compounds from the library to the pharmacophore model to identify potential binders [32]. MOE's docking algorithm (Affinity dG scoring function) was used to simulate the interaction between each compound and the target binding site. Post-docking, each compound was scored based on its docking pose and the degree of match with the pharmacophore model. The scoring function in MOE provided a quantitative measure of the binding affinity. Compounds were then ranked based on their scores, with higher-scoring compounds considered more likely to be active.

Experimental Testing and Assay Information

The assay has been previously published [33]. Briefly, 3 µL of enzyme (final concentration of 5 nM for ALDH3A1) or assay buffer (100 mM HEPES pH 7.5 with 0.01% Tween 20) were

dispensed into a 1,536-well solid-bottom black plate. Twenty nL of compounds (final concentration range 420 pM to 50 μM) or control (DEAB or ZM-39923 final concentration range 1.52 nM to 50 μM) were transferred via acoustic droplet ejection. Samples were incubated (room temperature, protected from light) for 15 minutes followed by an addition of 1 μL substrate mixture of $NADP^+$ and benzaldehyde (final concentrations of 1 mM and 200 μM, respectively; K_ms of 260 μM and 280 μM, respectively) [34, 35], in addition to coupling reagents Resazurin and Diaphorase (final concentrations of 100 μM and 0.7 U/mL) to right-shift the detection by monitoring the production of resorufin [36, 37]. Plates were centrifuged at 1,000 rpm (164 x g) for 15 seconds, then read (RT) in kinetic mode on a ViewLux High-throughput CCD imager equipped with standard Rhodamine optics (525 nm excitation, 598 nm emission) for 5 minutes (< 20% conversion). The change in fluorescence intensity over the 5-minute reaction period was normalized against no-inhibitor and no-enzyme controls.

Data from each assay were normalized plate-wise to corresponding intra-plate controls as noted above. The same controls were used for the calculation of the Z' factor, a measure of assay quality control. Concentration-response curves were fitted and classified as described previously [38–42]. IC_{50}'s were calculated using Prism software (version 8.1.2, GraphPad Software, Inc.), sigmoidal dose-response (variable slope). We used the Palantir Technologies (Washington, DC) data integration platform, which is configured to ingest all HTS results generated at NCATS and harmonized this data with other sources such as ChEMBL and OrthoMCL. All qHTS screening results are publicly available at PubChem**.** The chemical structures were standardized using the LyChI (Layered Chemical Identifier) program (version 20141028, https://github.com/ncats/lychi) [43].

Reaction-Based Compound Enumeration with KNIME

For the enumeration of potential compounds, we utilized the KNIME Analytics Platform [44, 45], leveraging its capabilities for integrating and automating cheminformatics processes [11]. Our approach focused on two key synthetic reactions: O-alkylation and amide coupling. We generated the reaction SMARTS for the two reactions and, using the RDKit two-component reaction node within KNIME, systematically generated a virtual library of compounds. This process was based on the chemotype we had previously identified, employing Enamine's building blocks to explore various compound permutations. The enumeration process yielded approximately 200,000 virtual compounds, thereby expanding our chemical space and facilitating the discovery of novel variants related to our target chemotype. This expansive virtual library serves as a resource for subsequent screening and experimental validation.

A QSAR model utilizing biochemical data from older protocols was then applied to these enumerated compounds to select the most promising candidates.

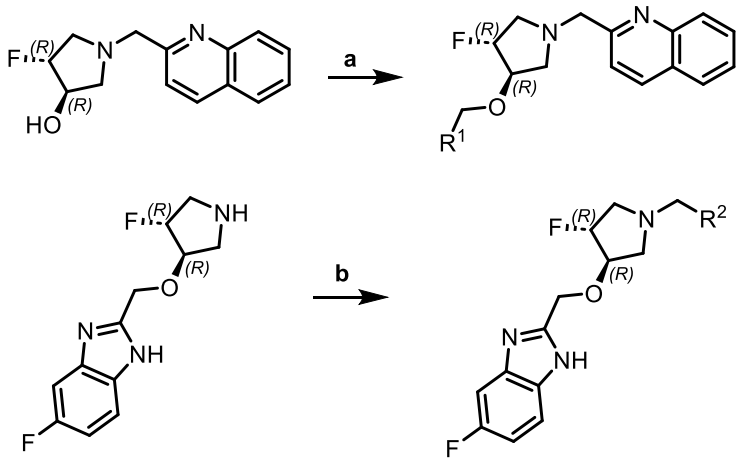
Quantitative Structure-Activity Relationship (QSAR) Modeling with KNIME

To refine the selection of our chemotype-specific virtual compound library, we developed a regression-based Quantitative Structure-Activity Relationship (QSAR) model [13, 46]. This model was built using the KNIME Analytics Platform, leveraging both physicochemical descriptors and Morgan fingerprints from RDKit to characterize the molecular structures. The model was built using the tree ensemble learning node within KNIME, which is well-suited for capturing complex non-linear relationships between descriptors and biological activity.

