Successful Virtual Screening of a Chemical Database for Farnesyltransferase Inhibitor Leads

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Virtual screening of chemical databases is an emerging approach in drug discovery that uses computers to dock chemicals into the active site of a drug target to identify leads through evaluation of binding affinities of the chemicals. However, there are concerns about the validity and scope of the reported virtual screens due to lack of studies to show that randomly selected chemicals are not equally active and due to the fact that metalloproteins were rarely used as drug targets. We have performed a virtual screening of a chemical database to identify prototypic inhibitors of farnesyltransferase (FT) with zinc present in the active site. Among the 21 compounds identified by computers, four inhibited FT in vitro with IC_{50} values in the range from 25 to 100 μM. The most potent inhibitor also inhibited FT in human lung cancer cells. In contrast, none of 21 randomly selected compounds have an IC_{50} lower than 100 μM. The results demonstrate the validity of virtual screening and the feasibility of applications of this approach to metalloprotein drug targets, such as matrix metalloproteinases, farnesyltransferase, and HIV-1 integrase, for the treatments of cardiovascular diseases, cancers, and AIDS.

Introduction

The number of chemicals generated by combinatorial chemistry has been increasing dramatically. This will result in the number of compounds available exceeding the number of agents that can be tested by high-throughput screening, since compounds generated for one drug target can be reused for others. To capitalize on large databases of accumulated compounds, computational (or virtual) screening can, in principle, be pursued by iteratively docking each compound of a database into the active site of a drug target to identify drug leads through evaluation of the binding affinity of the compound. This approach should reduce the burden of high-throughput screening by selecting subsets for experimental testing. It would also complement high-throughput screening in cases where a particular biological assay is not suitable for robotic screening, and it would afford screens of virtual libraries of chemicals not yet made or not currently available.

Although many virtual screenings have been reported,[1−11] for several reasons skepticism remains regarding the validity and scope of such virtual screenings. First, there is a paucity of theoretical ground for reconciling a conflict in most computational screenings: on one hand, the computational screenings are made tractable by assuming that receptors and ligands are rigid; on the other hand, receptor and ligands are seldom rigid and mutually adapt their conformations to effect binding. Second, except for one report,[11] all the reported screening methods were confined to proteins devoid of metal ions and/or cosubstrates in the active site. The reported screening methods have been generally considered not applicable to many important metalloproteins as drug targets for the treatments of cardiovascular diseases, cancers, and AIDS until now. For example, farnesyltransferase (FT),[12] matrix metalloproteinases,[13] and HIV-1 integrase[14] all contain a metal ion in the active site. Third, in all the computational screening examples reported so far, there has been a lack of control studies to prove that randomly selected chemicals are not equally active.

To address these issues, we performed a virtual screening of the Available Chemicals Directory (ACD) containing 219 390 chemicals[15] to identify prototypic inhibitors of zinc-bound FT and compared the hit rates of our virtual screening with the hit rates of random selection. FT catalyzes the transfer of the farnesyl group from the cosubstrate farnesyl pyrophosphate (FPP) to a Cys residue in the C-terminal fragment of pro-Ras proteins. The resulting protein farnesylation is a key step in the posttranslational modification of pro-Ras proteins.[12] FT consists of an α subunit (48 kDa) and a β subunit (46 kDa). The two subunits form a large cationic residue-rich active site in the middle of the enzyme (Figure 1).[12] The zinc divalent cation present in the active site is known to play a functional role in FT catalysis and to facilitate the binding of the substrates.[12,16] From the standpoint of ligand docking, it is challenging to work with FT since the active site of FT is very large. Additionally, the force field parameters of the zinc divalent cation required in docking studies had not been adequately tested until our recent studies of zinc proteins.[17−20] A structure-based approach has therefore not been considered applicable to the development of FT inhibitors (FTIs). Thus, although the first

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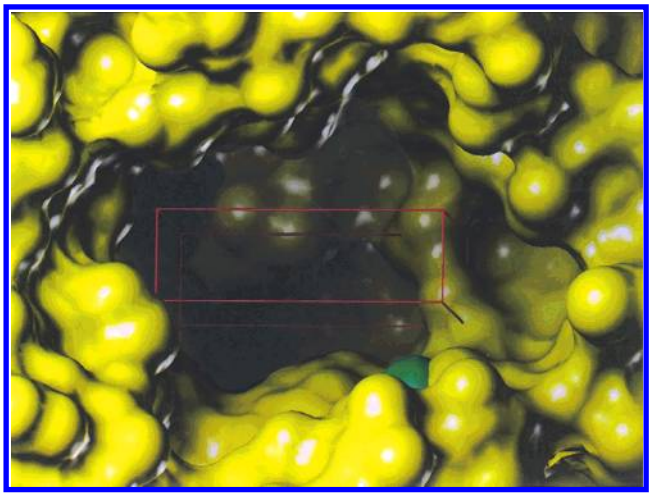

Figure 1. Top view of the large active site of FT with Zn^{2+} (green) and a rectangle box (red) that confines the translation of the mass center of compounds to be docked in the active site.

