In Silico Chemical Library Screening and Experimental Validation of a Novel 9-Aminoacridine Based Lead-Inhibitor of Human S-Adenosylmethionine Decarboxylase

Wesley H. Brooks,[†,#] Diane E. McCloskey,[‡,#] Kenyon G. Daniel,[†] Steven E. Ealick,[§] John A. Secrist, III,[||] William R. Waud,[||] Anthony E. Pegg,[‡] and Wayne C. Guida*[,†,⊥]

Drug Discovery Program, H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida 33612, Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033, Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853, Southern Research Institute, Birmingham, Alabama 35205, and Department of Interdisciplinary Oncology, University of South Florida College of Medicine, Tampa, Florida 33612

Received January 8, 2007

In silico chemical library screening (virtual screening) was used to identify a novel lead compound capable of inhibiting S-adenosylmethionine decarboxylase (AdoMetDC). AdoMetDC is intimately involved in the biosynthesis of polyamines, which are essential for tumor progression and are elevated in numerous types of tumors. Therefore, inhibition of this enzyme provides an attractive target for the discovery of novel anticancer drugs. We performed virtual screening using a computer model derived from the X-ray crystal structure of human AdoMetDC and the National Cancer Institute's Diversity Set (1990 compounds). Our docking study suggested several compounds that could serve as drug candidates since their docking modes and scores revealed potential inhibitory activity toward AdoMetDC. Experimental testing of the top-scoring compounds indicated that one of these compounds (NSC 354961) possesses an IC_{50} in the low micromolar range. A search of the entire NCI compound collection for compounds similar to NSC 354961 yielded two additional compounds that exhibited activity in the experimental assay but with significantly diminished potency relative to NSC 354961. In this report, we disclose the activity of NSC 354961 against AdoMetDC and its probable binding mode based on computational modeling. We also discuss the importance of virtual screening in the context of enzymes that are not readily amenable to high-throughput assays, thereby demonstrating the efficacy of virtual screening, combined with selective experimental testing, in identifying new potential drug candidates.

INTRODUCTION

The ubiquitous polyamines (primarily spermidine and spermine in eukaryotic cells) are essential in cell growth, differentiation, and proliferation.[1] However, few specific in vivo functions have been established for polyamines due to the difficulties in determining intracellular localization of polyamines and their concentrations. Variations in polyamine levels occur in the cell cycle and in response to cellular stressors such as hormones and UV light. Furthermore, the polyamines can exchange among pools of acetylated and unacetylated forms and among bound and unbound states. Additionally, polyamine levels can vary substantially due to the cellular pathways of polyamine synthesis, salvage, and transport. In spite of these difficulties, some putative roles for polyamines exist in cellular replication,[2] cell signaling,[3] modulation of transcription,[4,5] translation efficiency,[6,7] modulation of transmembrane channels and receptors,[8,9] free-radical scavenging,[10] apoptosis,[11] and angiogenesis.[12]

The synthesis, catabolism, and cellular influx/efflux of polyamines are tightly controlled. Excess or depletion of polyamine levels can lead to cellular abnormalities, such as cell death or uncontrolled growth. The importance of polyamines arises from their unique combination of length and polycationic charge distribution. This facilitates numerous putative molecular interactions. In particular, polyamines are known to interact with large polyanionic structures such as DNA, RNA, and phospholipids, aiding in organizing and condensing these structures and possibly stabilizing conformations and complexes.[13] An excess of polyamines with their ability to neutralize the self-repulsion of large polyanionic structures can explain the ability of these molecules to support abnormal synthesis and growth. It is also important for cellular polyamine levels to be controlled since polyamine synthesis can draw heavily on precursors required for other important functions in the cell. For example, S-adenosylmethionine (AdoMet) is used for methylation of both DNA and histones in transcriptional regulation.[14,15] AdoMet is decarboxylated as a key step in polyamine synthesis by S-adenosylmethionine decarboxylase (AdoMetDC), producing decarboxylated AdoMet (dcAdoMet). The cellular content of dcAdoMet is very tightly regulated according to the need for polyamine synthesis, and dcAdoMet levels are normally only a small fraction of the AdoMet pool unless polyamine synthesis is blocked.[16,17] Nonetheless, AdoMetDC

* Corresponding author phone: (813)745-6047; e-mail: wayne.guida@moffitt.org.
[†] H. Lee Moffitt Cancer Center & Research Institute.
[‡] Pennsylvania State University College of Medicine.
[§] Cornell University.
[||] Southern Research Institute.
[⊥] University of South Florida College of Medicine.
[#] These authors contributed equally to this work.