To ensure the relevance of our QSAR model's predictions, we introduced an applicability domain framework [47, 48]. This was performed by calculating the Tanimoto similarity between the enumerated compounds and those in the training set. We set a similarity threshold of 0.7, allowing us to focus on compounds that were structurally related to the biologically active compounds in our training data. This step ensured that the model predictions remained within the chemical space for which the model was applicable and reliable.

The incorporation of the applicability domain by similarity assessment served a dual purpose: it enhanced the robustness of our predictions and allowed us to identify which novel compounds were most likely to exhibit desirable levels of biological activity. Consequently, this approach facilitated a more targeted and efficient selection of candidates for subsequent experimental validation.

Synthetic Routes Assessing Pyrrolidine-Based ALDH3A1 Inhibitors



Reagents and conditions: (a) R^1-X (X = Cl or Br, K_2CO_3, DMF, rt to 60°C, 1−24 h. (b) R^2CHO, $NaBH(OAc)_3$, DCE, rt, 1-12 h.

*General Procedure for (**1**):* To a mixture of 4-fluoro-1-(quinolin-2-ylmethyl)pyrrolidin-3-ol (10 mmol, 1 eq) and potassium carbonate (K_2CO_3, 15 mmol, 1.5 eq) was added N,N-dimethylformamide (DMF, 0.6 M). The mixture was stirred at room temperature for 5 minutes, before the addition of R^1-X (12 mmol, 1.2 eq). The reaction mixture was stirred at room temperature for 30 minutes and then heated at 60°C for 2 hours. After the reaction was complete, the mixture was filtered to remove the solids. The filtrate containing the product was purified on a preparative HPLC to obtain pure products after lyophilization.

*General Procedure for (**2**):* "To a mixture of 5-fluoro-2-((4-fluoropyrrolidin-3-yloxy)methyl)-1H-benzo[d]imidazole (10 mmol, 1 eq) and an appropriate aldehyde (12 mmol, 1.2 eq) in dichloroethane/MeOH (0.6 M) was added sodium triacetoxyborohydride (15 mmol, 1.5 eq). The reaction mixture was stirred at room temperature for 1-12 hours. After completion of the reaction, the excess solvent was removed by evaporation (instead of 'forced air'). The crude mixture was then dissolved in DMSO and purified using preparative HPLC to obtain pure products.

Results:

Identification of Compounds through Pharmacophore-Based Docking

Our pharmacophore-based docking approach, utilizing the MOE software, successfully identified 1,692 compounds that aligned with our pharmacophore hypothesis. From this set, we carefully selected 255 top-ranked compounds for further analysis. This selection was made based on criteria such as docking scores, how well each compound fit the pharmacophore model, and their chemical diversity. These compounds were then advanced to *in vitro* assays to assess their biological activity against the ALDH3A1 enzyme.

Experimental Validation and Activity Screening

Experimental validation of the 255 compounds resulted in the identification of 47 compounds with significant biological activity. This activity was characterized by their ability to inhibit the enzymatic function of the ALDH3A1 target, reinforcing the predictive power of our pharmacophore-based docking strategy.

From these active compounds, we were able to distinguish five unique chemotypes, each showing a promising profile of activity. Focusing on one of these chemotypes, we then performed a reaction-based enumeration to further explore its potential.

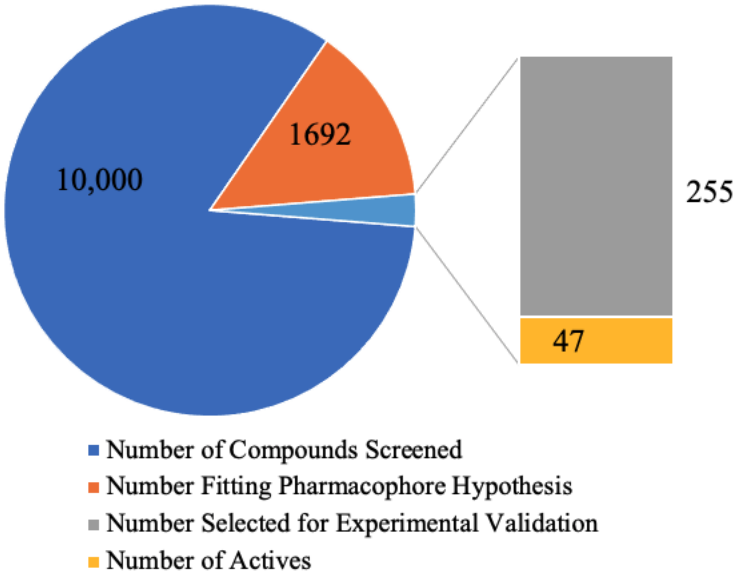


Figure 2: Docking and Activity Results: A pie chart showing the number of compounds screened, the number fitting the pharmacophore hypothesis, the number selected for experimental validation, and the number showing activity.

Compound Expansion through Reaction-Based Enumeration

We expanded our chemical exploration by employing reaction-based enumeration using one million building blocks provided by Enamine. This process included both amide coupling and O-alkylation reactions, culminating in a substantial library of 250,000 enumerated compounds. Subsequent application of a QSAR model, which was trained using biochemical data, enabled us to shortlist the most promising candidates from this extensive compound set.