X-ray structure of FT was reported in early 1997,[12] to date, there has not been a single report of a structure-based approach to FTIs. On the other hand, FT is a promising anticancer drug target[21] and selective inhibitors of FT have been found to be able to inhibit tumor cell proliferation without substantially interfering with normal cell growth.[22−24] Several FTIs are currently in clinical trials.[25,26] Use of FT as a drug target in our virtual screening would allow us to rigorously test the validity of our virtual screening approach and to develop second generation FTIs that supplement the ones in clinical trials. Herein, we report the details of a virtual screening of a chemical database that identified a prototypical inhibitor capable of penetrating cell membranes and inhibiting FT in human lung cancer cells.

Experimental Section

Materials. Compounds **1**, **2**, **6**, **8**−**10**, **12**−**13**, **16**, **21**−**27**, **29**, **30**, and **38** were purchased from SALOR (Sigma Aldrich Library of Rare Chemicals, Aldrich Chemical Co. Inc., Milwaukee, WI), compounds **3**−**5**, **7**, **11**, **15**, **17**, **18**, **28**, **32**, **35**−**37**, and **39**−**42** were purchased from Maybridge (Cornwall PL34 0HW, U.K.), compounds **14**, **19**, **20**, **24**, and **34** were purchased from Bionet (Cornwall PL32 9QZ, U.K.), compound **31** was purchased from Bachem (King of Prussia, PA), and compound **33** was purchased from Fluka (Milwaukee, WI). B581, {*N*-[2(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-3-methylbutyl]-Phe-Met-OH}, was purchased from CALBIOCHEM (La Jolla, CA). The purified recombinant rat FT was a generous gift from P. J. Casey of Duke University.[27] The farnesyl transferase [^{3}H] SPA (scintillation proximity assay) enzyme kit was purchased from Amersham (Piscataway, NJ). The BCA assay kit was obtained from Pierce (Rockford, IL).

IBS Virtual Screening Method. To computationally screen large databases containing millions of compounds by a docking study, we proposed to use a nonphysical mechanism of ligand−receptor binding termed "conformation substitution" to justify the use of one conformation of each ligand in a docking study with a large number of structurally diverse ligands.[28] In this strategy, the mutually complementary conformations of a ligand and its receptor are not obtained from adaptation,[29] namely, changing the conformation of the ligand according to the receptor and vice versa, rather they can be obtained from substitution, namely, replacing the unfit stable conformation of a molecule as a test ligand with a fit stable conformation of another molecule in a database, as long as the number of structurally different molecules to be docked in the database is large enough. In other words, if one conformation of a compound is found to be not complementary to the receptor, instead of docking alternative conformations of that compound that are generated in advance or can be generated on the fly to judge if it can bind the receptor or not, the docking program discards that compound and docks the next compound in the database that may have a conformation complementary to the receptor, since the goal of our virtual screening is to identify a subset enriched in active compounds not to identify all the active compounds in the database. This mechanism offers theoretical ground for reconciling the conflict between the conformational flexibility of ligands and receptors found in biological systems and the conformational rigidity of ligands and receptors used in the algorithm for docking compounds in a large chemical database.

To achieve this, we have developed a new docking program, EUDOC. This program is an extension of our previous docking program SYSDOC (devised by Y.-P. Pang)[30] whose algorithm has been validated by (1) predictions of the exact atomic loci and orientations of huperzine A[30] and the highly flexible E2020[31] binding in acetylcholinesterase (AChE) reported *before* the confirmatory X-ray crystal structures[32,33] and (2) a prediction of a low-affinity binding site of THA that was not obvious in the crystal structures of AChE but confirmed subsequently by use of synthetic molecular probes.[34] Important features of the EUDOC program include (1) incorporation of the Cornell et al. AMBER force field,[35] (2) the ability to calculate the intermolecular interactions of metal ions such as Zn^{2+}, Ca^{2+}, and Mg^{2+} that mediate the binding of ligands to receptors, and (3) automation for rapidly docking millions of chemicals into a macromolecular receptor to screen for complementary ligands employing "spatial decomposition" to achieve 100% parallelism in computing.