10.1021/ci700005t CCC: $37.00
Published on Web 08/04/2007

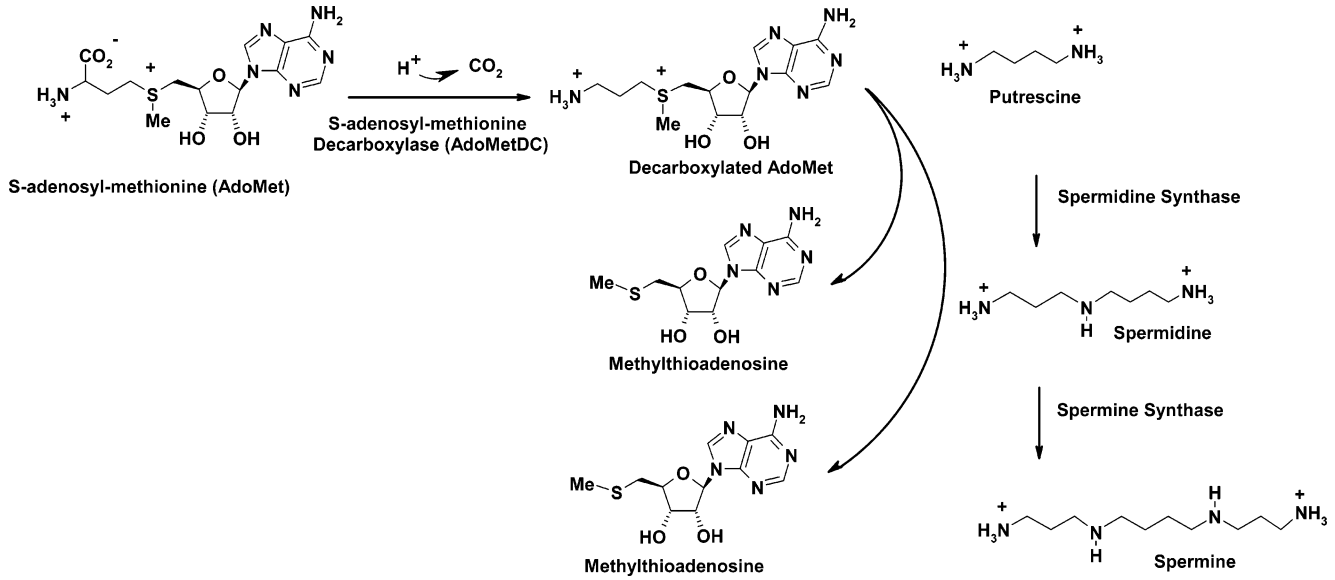


Figure 1. Biosynthetic pathway of the polyamines. S-Adenosyl-methionine decarboxylase (AdoMetDC) converts S-adenosyl-methionine (AdoMet) to decarboxylated AdoMet (dcAdoMet). This is then used to provide aminopropyl groups in the synthesis of spermidine from putrescine by spermidine synthase and in the synthesis of spermine from spermidine by spermine synthase.

is an attractive target for inhibition of polyamine synthesis due to the absolute requirement of dcAdoMet to provide the aminopropyl groups for both spermidine and spermine synthesis (Figure 1).

Neoplastic cells have increased levels of polyamines and polyamine synthesis.[18,19] Urinary polyamines are elevated in many cancers, especially solid tumors like breast, ovarian, and testicular cancer, and in non-Hodgkin's lymphoma.[20] In some cancers, such as ovarian cancer, urinary polyamine levels rise with tumor progression and fall with tumor response to therapy.[21,22]

It has also been reported that tissue levels of polyamines in human ovarian, cervical, and endometrial carcinomas are elevated compared to surrounding normal tissues.[23] Tumor progression requires elevated levels of polyamines to facilitate the condensation of newly synthesized DNA and for other purposes, such as translational efficiency.[6] Based on these observations relating polyamines to cancers and the likelihood that tumor cells will be more sensitive to apoptosis induction when polyamine levels are reduced,[11] drugs that interfere with polyamine synthesis should be highly effective as antitumor agents.[19]

Compounds have been discovered that can inhibit enzymes involved in polyamine synthesis thereby reducing intracellular polyamine levels. In some cases these compounds have shown clinical efficacy.[24,25] For example, difluoromethylornithine (DFMO), which inhibits ornithine decarboxylase (ODC) a key enzyme in polyamine synthesis, is currently in clinical trials as an agent for cancer prevention.[26−28]

As an antitumor agent, however, DFMO has not proven as effective as initially anticipated since polyamine uptake and salvage pathways can circumvent some of the effects of DFMO. In addition, some potential side effects of DFMO have emerged, such as dermal and intestinal lesions, hematological responses, and reversible hearing loss.[29] Another compound that has been tested in clinical studies is SAM486A (also referred to as CGP48664 or simply CGP486), an inhibitor of AdoMetDC.[30,31] In phase I clinical trials, SAM486A showed promising results in advanced solid malignancies and, when used in combination with 5-fluorouracil/leucovorin, in metastatic colorectal cancer.[32,33] In a phase II study, SAM486A was demonstrated to be effective in patients with non-Hodgkin's lymphoma.[25] In these studies, side effects including nausea, diarrhea, vomiting, abdominal pain, thrombocytopenia, and neutropenia were observed in some patients. These side effects appear to be unrelated to inhibition of AdoMetDC or the subsequent reduction in polyamine levels. These studies provide proof of concept that depleting polyamines, by inhibition of polyamine synthesis enzymes, can yield effective clinical results. However, there is a need for more efficacious therapeutic agents with fewer side effects. Therefore, new antitumor agents targeting polyamine biosynthesis are needed. It is now possible to take advantage of automated computational techniques like in silico chemical library screening (virtual screening) applied to structure-based drug design to identify novel AdoMetDC inhibitors.

The purpose of the present study was to find new potential drug candidates that will inhibit AdoMetDC in an effort to reduce polyamine levels as a means of attenuating tumor growth and progression. The method we employed was a synergistic approach combining the benefits of rapid high-throughput structure-based virtual screening and experimental testing with a validated AdoMetDC inhibition assay to subsequently screen only a limited number of the top ranking compounds chosen from the virtual screening results. Our methodology yielded not only an enriched subset of compounds for subsequent analysis but also uncovered a promising lead candidate that is proceeding to the next steps of drug discovery and development.

Additionally, our results provide a practical example of the synergy between experimental high-throughput screening and virtual screening. The available biochemical assay for AdoMetDC requires the use of radioactive substrates, which is problematic in a high-throughput screening environment. Our results demonstrate the unique ability of virtual screening to complement experimental high-throughput screening in

assays that are not readily amenable to high-throughput protocols.

MATERIALS AND METHODS

Materials. The NCI Diversity Set of 1990 compounds was obtained (as solutions in DMSO) from the National Cancer Institute.[34] *S*-Adenosyl-L-[carboxy-^{14}C]methionine (58 mCi/mmol) was obtained from Amersham Pharmacia Biotech. Recombinant human AdoMetDC was produced in *E. coli* from a pQE30 vector and purified by immobilized metal affinity chromatography essentially as previously described.[35] However, the construct used for AdoMetDC expression had an $(H)_6$ tag at the carboxyl end of the protein replacing the C-terminal −QQQQQS sequence rather than the amino-terminal $(H)_6$ tag used previously.

Hardware. Molecular modeling and virtual screening studies were performed using a Dell Dimension 450 workstation running Red Hat Linux 9.0 with dual Intel Xeon 3.06 GHz processors, 2 Gb RAM, 120 Gb hard drive, and an nVidia FX 500 graphics card.