QSAR Model Performance and Compound Selection

The QSAR model, built on regression analysis using RDKit descriptors and fingerprints, demonstrated an R-squared value of 0.52, indicating a moderate predictive ability. Leveraging this model, we selected the top 500 compounds for synthesis.

Synthesis and Bioactivity of Selected Compounds

From the top 500 compounds, we further selected and were able to synthesize 50. Testing of these synthesized compounds revealed that 21 exhibited measurable activity, as indicated by negative curve class values. Further scrutiny within this active group identified 6 compounds with IC_{50} values below 30 µM and efficacy (Eff) values of less than -50%. The most potent compound within this selection stood out with an IC_{50} of 447 nM, showcasing the strength of our integrated approach in discovering highly active compounds for potential therapeutic use.

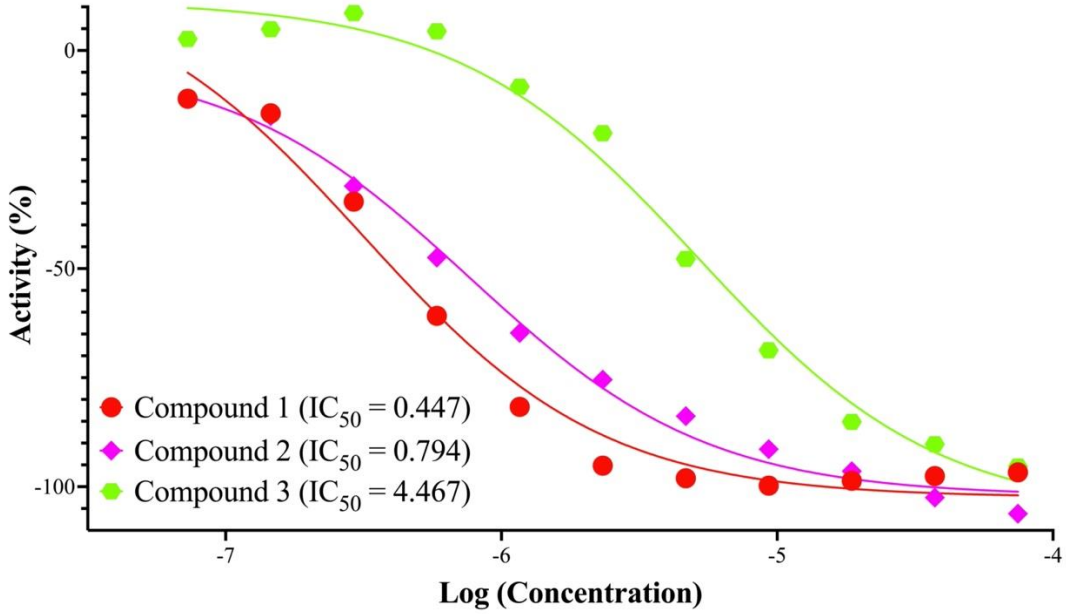


Figure 3: Lead Compound Potency: Dose-response curve for top 3 lead compounds.

Discussion

The results obtained from our integrated drug discovery approach illustrate the effectiveness of combining pharmacophore-based docking with reaction-based enumeration and QSAR modeling. The successful identification of 47 active compounds from an initial pool of 1,692, derived via pharmacophore-based docking, validates our hypothesis-driven selection criteria. Notably, the reduction to 255 compounds for experimental validation underscores the importance of a multi-parameter selection process that balances docking scores with pharmacophore fit and chemical diversity.

Our reaction-based enumeration strategy, leveraging the extensive Enamine building block repository, demonstrates the utility of combinatorial chemistry in modern drug discovery. By generating a vast library of 250,000 compounds through targeted reactions, we effectively expanded the chemical space under investigation. The subsequent application of a QSAR model,

with a moderate R-squared value of 0.52, was instrumental in prioritizing 500 compounds with the highest potential for biological activity.

The synthesis and experimental validation of these compounds led to a substantial hit rate of 42%, with 21 active compounds emerging from a synthesized set of 50. This high success rate demonstrates the robustness of our reaction enumeration and QSAR predictions and the chemical intuition underlying the selection of these molecules for synthesis. The further delineation of 6 compounds with IC_{50} values lower than 30 μM and efficacy values less than -50% reinforces the potential of these compounds as potent inhibitors of ALDH3A1. Finding a lead compound with an IC_{50} value of 447 nM is a noteworthy step forward in our study. It exemplifies the transformative potential of a methodical and data-driven approach to drug discovery. This compound, alongside the other active compounds identified, provides a promising foundation for the development of new therapeutic agents.

In conclusion, our study demonstrates the power of an integrated approach that combines computational and experimental methodologies. This hybrid strategy maximizes the efficiency of the drug discovery process and paves the way for the rapid development of novel therapeutic compounds. Future work will focus on further optimization of these active compounds and validation of their efficacy in relevant biological models, with the ultimate goal of contributing meaningful advances to the field of medicinal chemistry and drug development.

Acknowledgments

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