Using the fast affine transformation,[30] the EUDOC program systematically and efficiently translates and rotates a ligand in the putative binding pocket of a receptor to search for energetically favorable orientations and positions. The docking region is defined by a box within the binding pocket to confine translations of the mass center of the ligand. The resolution of the docking study is governed by the translational and rotational increments and the size of the box employed. The intermolecular interaction energy is calculated from the potential energy of the ligand−receptor complex relative to the potential energies of the two partners in their free state. The potential energies are calculated according to eqs 1−2 with the additive, all-atom force field by Cornell et al.[35]

$$E = \sum_{i<j} \epsilon_{ij}^{*} \left(\frac{r_{ij}^{*12}}{R_{ij}^{12}} - 2\frac{r_{ij}^{*6}}{R_{ij}^{6}} \right) + \sum_{i<j} \frac{q_i q_j}{\epsilon_0 R_{ij}} \qquad (1)$$

$$\epsilon_{ij} = (\epsilon_i \epsilon_j)^{1/2}, \quad r_{ij}^{*} = r_i^{*} + r_j^{*}, \quad R_{ij} = R_i + R_j \qquad (2)$$

A distance-dependent dielectric function is used for calculating the electrostatic interactions.[36] The cutoff for steric and electrostatic interactions was set to 8.0 Å[37] in this work. The zinc force field parameters used in this study and the docking studies of known potent and weak FTIs using such parameters have been reported elsewhere.[19,20] A detailed description of the use and evaluation of the EUDOC program will be reported separately, and the program will thereafter be freely available upon request.

To improve the efficiency of a virtual screening of a chemical database, we further proposed an integrated, broad, conformation substitution-based (IBS) virtual screening approach. This approach uses a number of successive filters to remove unwanted compounds, namely, it identifies drug leads by removing unwanted compounds in a stepwise fashion employing a number of computer programs serving as "filters", rather than docking all the structures in a chemical database which wastes computing time spent on docking compounds that should be removed according to other criteria prior to docking. This approach requires a two-step preparation. The first step is to determine a region in the active site of an enzyme where

chemicals will be docked. It is crucial at this step to determine the protonation states of the residues in the active site and to determine whether cosubstrates, cofactors, and/or metal ions need to be included in the active site. If so, these molecules will be docked into the active site first. The second step is to gather chemicals from different 3D databases and assign atomic charges to all the chemicals. This step can be skipped if a database of chemicals with atomic charges exists. Using a suite of commercially available computer programs as well as programs that we developed, the IBS approach performs "filtration" with (1) a chemical property filter to remove those chemically unstable or reactive compounds and compounds with molecular weights outside the range of most clinical drugs (300−700);[38] (2) a drug target-dependent filter to effectively select the chemicals with desired structural properties (e.g., neutral, nonzwitterionic compounds); (3) a steric and electrostatic complementarity filter (the EUDOC program) to remove chemicals that do not fit the cavity of the drug target; and (4) a biological property filter to remove the chemicals that are either too hydrophilic or too hydrophobic to ensure bioavailability and/or cell membrane penetration. It should be noted that there are no absolute sets of filtering rules in virtual screening. Some filters may not be necessary in some cases but mandatory in others. It all depends on the eagerness of the chemists and biologists for a drug lead. For example, if a medicinal chemist is eager to obtain a drug lead upon which one may evolve several generations of analogues to yield a compound with desired properties, the drug target-dependent filter should not be used to remove compounds bearing the charged carboxylate group. Although the carboxylate group has been shown to impede cell membrane penetration,[39] a medicinal chemist may be able to solve the penetration problem by converting the carboxylate group to an ester group.

It is expected that the IBS approach may miss active compounds in the screened database and identify some compounds that may not be active. This is because the conformation of a compound recorded in the database happens to be the unbound stable conformation and the conformation substitution mechanism does not allow the compound to change its conformation according to the receptor. This is also because the conformation of the identified compound happens to be incorrectly generated by the 3D generator program used to develop the 3D database. However, as long as the percentage of the desired compounds identified by the IBS approach is significantly larger than the one achieved by a random selection, the IBS approach is considered successful.