Software. Schrödinger's Maestro 6.0 and 6.5 were used as the primary graphical user interfaces.[36] Schrödinger's LigPrep 1.0[36] was used for conversion of the NCI Diversity Set from the original set of 3D models in SDF file format obtained from the NCI (http://dtp.nci.nih.gov/docs/3d database/Structural information/structural data.html) to refined 3D models in Maestro file format. Swiss PBD Viewer[37] and Schrödinger's MacroModel 8.5[36] were used in preparation of the enzyme coordinates for docking studies. Schrödinger's GLIDE 2.7[36] was used for the generation of grid files and automated in silico docking (virtual screening). PyMol from DeLano Scientific was used for graphical presentation of the results.[38]

Structures. The NCI Diversity Set, consisting of 1990 3D structures in SDF file format, was used as our small molecule database. The NCI Diversity Set is a subset of the entire NCI chemical library. As previously mentioned, ligand refinement was done using Schrödinger's LigPrep, which increased the number of 3D structures to 2392. The additional structures represented different tautomeric states, ring conformations, and protonation states of the original 1990 compounds. Energy minimization of these structures employing the MMFF force field was also performed using LigPrep.

We used the crystal structure of human S-adenosylmethionine decarboxylase (EC no. 4.1.1.50; complexed with 4-amidinoindan-1-one-2′-amidinohydrazone (SAM486A)) solved in our laboratories[35] (entry 1I7M.pdb in the Protein DataBank,[39] http://www.rcsb.org). This structure was solved at 2.24 Å resolution. This particular structure was selected since SAM486A is relatively potent, has been in clinical trials (see the Introduction), and is not a covalent inhibitor (and, therefore, would not require removal of a covalently bound ligand as part of the protein preparation process for virtual screening). Although there are two identical copies (within experimental error) of AdoMetDC in the asymmetric unit of the crystal structure, each with SAM486A bound, the B-factors are somewhat lower for the SAM486A molecule when it is in association with the AdoMetDC monomer comprised of the B and D chains than when it is associated with the A and C chains. Thus, the B and D chains of AdoMetDC were selected for virtual screening.

In Silico Docking. To prepare the AdoMetDC structure for use with the GLIDE program, the GLIDE Protein Prep module[36] from Schrödinger was used. For compatibility with the OPLS all atom force field used by GLIDE, selenium atoms in the X-ray structure were changed to sulfur atoms. All explicit water molecules were removed. The Protein Prep module was then used to add explicit hydrogen atoms, appropriate charges, and to perform restrained energy minimization of the protein structure. GLIDE was then used for grid-based docking of the small-molecule ligands to the protein target. Default grids centered on SAM486A in the X-ray structure and default docking parameters were employed for automated docking. In order to validate our docking simulations with GLIDE, the SAM486A molecule was contorted into a docking mode that differed significantly from the X-ray structure, and a docking simulation was performed using GLIDE. With both GLIDE 2.7 SP and XP, the docking mode (pose) with the best GLIDE docking score (GScore) for SAM486A matched the original crystal structure quite well. The docking mode for XP was virtually identical to the one observed with SP giving rise to an rms of 1.1 Å for both poses relative to the X-ray structure. Compounds from the NCI Diversity Set were docked against AdoMetDC and sorted by GScore.

Scoring. The GLIDE docking scores (GScores) represent approximate relative binding free energies between each docked compound and the enzyme. These GScores are calculated and used to rank the structures relative to each other in a GLIDE docking run. Those structures with the better GScores (more negative free energy) are considered to be the better potential inhibitors of the enzyme.

AdoMetDC Assay. AdoMetDC was assayed by measuring the release of $^{14}CO_2$ from S-adenosyl-L-[carboxy-^{14}C]methionine.[40] The NCI Diversity Set compounds were received from the NCI as frozen plated samples in 96-well microtiter plates. Each plate contained 80 samples that were each 20 μL of a 10 mM solution in 100% DMSO. For the initial screening of compounds picked from the virtual screening process, each compound was tested in duplicate at 100 μM concentration for its ability to inhibit 30 ng of C-terminally His-tagged AdoMetDC using the standard 30 min AdoMetDC assay at pH 6.8.[40] In this assay, the final assay volume was 250 μL. To achieve the 1:100 dilution necessary for the final 100 μM concentration of the NCI compounds, 2.5 μL of the 10 mM solution in DMSO from the microtiter plate was added to the assay tube. This resulted in a total of 1% DMSO in the final assay volume, and therefore 1% DMSO was added to the no inhibitor controls. This amount of DMSO did not affect the enzymatic activity. Assaying 30 ng of C-terminal his-tagged AdoMetDC under these conditions resulted in ∼7000 cpm with a background of 30 and an activity of ∼1.5 pmol/min/ng protein. In our assay, SAM486 inhibited the enzyme by 99% when assayed at a concentration of 5 nM, which is consistent with the reported IC_{50} for this compound.[30] Triton X-100 (0.01%) was included in some of the assays carried out to determine whether compounds might be acting as nonspecific, promiscuous inhibitors.[41] For those compounds that demonstrated the greatest percent inhibition, dose response studies were performed, and the concentration dependence of inhibition was assessed under the same assay conditions.

Table 1. Results of Assaying for Inhibition of AdoMetDC at 100 μM by the 133 Top-Scoring Compounds from Virtual Screening

range of inhibition (%)	number of compounds	% of compounds
0−10	61	46
11−20	21	16
21−30	11	8
31−40	15	11
41−50	7	5
51−60	9	7
61−70	7	5
71−80	2	2
Randomly Chosen Controls[a]		
range of inhibition (%)	number of compounds	% of compounds
0−10	26	87
11−20	4	13

[a] Thirty compounds were chosen at random from the NCI Diversity Set supplied in 96-well plates.

RESULTS

Docking of NCI Diversity Set in AdoMetDC and Selection of Top Scoring Unique Compounds for Experimental Screening. As described above, the NCI Diversity Set (2392 3D structures obtained from 1990 compounds) was docked in AdoMetDC using Schrödinger's GLIDE software, which generates a ranked list of structures based on docking scores. The entire NCI Diversity Set was screened using both GLIDE 2.7 SP (standard precision) mode and XP (extra precision) mode. In prior work with other biomolecular targets, we noticed that occasionally GLIDE 2.7 SP outperforms XP in terms of observed enrichment factors when these targets were screened against the NCI Diversity Set. In a recent report[42] in which the performance of GLIDE 4.0 was assessed for 198 complexes, very occasionally the enrichment factors for SP were observed to be better than XP (for example with cyclooxygenase-2). Thus, in addition to selecting for the top ranked 100 compounds from GLIDE 2.7 XP docking, this list was augmented by unique compounds that could be found in the top 100 compounds from GLIDE 2.7 SP docking. This resulted in 133 structures that were assayed experimentally as previously described.

