Straightforward hit identification approach in fragment-based discovery of bromodomain-containing protein 4 (BRD4) inhibitors

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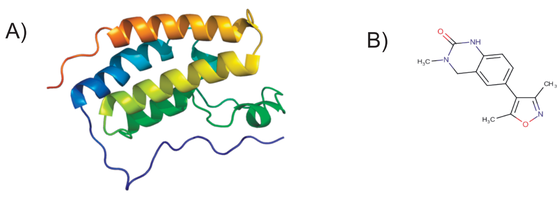
INTRODUCTION

A combination of fragment-based approach and “SAR by catalog” were employed for the discovery of bromodomain-containing protein 4 (BRD4) inhibitors. A screening of 3,695-fragment library against bromodomain 1 of BRD4 using protein Thermal Shift Assay (TSA), followed by initial hit validation, resulted in 73 fragment hits. Based on these findings, a follow-up library of analogs available in Enamine screening collection was created, as well as two other control sets containing analogs of inactive fragments and randomly selected compounds (3,200 compounds each). Screening by TSA of these 3 compound sets, followed by dose-dependence screen, counter-screen, and TR-FRET confirmation assay for BRD4-substrate binding inhibition, resulted in 18 hits. Compounds derived from the initial fragment hits exhibited substantially higher hit rate (>3-fold) compared to the two reference sets. For three compounds with IC_{50} = 1.9–7.4 uM docking poses in the BRD4 (4UYD) bromodomain 1 binding site were determined.

THERMAL SHIFT ASSAY FOR hBRD4

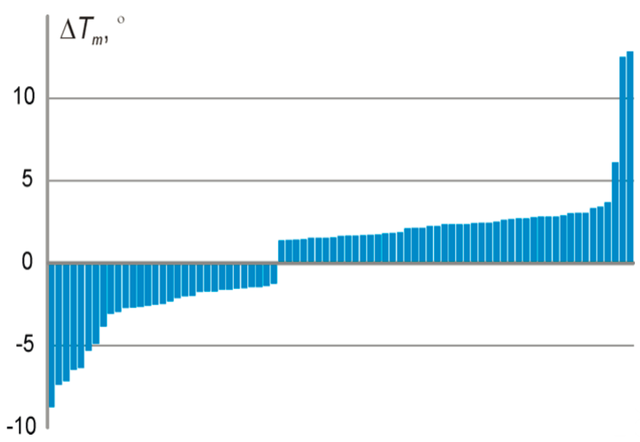
Bromodomain-containing protein 4 (BRD4) is the most extensively studied member of bromo- and extraterminal domain (BET) family, known for its role in epigenetic regulation and chromatin remodeling (A). BRD4 has been implicated in a number of diseases including cancer, inflammation, human immunodeficiency virus (HIV) infection, cardiovascular diseases, and central nervous system (CNS) disorders[1].



A. BRD4 structure; “Ligandability” pre-screen[2] was performed with BRD4 reference binder CNEMBL1828978[3] (B), resulting in positive dTm shift in Thermal Shift Assay (TSA) (C).

FRAGMENT SCREENING

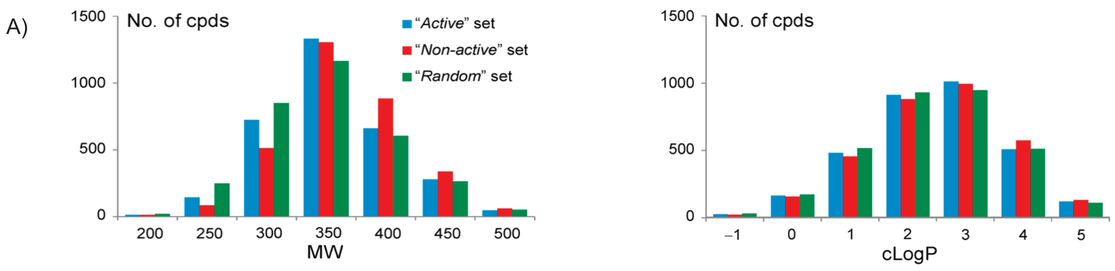
The initial fragment set for the screening was selected from Enamine’s Ro3-compliant fragment collection (7,450 compounds) with experimentally confirmed solubilities using 2D fingerprint-based diversity filtering. The resulting set of 3,695 fragments was subjected to protein thermal shift assay (TSA) using recombinant, truncated, His6-tagged bromodomain 1 of BRD4 at 100 μM, resulting in 79 initial fragment hits (2.14% hit rate; 48 positive and 31 negative shifters).