Drug Target. In the present study, we used the zinc-bound, FPP-free crystal structure of FT (PDB code: 1ft1) as a drug target[12] with the following modifications: (1) addition of hydrogen atoms and (2) protonation or deprotonation of the Arg, Lys, Asp, Glu, His, and Cys residues. To determine the protonation states, all the Arg, Lys, Asp, Glu, His, and Cys residues were visually inspected. Asp and Glu were treated as deprotonated unless they were located in a hydrophobic environment. Arg and Lys were treated as protonated unless they were surrounded by hydrophobic residues. His was treated as histidinate when coordinating to zinc.[18] His not coordinated to zinc was treated as protonated if it was less than 8 Å away from an acidic residue, otherwise it was treated as neutral. In the structure of the neutral His, one proton was attached to the δ nitrogen atom of the imidazole ring if the resulting tautomer formed more hydrogen bonds in the protein. Otherwise the proton was attached to the ϵ nitrogen atom. Cys was treated as deprotonated when it formed a disulfide bond or coordinated to the zinc ion.[40] The water molecules in the active site, including the water molecule coordinating to zinc, were removed from FT prior to docking.

In Vitro Inhibition Assay. The in vitro inhibition of FT by the selected compounds was measured by using the FT [^{3}H] SPA kit. In this assay recombinant rat FT at a final concentration of 0.6 to 1.2 ng/μL was incubated for 1 h in the presence of [^{3}H] FPP, a human lamin-B carboxy-terminal sequence peptide (biotin-YRASNRSCAIM), and a testing compound or control. The peptide was [^{3}H] farnesylated at the cysteine near the C-terminus when processed by FT. The resultant [^{3}H] farnesyl-(CYS)-biotin lamin B was captured by a streptavidin-linked SPA bead. Radioactivity of the peptide in a 150 μL Eppendorf tube was measured on a Beckman LS 6000IC scintillation counter. The test compounds were dissolved in DMSO and diluted 1:10 in the final assay solution. The solvent effect on FT activity was corrected by using a 10% DMSO-containing control. Each assay was carried out in duplicate with deviations less than 10%.

Ex Vivo Inhibition Assay. To determine effects of agents on FT in intact cells, the processing of prelamin A to mature lamin A was examined. Previous studies have demonstrated that removal of the carboxyl terminal prepeptide requires sequential farnesylation followed by proteolysis.[41,42] Inhibition of FT results in accumulation of prelamin A, which can be detected using antisera raised against the prepeptide.[41] An antiserum that recognizes the lamin A prepeptide was generated and characterized[41] as described elsewhere.[43] Log phase A549 human lung cancer cells in RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum, 100 U/mL penicillin G, 100 μg/mL streptomycin and 2 mM glutamine were incubated for 24 h with diluent or various drug concentrations. At the completion of the incubation, cells were washed twice with serum-free RPMI medium and lysed in 6 M guanidine hydrochloride under reducing conditions as previously described.[44] After treatment of samples as described,[44] aliquots containing 50 μg of protein (determined by the bicinchoninic acid method[45]) were subjected to SDS−PAGE and electrophoretically transferred to nitrocellulose. Blots were probed with anti-prelamin A followed by peroxidase-coupled anti-rabbit IgG using standard techniques.[44] To confirm equal loading of the samples, blots were reprobed with anti-lamin B[46] followed by peroxidase-coupled anti-chicken IgG.

Results

Screen of the ACD for FTIs. We used the chemical property filter, complementarity filter, and solvation filter to remove unwanted compounds from the 1998 release of the ACD,[15] a database containing the 3D structures of 219 390 commercially available compounds. The chemical property filter (the ISIS Base program by MDL[47] in conjunction with a program devised by Y.-P. Pang) removed those compounds with molecular weights outside the range 300−700 and the charged or zwitterionic compounds in order to identify compounds that might be more effective in penetrating cell membranes.[39] This filter also removed all chemicals devoid of an aromatic ring because such compounds cannot form cation−π interactions with the FT active site that is rich in cationic residues. As a result, a total of 67 928 compounds in the RD format[47] were selected from the ACD containing compounds with single conformation. After assigning the Gasteiger−Marsili empirical point charges[48,49] to the selected compounds using the SYBYL program[50] with a script written in the SYBYL programming language, we used the EUDOC program as the complementarity filter to remove all the unfit structures. The docking box (12.5 × 10.5 × 4.0 Å^3) in the active site of FT is depicted in Figure 1. A low-resolution docking, performed using 30° of arc and 3.0 Å as rotational and translational increments, respectively, selected 1186 molecules with intermolecular interaction energies lower than −35 kcal/mol. We then re-docked these molecules at high resolution (10° of arc and 1.0 Å as rotational and translational increments, respectively) and selected 313 molecules with intermolecular interaction energies lower than −45 kcal/mol.