AdoMetDC Inhibition Assays. The results of experimental testing of the abilities of the 133 top scoring compounds to inhibit AdoMetDC at 100 μM concentration are shown in Table 1. Table 2 shows the % inhibition of compounds that exhibited >50% inhibition along with their chemical structures and GLIDE scores and ranks. As has previously been described, the experimental assay for AdoMetDC requires use of a radiolabeled substrate, is quite laborious, and currently is not amenable to high-throughput screening. Thus, we have been unable to screen the entire Diversity Set to obtain enrichment factors for our virtual screening protocol. Nonetheless, in order to determine whether the results we obtained would be different from those that would be attained from simply randomly sampling the NCI Diversity Set, 30 compounds were chosen at random, and their ability to inhibit AdoMetDC was also assessed (Table 1). This number of compounds was necessary and sufficient to obtain the statistics reported below. None of the randomly chosen compounds gave more than 20% inhibition, whereas 51 of the compounds chosen by virtual screening, or 38% of those tested, produced greater than 20% inhibition of AdoMetDC activity. This inherent separation of the data into two distributions was subjected to Chi square analysis, which demonstrated that the difference between the two distributions was statistically significant (Chi square = 16.7, $p = 0.001$). Figure 2 shows a bar graph of the distributions. This analysis strongly suggests that our virtual screening campaign had, in fact, enriched the number of authentic experimentally validated AdoMetDC inhibitors located within the top ranking compounds. As an additional measure of the effectiveness of the virtual screening process, Chi square analysis was also performed with a 50% inhibition cutoff, and again the distributions were significantly different using this statistical measure (Chi square = 4.6, $p = 0.05$).

Because of concerns that some of the compounds could potentially be acting as promiscuous, nonspecific inhibitors, the compounds that were identified in the initial screen as having greater than 20% inhibition of AdoMetDC (51 of 133 or 38%) were reassayed for inhibition of AdoMetDC activity in the presence of 0.01% Triton X-100, a condition that has been reported to eliminate nonspecific inhibition of many small molecules, particularly when assayed at high concentration.[41] In the presence of Triton X-100, two compounds exhibited similar inhibition as seen initially, while 49 of the 51 compounds exhibited <20% inhibition. Thus, it is possible that most of the 51 compounds are indeed promiscuous inhibitors, but as discussed below we cannot conclude at this point that this is indeed the case. The use of Triton X-100 may not be a suitable diagnostic test for AdoMetDC, and additional experiments will be required to resolve this issue. Of the two compounds where nonpromiscuous inhibition of AdoMetDC was definitively indicated experimentally, one (NSC 99799) exhibited 29% inhibition at 100 μM concentration and the other (NSC 354961) showed 73% inhibition at 100 μM concentration. The better inhibitor of those two compounds, NSC 354961, was further tested using powder obtained from the NCI that we determined to be 90% pure by NMR and demonstrated to have an IC_{50} of 12 μM (Figure 3). The structure of NSC 354961, its modeled interaction with the AdoMetDC active site, and its modeled interaction compared to the X-ray structure of SAM486A are shown in Figure 4 (parts A, B, and C, respectively).

DISCUSSION

Using GLIDE 2.7 for virtual screening, 133 top scoring compounds from the NCI Diversity Set were selected for experimental testing. Since the experimental assay for AdoMetDC is not amenable to high-throughput screening, we have not been able to screen the entire Diversity Set and have been unable to obtain enrichment factors. In lieu of the calculation of such enrichment factors, we screened 30 compounds selected at random from the Diversity Set against AdoMetDC as described previously. None of the randomly chosen compounds showed more than 20% inhibition, whereas 51 of the compounds chosen by virtual screening did exhibit greater than 20% inhibition suggesting that enrichment had occurred. A Chi square analysis was able to validate this premise and indicated that the difference between the two distributions is statistically significant. Moreover, a Chi square analysis using a 50% inhibition cutoff was also statistically significant. Thus it is highly likely that our virtual screening effort did, in fact, enrich the number

Table 2. GLIDE Scores and Ranks for Compounds Exhibiting >50% Inhibition at 100 μM

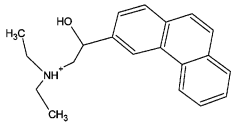
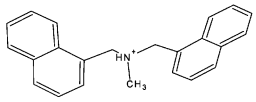
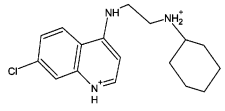
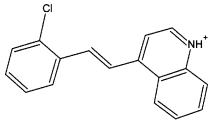
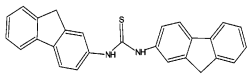
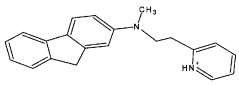
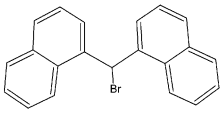
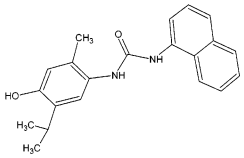
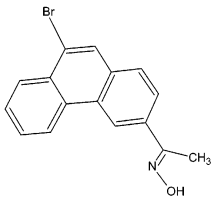
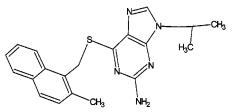
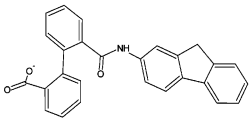
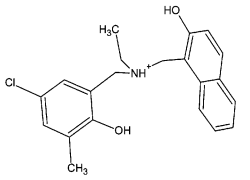
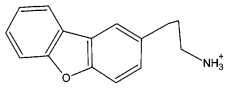
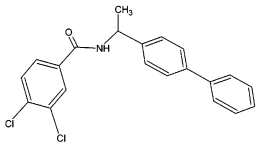
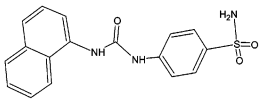
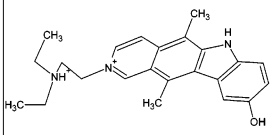
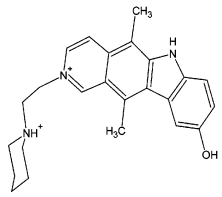
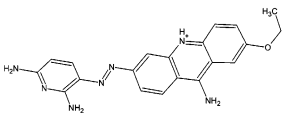
NSC #	Structure	% Inhibition @ 100 μM	GLIDE Score[a] XP	GLIDE Score[a] SP	Rank XP	Rank SP
2052		56	-8.02	-10.78	111	1
10424		60	-5.45	-8.47	430	88
13028		55	-10.40	-9.23	6	21
15784		67	-8.63	-9.29	52	14
23217		69	-8.84	-8.73	41	57
24047		57	-9.72	-10.52	15	3
28081		59	N/A[b]	-8.95	N/A[b]	35
32673		61	-5.16	-8.40	463	95
44477		75	-9.21	-7.29	26	448