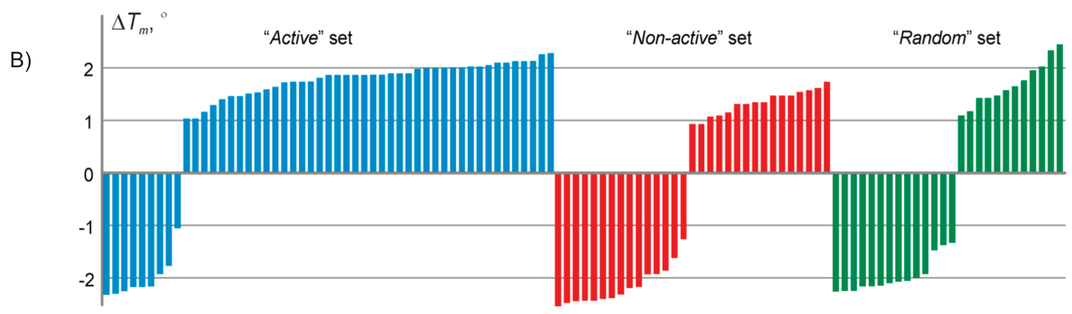
Thermal shift values (dTm) for the initial 79 fragment hits

HIT EXPANSION AND DOCKING

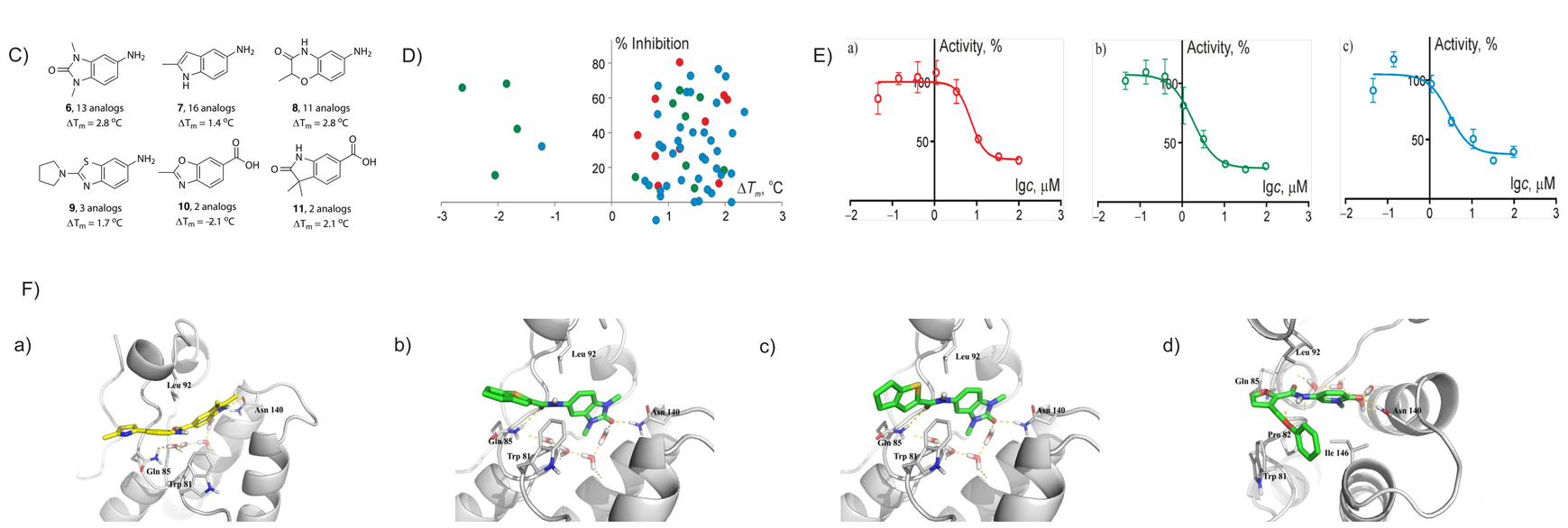
Validated fragment hits were subjected to substructure search within 337 Million+ feasible compounds from Enamine’s REAL database, using physico-chemical filtering (lead-likeness criteria) and fingerprint-based diversity selection procedure (Morgan2 fingerprints, 1,024 bit[4]), resulting in the selection of three sets of 3,200 compounds each: “Active” set included selected fragments substructures, ”Non-active” set- of non-hit fragments , and ”Random” set (A).