Next, we used the solvation filter to remove the chemicals that were too hydrophilic or too hydrophobic

Table 1. Predicted Binding Energies and in Vitro FT Inhibitions by the 21 Identified Compounds

cpd	predicted affinity (kcal/mol)	FT inhibition
1	−49	$IC_{50} = 25\ \mu M$
2	−43	$IC_{50} = 80\ \mu M$
3	−47	$IC_{50} = 96\ \mu M$
4	−37	$IC_{50} = 100\ \mu M$
5	−41	$IC_{50} = 110\ \mu M$
6	−41	42% at 100 μM
7	−56	41% at 100 μM
8	−37	30% at 100 μM
9	−41	28% at 100 μM
10	−39	25% at 100 μM
11	−39	20% at 100 μM
12	−47	17% at 100 μM
13	−44	60% at 500 μM
14	−42	51% at 500 μM
15	−34	27% at 500 μM
16	−44	25% at 500 μM
17	−43	17% at 500 μM
18	−42	17% at 500 μM
19	−40	inactive at 500 μM
20	−43	inactive at 500 μM
21	−33	inactive at 500 μM

and selected 128 compounds with estimated binding energies lower than −33 kcal/mol. This filtration was carried out by using an interface (devised by Y.-P. Pang) that automatically calculates the solvation energies of the selected compounds using the commercially available AMSOL program with the SM5.4 PDA-AM1 method.[51] The binding energy was estimated as the EUDOC intermolecular interaction energy minus the AMSOL solvation energy. A compound was considered too hydrophobic if its solvation energy was greater than the solvation energy of neopentane (2.5 kcal/mol).[52]

Last, we visually examined 128 compounds and purchased 27 compounds with the highest binding energies after removing those compounds with obviously incorrect conformations (such as two face-to-face stacked aromatic rings at a separation of 2.0 Å), compounds with more than 10 rotatable bonds, compounds containing unwanted functional groups, and compounds that were no longer commercially available. The unwanted functional groups included thiols, azides, aldehydes, and hydroxamic acids that all have low occurrence in launched drugs documented in the MDDR.[53] To ensure that the compounds tested were the compounds identified by the virtual screen, we verified the structures of all 27 compounds and the purity of these compounds by means of ^{1}H NMR and, if necessary, MS. Six compounds were removed, either because their structures were incorrect or because they contained more than 50% impurities according to the NMR integration. The final 21 structures selected are depicted in Figure 2. Surprisingly, almost one-quarter of the purchased compounds had to be discarded. This underscores the importance of purity check and structure confirmation in virtual screenings. The predicted binding energies of the 21 structures are listed in Table 1. None of these structures have been previously reported as FTIs.

Experimental Evaluation. The in vitro FT inhibitory activities of the selected compounds are listed in Table 1. Eighteen compounds inhibited FT in vitro at the concentration of 500 μM, and 12 had inhibitory activities at the concentration of 100 μM. Four compounds had IC_{50} values in the range from 25 to 100 μM. Furthermore, **1** also inhibited FT in human lung cancer cells (Figure 3), indicating that the compound identified by the IBS virtual screening was able to penetrate cell membranes and was a functionally relevant inhibitor of FT. Consistent with the observed FT inhibitory activity of **1**, the EUDOC program used in the IBS virtual screening did suggest that **1** had specific interactions with FT including coordination to the zinc ion and hydrogen bonding, $\pi-\pi$, cation$-\pi$, and van der Waals intermolecular interactions as illustrated in Figure 4.

To demonstrate the predictive power of our IBS virtual screening, we purchased 21 compounds randomly selected, using a random number generator provided with Excel 97, from the same subset of the ACD as used in the docking step of the IBS virtual screening. These compounds went through the same filter of the above-mentioned unwanted functional groups as well. These structures are shown in Figure 5. Interestingly, the [^{3}H] SPA showed that nine and five randomly selected compounds inhibited FT in vitro at the concentrations of 500 and 100 μM, respectively. Nevertheless, none of these compounds have an IC_{50} lower than 100 μM. This result illustrates the predictive power of our IBS virtual screening in the case of the search of FTIs and demonstrates the importance of control studies in evaluating virtual screening approaches.