Table 2 (Continued)

NSC #	Structure	% Inhibition @ 100 μM	GLIDE Score[a] XP	GLIDE Score[a] SP	Rank XP	Rank SP
56452		64	-8.57	-6.81	58	677
67690		74	-8.40	-8.58	77	71
88850		59	-8.32	-8.83	81	45
89110		57	-8.13	-9.05	96	28
201863		58	-5.68	-8.45	409	90
205628		57	-9.03	-8.44	34	92
311152		67	-6.73	-9.80	264	7
311153		69	-6.40	-8.78	309	52
354961		64	-9.77	-8.57	14	74

[a] kcal/mol. [b] No reasonable poses were found by GLIDE 2.7 XP.

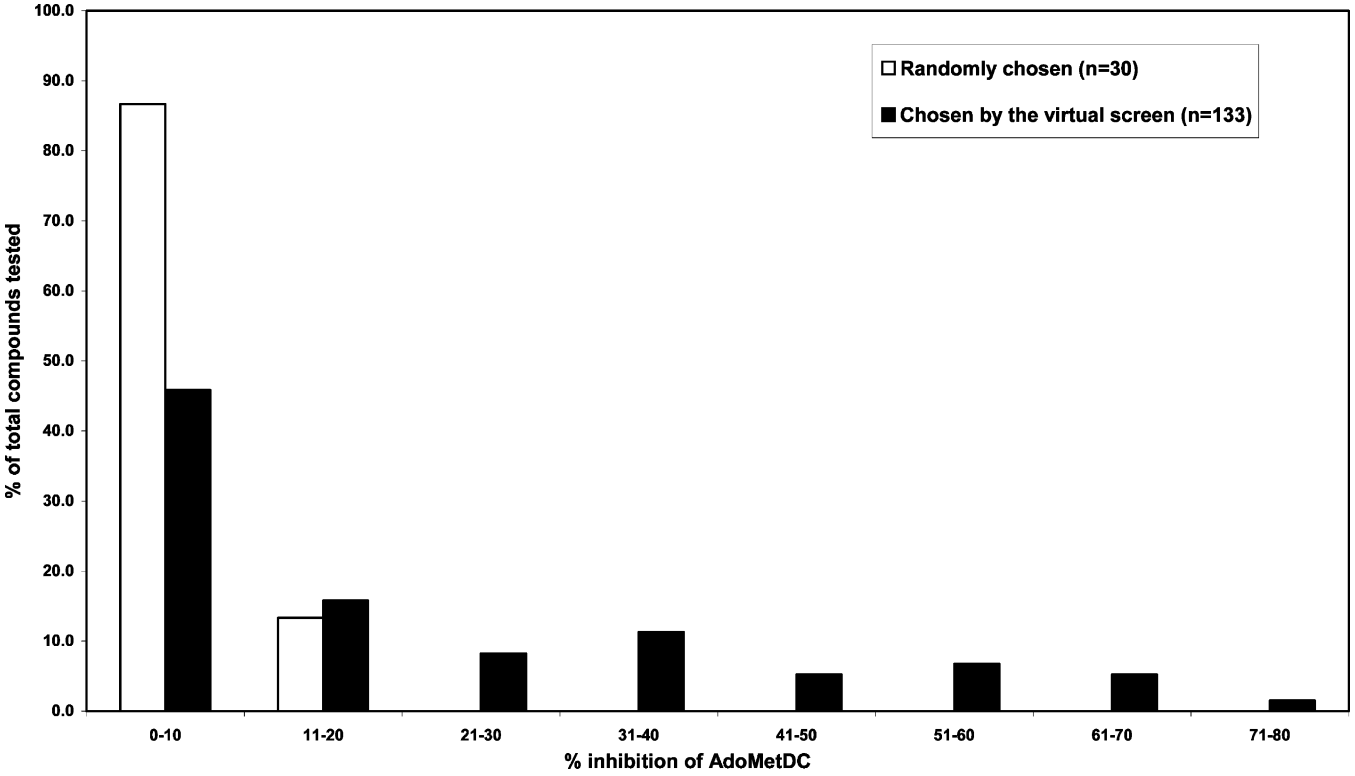


Figure 2. Comparison of the inhibition of AdoMetDC by compounds selected from virtual screening vs compounds selected at random. Bar graph of the % inhibition of AdoMetDC by NCI Diversity Set compounds either selected at random or selected by the virtual screening process.

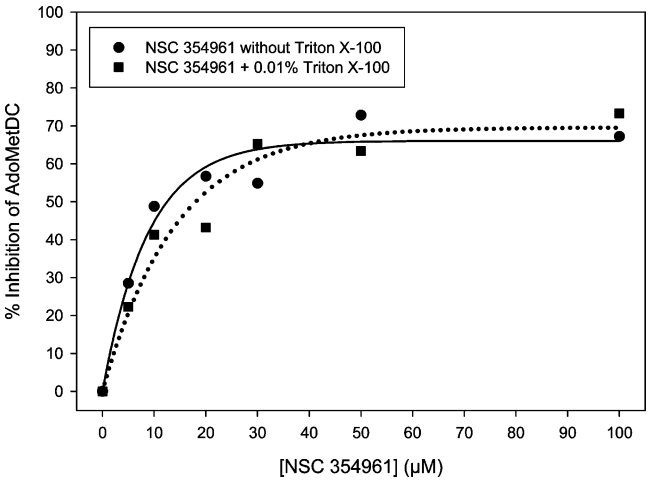


Figure 3. Concentration dependence of inhibition of AdoMetDC by NSC 354961. The ability of NSC 354961 to inhibit AdoMetDC was determined at increasing concentrations up to 100 μM in both the absence and presence of 0.01% Triton X-100 as described in the "Materials and Methods" section. The IC_{50} values as determined from the curve fits (solid line: no Triton X-100, dotted line: + Triton X-100) were 12 μM in the absence of Triton X-100 and 18 μM in the presence of Triton X-100.

of AdoMetDC inhibitors located within the top scoring compounds, although the precise enrichment factors remain unknown.