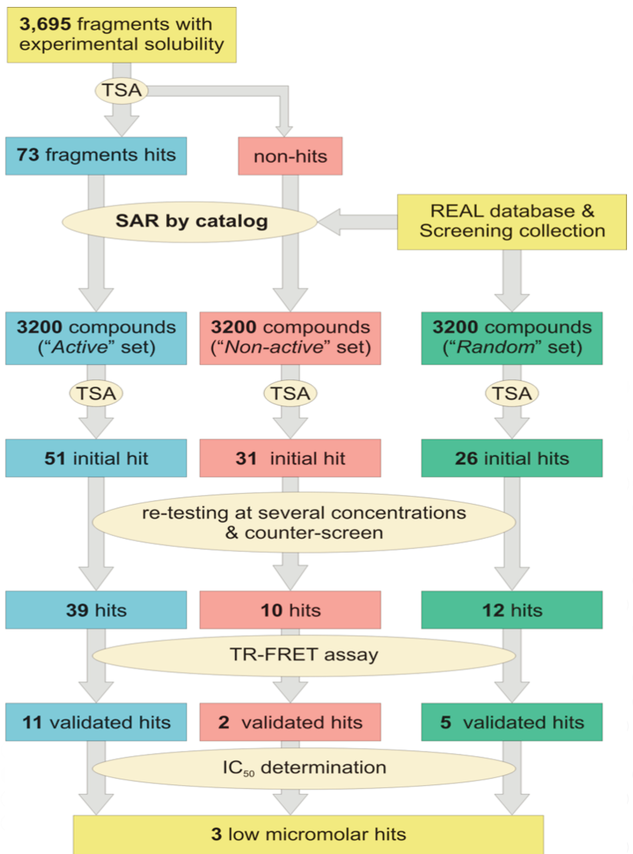
All three sets were subjected to the TSA against BRD4 at 20 μM (B). The “Active” set demonstrated 3.2–3.9-fold higher hit rate compare to two other sets with 82% of positive shifters within the “Active” set. 47 out of 51 hits from the “Active” set were derived from only six structurally similar fragments 6–11 (C). Next, 61 validated hits from all 3 sets were subjected to time-resolved fluorescence resonance energy transfer (TR-FRET) assay against BRD4 at 20 μM[5] (D).



For the 3 most active hits, dose-response curves were obtained, and IC_{50} values were calculated (E). In addition, docking calculations were made and docking poses of the 3 most active compounds were determined.



A. Physical chemical profile of the compound sets selected for the fragment hit expansion: MW (left panel), cLogP (right panel). B. Thermal shift values (dTm) for the hits obtained after “SAR by catalog” studies. C. Fragments which provided the largest numbers of active analogs after the hit expansion. D. Inhibition of BRD4 (TR-FRET assay) plotted against the TSA data (mean dTm after re-testing, n=4) for 61 validated hits at 20 μM (blue dots: “Active” set, red: “Non-Active” set; green: “Random” set). E. Dose-response curves for the compounds 12 (a), 18 (b), and 20 (c). F. Docking poses of the most active compounds in the BRD4 (4UYD) binding site: (a) 12; (b) 18, pose 1; (c) 18, pose 2; (d) 20



CONCLUSIONS

Workflow of this project shows that using initial fragment hits to extract their structural derivatives from the screening compound collection (“Active” set) results in hit enrichment in subsequent screens, as compared to the compounds derived from fragment non-hits or selected by diversity algorithms (“Non-active” and “Random” sets). Specifically, up to 3–5-fold better productivity was achieved for such derivatives at the final steps in this particular study.

This work underlines the effectiveness of fragment-based approach in early drug discovery programs. A combination of an appropriate fragment library and a physical screening collection containing sufficient number of compounds incorporating these fragments and/or a virtual database of feasible rapidly synthesizable screening compounds is a powerful tool to discover starting points for further optimization.

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