Discussion

The in vitro FT inhibition data and the estimated binding energies listed in Table 1 indicate that the IBS virtual screening did identify a few inactive compounds due to the aforementioned reasons (see IBS Virtual Screening Method). In addition, it is evident in Table 1 that the predicted rank order of inhibitory potency is not identical to the observed one. The reasons that contributed to the identifications of inactive compounds would also be expected to cause discrepancy between the two rank orders. Despite the false hits and the discrepancy of the rank order, the IBS virtual screening method is considered successful because the IBS screen identified four compounds with IC_{50} values in the range from 25 to 100 μM (hit rate: 19%), 12 compounds active at 100 μM (hit rate: 57%), and 18 compounds active at 500 μM (hit rate: 86%). In contrast, the random number generator identified no compounds with IC_{50} values in the same range (hit rate: 0%), five compounds active at 100 μM (hit rate: 23%), and nine compounds active at 500 μM (hit rate: 43%). This method is effective, since the most potent inhibitor lead, **1** ($IC_{50} = 25\ \mu M$), identified by the structure-based virtual screening is more active in inhibiting FT in vitro than the most potent inhibitor lead, kurasoin A ($IC_{50} = 59\ \mu M$), identified from a brute force screening of compounds generated from fermentation.[54]

The present work has demonstrated the validity of the virtual screening approach as a tool for identifying lead compounds and expanded its scope to the zinc-containing protein targets. The results now pose a new question: Can we identify even more potent, new inhibitors with virtual screens? Although a definite answer requires further experiments, several considerations suggest that more active compounds can indeed be discovered by virtual screens. First, only the ACD was used in the present study. Screens of more data-

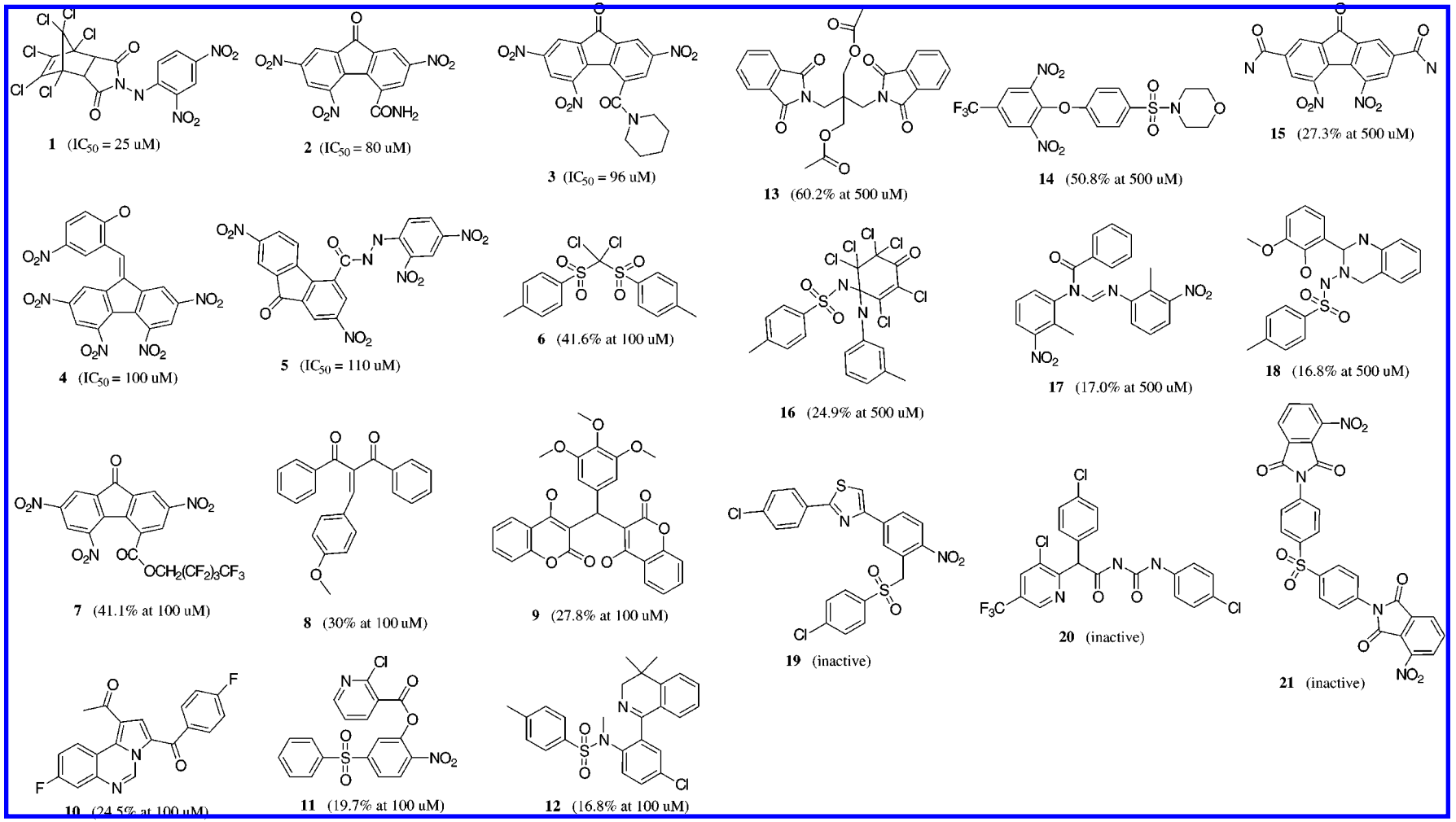

Figure 2. Twenty-one chemical structures identified by the IBS virtual screening. (The in vitro FT inhibitory activity is given in parentheses. Some polar hydrogen atoms are omitted for clarity.)