It is surprising that none of the 30 compounds selected at random showed >20% inhibition at 100 μM if promiscuous inhibition at this high concentration is operative for AdoMetDC. On the other hand, 49 out of 51 of the compounds selected by virtual screening showed a decrease in activity in the presence of Triton-X, which is assumed to abolish promiscuous inhibition that is due to aggregation of small molecules at high concentration followed by adsorption (or absorption) of the protein by these aggregates.[41] In fact, we recently observed that NSC 311152, which showed 67% inhibition of AdoMetDC in the absence of Triton-X (Table 2) but only 14% in its presence, is unable to inhibit ornithine decarboxylase (ODC) in the absence of Triton-X at 100 μM concentration (data not shown). If promiscuous inhibition via (presumably nonspecific) protein association with aggregates is operative for AdoMetDC, why would it be absent for ODC? As previously mentioned, further experiments will be required to resolve these issues.

AdoMetDC is an attractive target for chemotherapy in cancer treatment, and the NCI Diversity Set provides a chemically diverse set of compounds for lead identification. In the current study, the crystal structure of AdoMetDC bound with the inhibitor SAM486A was used for our virtual screening study. This structure reveals the binding characteristics that are necessary for superior inhibition of AdoMetDC. The features noted are hydrogen bond formation with Glu247, placement of an aromatic moiety between Phe223 and Phe7, and interaction with the pyruvoyl group in the active site.

Our experimental assays revealed that one of the compounds from the NCI Diversity Set selected for experimental testing based upon our virtual screening results, NSC 354961, possessed significant inhibitory activity (IC_{50} = 12 μM). This compound, whose activity was not diminished in the presence of Triton-X, appears to be a genuine inhibitor of AdoMetDC. In our virtual screening studies, NSC 354961 found an appropriate docking pose, with the acridine ring poised between Phe223 and Phe7, and a hydrogen bond formed

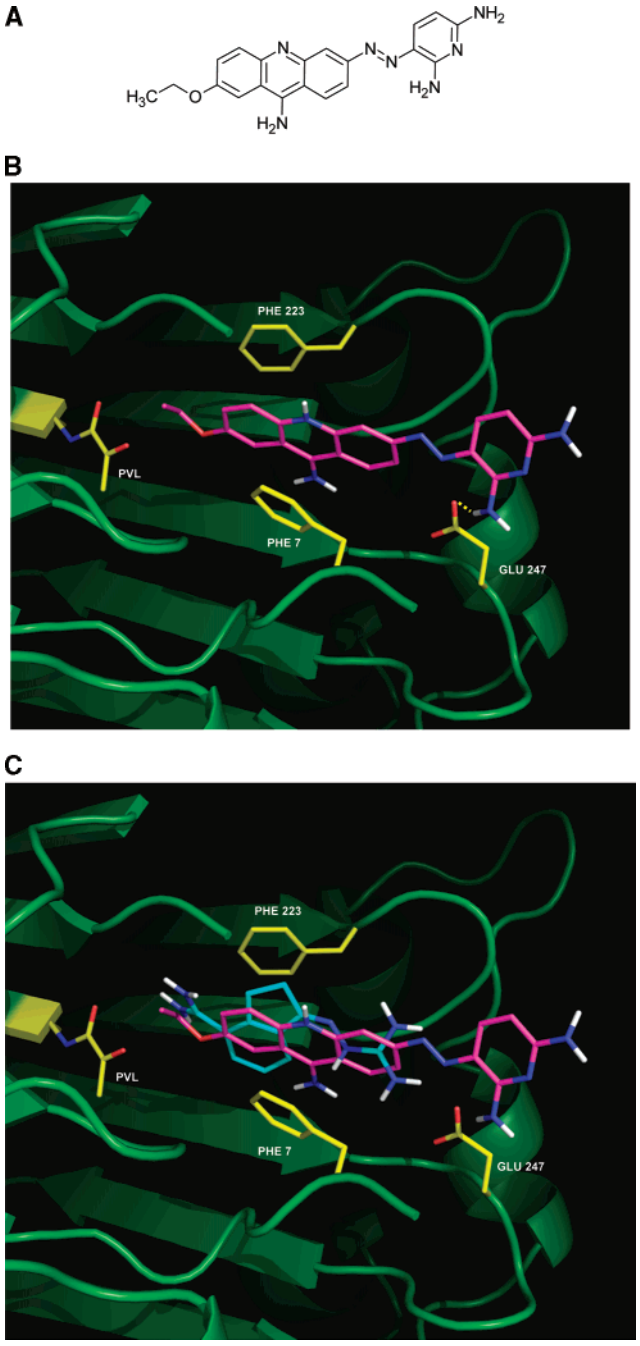


Figure 4. Structure, docking pose, and X-ray structure of NSC 354961. (A) Chemical structure of NSC 354961. (B) Docking pose of NSC 354961 bound to AdoMetDC: this pose interacts with landmark residues of the AdoMetDC active site including the hydrogen bond with Glu247 (indicated by dashed yellow line) and placing the acridine ring between Phe223 and Phe7; the pyruvoyl group (PVL) that is used by AdoMetDC as a cofactor for the decarboxylation reaction is also shown. (C) Superimposition of SAM486A (from the X-ray structure; cyan carbon atoms) and NSC 354961 docked to AdoMetDC.

between one of the amino groups attached to the pyridine ring and Glu247 (Figure 4B). Interestingly, searching the entire NCI collection for compounds similar to NSC 354961 produced three compounds that were ordered from the NCI and tested in our assay. Two of the compounds (NSC 381408 and NSC 163296) demonstrated nonpromiscuous inhibition of AdoMetDC (though significantly less than that of NSC 354961) further supporting the validity of our computational approach.

CONCLUSION

Virtual screening is a tool that allows for the rapid screening of compound databases that can contain hundreds of thousands to millions of members. The advantage of this method is that subsets of compounds can be generated that are enriched for desired properties, such as potential inhibitory activity as reported here. By performing virtual screening, time and materials can be saved especially when specific assays may be impractical or costly for large libraries. It is important to note that virtual screening will not, necessarily, accurately predict the binding affinity of a specific inhibitor but can dramatically increase the probability that a resulting subset will contain one or more experimentally validated inhibitors, and this enrichment is statistically significant. Thus, virtual screening has the potential to significantly complement experimental high-throughput screening especially when the physical assay is not amenable to a high-throughput environment, as is the case with the biochemical assay used to measure AdoMetDC activity that relies upon a radiolabeled substrate. In the studies reported here, the virtual screening protocol allowed us to identify an inhibitor of AdoMetDC with sufficient potency to justify its further investigation as a lead compound for drug discovery and development, underscoring the value of virtual screening in this important arena. Moreover, our results, along with prior work reported in the literature,[43] clearly demonstrate the ability of virtual screening to produce an enriched population of compounds for experimental testing that will result in the discovery of active compounds.

In summary, virtual screening, combined with experimental testing, allowed us to identify a lead inhibitor (NSC 354961) for S-adenosylmethionine decarboxylase. These results allow us to pursue the development of analogs based on this compound with the aim of improving efficacy. Currently, we are preparing analogs using the 9-aminoacridine moiety as a template for lead optimization and anticipate that novel, potent, and selective AdoMetDC inhibitors will emerge.

ACKNOWLEDGMENT

This research was support by grant PO1 CA-94000 from the National Cancer Institute, Bethesda, MD. The 1990 compound NCI Diversity Set was provided by the Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, 6130 Executive Blvd., Room 8020, Rockville, MD 20852.

REFERENCES AND NOTES

(1) Wallace, H. M.; Fraser, A. V.; Hughes, A. A perspective of polyamine metabolism. *Biochem. J.* **2003**, *376*, 1−14.

(2) Ackermann, J. M.; Pegg, A. E.; McCloskey, D. E. Drugs affecting the cell cycle via actions on the polyamine metabolic pathway. *Prog. Cell Cycle Res.* **2003**, *5*, 461−468.

(3) Bachrach, U.; Wang, Y. C.; Tabib, A. Polyamines: new cues in cellular signal transduction. *News Physiol. Sci.* **2001**, *16*, 106−109.

(4) Childs, A. C.; Mehta, D. J.; Gerner, E. W. Polyamine-dependent gene expression. *Cell Mol. Life Sci.* **2003**, *60*, 1394−1406.

(5) Wang, Y.; Devereux, W.; Stewart, T. M.; Casero, R. A., Jr. Polyamine-modulated factor 1 binds to the human homologue of the 7a subunit of the Arabidopsis COP9 signalosome: implications in gene expression. *Biochem. J.* **2002**, *366*, 79−86.

(6) McMurry, L. M.; Algranati, I. D. Effect of polyamines on translation fidelity in vivo. *Eur. J. Biochem.* **1986**, *155*, 383−390.