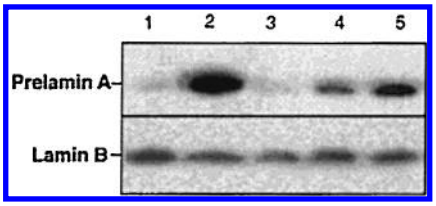

Figure 3. Inhibition of FT by **1** in human lung cancer cells (lane 1: treated with vehicle; lane 2: treated with a known potent, cell-permeable FTI, B581,[62] at 100 μM; lanes 3, 4, and 5: treated with **1** at 100, 200, and 500 μM, respectively).

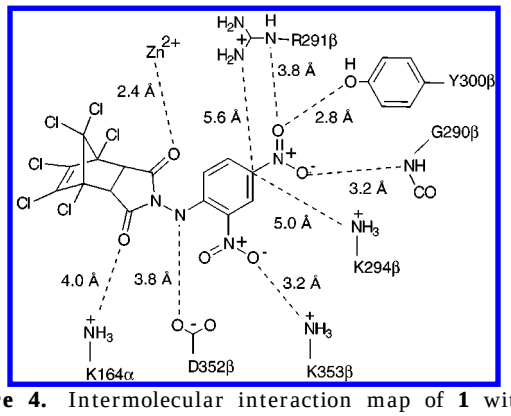

Figure 4. Intermolecular interaction map of **1** with FT predicted by the EUDOC program.

bases will undoubtedly increase the chance of finding more active compounds. Other databases that can be screened with the same method described here include the Cambridge Structural Database (197 481 compounds),[55] the MACCS-II Drug Data Report (96 730 compounds),[53] the National Cancer Institute Database (126 554 compounds),[56] and the Available Chemicals Directory-Screening Compounds (1 206 141 compounds).[47] Additionally, more new chemical databases containing the products of current combinatorial chemistry will be developed soon. Second, the present study was focused to prove the concept and therefore used a low-resolution docking (30° of arc and 3 Å rotational and translational increments, respectively) at which some compounds that actually bind well may not be identified. Effective screenings can be practically pursued with medium-resolution dockings with 10° or 20° of arc rotational increment and 1.0 Å translational increment followed by a second round, high-resolution docking with 10° of arc and 0.5 Å rotational and translational increments, respectively. Third, the 3D structures in the ACD were generated without consideration of electrostatic interaction. In visual inspections of all the EUDOC-identified compounds, we found several 3D structures with close intramolecular van der Waals contacts. It is likely that energy minimizations of all the selected structures with semiempirical charges prior to docking will increase the chance of finding more active compounds while reducing the number of false hits. Last, the cosubstrate FPP was not included in the FT drug target in the present screen