(7) Kahana, C.; Nathans, D. Translational regulation of mammalian ornithine decarboxylase by polyamines. *J. Biol. Chem.* **1985**, *260*, 15390−15393.
(8) Scott, R. H.; Sutton, K. G.; Dolphin, A. C. Interactions of polyamines with neuronal ion channels. *Trends Neurosci.* **1993**, *16*, 153−160.
(9) Williams, K. Modulation and block of ion channels: a new biology of polyamines. *Cell Signalling* **1997**, *9*, 1−13.
(10) Ha, H. C.; Sirisoma, N. S.; Kuppusamy, P.; Zweier, J. L.; Woster, P. M.; Casero, R. A., Jr. The natural polyamine spermine functions directly as a free radical scavenger. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11140−11145.
(11) Schipper, R. G.; Penning, L. C.; Verhofstad, A. A. Involvement of polyamines in apoptosis. Facts and controversies: effectors or protectors. *Semin. Cancer Biol.* **2000**, *10*, 55−68.
(12) Nishioka, K.; Mitchell, M. F.; Ajani, J. A. Clinical Studies of Polyamines and Their Antimetabolites. In *Polyamines in cancer: basic mechanisms and clinical approaches*; Nishioka, K., Ed.; Springer-Verlag: Heidelberg, 1996; pp 251−278.
(13) Feuerstein, B. G.; Williams, L. D.; Basu, H. S.; Marton, L. J. Implications and concepts of polyamine-nucleic acid interactions. *J. Cell Biochem.* **1991**, *46*, 37−47.
(14) Chiang, P. K.; Gordon, R. K.; Tal, R. K.; Tal, J.; Zeng, G. C.; Doctor, B. P.; Pardhasaradhi, K.; McCann, P. P. S-adenosylmethionine and methylation. *FASEB J.* **1996**, *10*, 471−480.
(15) Rice, J. C.; Allis, C. D. Histone methylation versus histone acetylation: new insights into epigenetic regulation. *Curr. Opin. Cell Biol.* **2001**, *13*, 263−273.
(16) Hibasami, H.; Hoffman, J. L.; Pegg, A. E. Decarboxylated S-adenosylmethionine in mammalian cells. *J. Biol. Chem.* **1980**, *255*, 6675−6678.
(17) Haegele, K. D.; Splinter, T. A. W.; Romjin, J. C.; Schechter, P. J.; Sjoerdsma, A. Decarboxylated-S-adenosylmethionine excretion: a biochemical marker of ornithine decarboxylase inhibition by α-difluoromethylornithine. *Cancer Res.* **1987**, *47*, 890−895.
(18) Janne, J.; Poso, H.; Raina, A. Polyamines in rapid growth and cancer. *Biochim. Biophys. Acta* **1978**, *473*, 241−293.
(19) Pegg, A. E. Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res.* **1988**, *48*, 759−774.
(20) Russell, D. H.; Levy, C. C.; Schimpff, S. C.; Hawk, I. A. Urinary polyamines in cancer patients. *Cancer Res.* **1971**, *31*, 1555−1558.
(21) Lawton, F.; Griffin, M.; Slack, J.; Blackledge, G. Urinary polyamine excretion patterns in patients with epithelial ovarian cancer. *Gynecol. Obstet. Invest.* **1989**, *28*, 212−214.
(22) Hayase, R.; Eguchi, K.; Sekiba, K. Polyamine levels in gynecologic malignancies. *Acta Med. Okayama* **1985**, *39*, 35−45.
(23) Chandra, R.; Ganguly, A. K. Diamine-oxidase activity and tissue di- and polyamine contents of human ovarian, cervical and endometrial carcinoma. *Cancer Lett.* **1995**, *89*, 23−28.
(24) Love, R. R.; Jacoby, R.; Newton, M. A.; Tutsch, K. D.; Simon, K.; Pomplun, M.; Verma, A. K. A randomized, placebo-controlled trial of low-dose alpha-difluoromethylornithine in individuals at risk for colorectal cancer. *Cancer Epidemiol., Biomarkers Prev.* **1998**, *7*, 989−992.
(25) Pless, M.; Belhadj, K.; Menssen, H. D.; Kern, W.; Coiffier, B.; Wolf, J.; Herrmann, R.; Thiel, E.; Bootle, D.; Sklenar, I.; Muller, C.; Choi, L.; Porter, C.; Capdeville, R. Clinical efficacy, tolerability, and safety of SAM486A, a novel polyamine biosynthesis inhibitor, in patients with relapsed or refractory non-Hodgkin's lymphoma: results from a phase II multicenter study. *Clin. Cancer Res.* **2004**, *10*, 1299−1305.
(26) Basuroy, U. K.; Gerner, E. W. Emerging concepts in targeting the polyamine metabolic pathway in epithelial cancer chemoprevention and chemotherapy. *J. Biochem. (Tokyo)* **2006**, *139*, 27−33.
(27) Fabian, C. J.; Kimler, B. F.; Brady, D. A.; Mayo, M. S.; Chang, C. H.; Ferraro, J. A.; Zalles, C. M.; Stanton, A. L.; Masood, S.; Grizzle, W. E.; Boyd, N. F.; Arneson, D. W.; Johnson, K. A. A phase II breast cancer chemoprevention trial of oral alpha-difluoromethylornithine: breast tissue, imaging, and serum and urine biomarkers. *Clin. Cancer Res.* **2002**, *8*, 3105−3117.
(28) Gerner, E. W.; Meyskens, F. L., Jr. Polyamines and cancer: old molecules, new understanding. *Nat. Rev. Cancer* **2004**, *4*, 781−792.
(29) Meyskens, F. L., Jr.; Gerner, E. W. Development of difluoromethylornithine (DFMO) as a chemoprevention agent. *Clin. Cancer Res.* **1999**, *5*, 945−951.
(30) Regenass, U.; Mett, H.; Stanek, J.; Mueller, M.; Kramer, D.; Porter, C. W. CGP 48664, a new S-adenosylmethionine decarboxylase inhibitor with broad spectrum antiproliferative and antitumor activity. *Cancer Res.* **1994**, *54*, 3210−3217.
(31) Svensson, F.; Persson, L.; Mett, H. CGP 48664, a potent and specific S-adenosylmethionine decarboxylase inhibitor: effects on regulation and stability of the enzyme. *Biochem. J.* **1997**, *322*, 297−302.
(32) Siu, L. L.; Rowinsky, E. K.; Hammond, L. A.; Weiss, G. R.; Hidalgo, M.; Clark, G. M.; Moczygemba, J.; Choi, L.; Linnartz, R.; Barbet, N. C.; Sklenar, I. T.; Capdeville, R.; Gan, G.; Porter, C. W.; Von Hoff, D. D.; Eckhardt, S. G. A phase I and pharmacokinetic study of SAM486A, a novel polyamine biosynthesis inhibitor, administered on a daily-times-five every-three-week schedule in patients with Advanced solid malignancies. *Clin. Cancer Res.* **2002**, *8*, 2157−2166.
(33) van Zuylen, L.; Bridgewater, J.; Sparreboom, A.; Eskens, F. A.; de Bruijn, P.; Sklenar, I.; Planting, A. S.; Choi, L.; Bootle, D.; Mueller, C.; Ledermann, J. A.; Verweij, J. Phase I and pharmacokinetic study of the polyamine synthesis inhibitor SAM486A in combination with 5-fluorouracil/leucovorin in metastatic colorectal cancer. *Clin. Cancer Res.* **2004**, *10*, 1949−1955.
(34) The Diversity Set is available from the Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute (NCI). The Diversity set was selected from a larger repository of compounds at the NCI. See: http://dtp.nci.nih.gov/branches/dscb/diversity_explanation.html.
(35) Tolbert, D. W.; Ekström, J. L.; Mathews, I. I.; Secrist, J. A., III; Kapoor, P.; Pegg, A. E.; Ealick, S. E. The structural basis for substrate specificity and inhibition of human S-adenosylmethionine decarboxylase. *Biochemistry* **2001**, *40*, 9484−9494.
(36) Schrödinger, L. L. C. New York, NY, 2004.
(37) Guex, N.; Peitsch, M. C. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* **1997**, *18*, 2714−2723.
(38) *PyMOL Molecular Graphics System, version 0.99*; DeLano Scientific, L.L.C.: San Carlos, CA, 2006.
(39) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235−242.
(40) Shantz, L. M.; Pegg, A. E. Assay of Mammalian S-Adenosylmethionine Decarboxylase Activity. In *Polyamine Protocols*; Morgan, D. M. L., Ed.; Humana Press: Totowa, 1998; Vol. 79, pp 45−50.
(41) McGovern, S. L.; Helfand, B. T.; Feng, B.; Shoichet, B. K. A specific mechanism of nonspecific inhibition. *J. Med. Chem.* **2003**, *46*, 4265−4272.
(42) Friesner, A. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J. Med. Chem.* **2006**, *49*, 6177−6196.
(43) Ghosh, S.; Nie, A.; An, J.; Huang, Z. Structure-based virtual screening of chemical libraries for drug discovery. *Curr. Opin. Chem. Biol.* **2006**, *10*, 1−9.

CI700005T