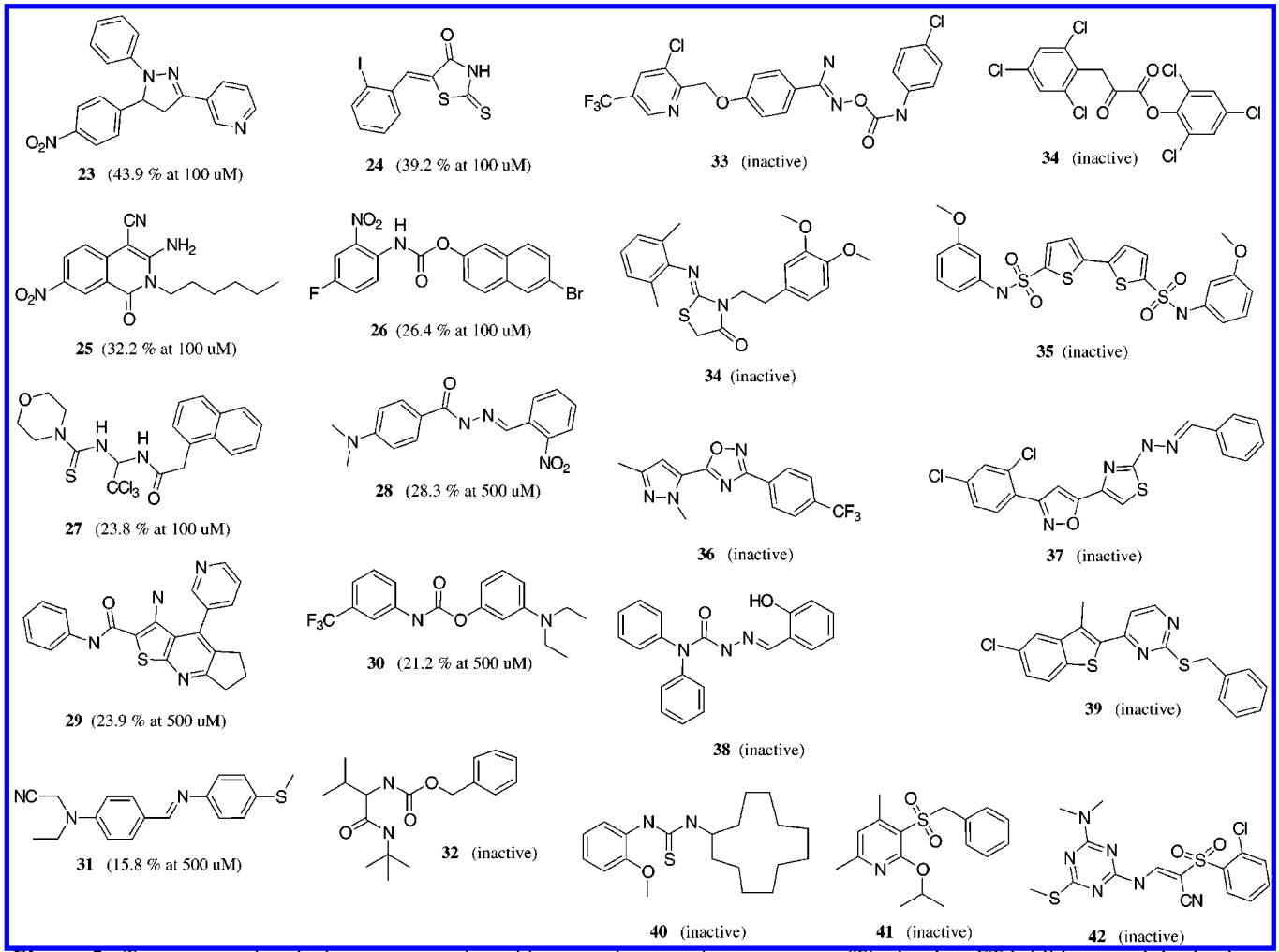

Figure 5. Twenty-one chemical structures selected by a random number generator. (The in vitro FT inhibitory activity is given in parentheses. Some polar hydrogen atoms are omitted for clarity.)

due to the uncertainty of the bound conformation of FPP in FT revealed by the conflicting crystal structures of the FPP−FT complex reported by two independent groups.[57,58] However, according to our docking studies with known FTIs[20] and the reported X-ray structures of inhibitor-bound FT complexes,[59,60] it is possible that more active inhibitors can be identified using an FPP−FT binary complex as a drug target. The presence of the endogenous, high-affinity cosubstrate FPP ($K_D = 2.8$ nM on rat recombinant FT)[61] in the active site reduces the net positive charge of the pocket because of the three negative charges on FPP, thus enabling neutral molecules to more effectively anchor at the active site.[20] In addition, the presence of FPP significantly reduces the volume of the large binding site of FT that accommodates two large substrates, thus enabling relatively smaller molecules with molecular weights close to 400 to fully occupy the remaining region of the active site of the FPP−FT complex.[20] We are currently performing a 3.0 ns molecular dynamics (MD) simulation of the FPP−FT binary complex to determine the bound conformation of FPP in the FT active site. Thereafter we will launch a second round virtual screening of the ACD and other databases using the MD-determined FPP−FT structure as a putative drug target.

Conclusions

The present work offers a successful example of virtual screening of chemical databases. Particularly important is that it allows screening of the zinc-containing protein drug targets for drug leads. In addition, the present work demonstrates the importance of control studies in evaluating different virtual screening methods. The emerging virtual screening approach will likely become an indispensable, complementary tool in drug discovery and will facilitate optimal application of combinatorial chemistry and high-throughput screening for drug discovery and development.

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Supporting Information Available: Spectral data (^{1}H NMR and MS) for compounds **1**−**21** (four pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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