Discovering New Casein Kinase 1d Inhibitors with an Innovative Molecular Dynamics Enabled Virtual Screening Workflow

Simone Sciabola,^{†,‡} Paolo Benedetti,^{§,||} Giulia D'Arrigo,[⊥] Rubben Torella,[†] Massimo Baroni,[#] Gabriele Cruciani,^{*,§,||} and Francesca Spyarakis^{*,⊥}[†]Pfizer Worldwide Research and Development, 1 Portland Street, Cambridge, Massachusetts 02139, United States[‡]Biotherapeutics and Medicinal Sciences, Biogen Inc., Cambridge, Massachusetts 02139, United States[§]Department of Chemistry, Biology and Biotechnology, University of Perugia, Via Elce di Sotto 8, 06123 Perugia, Italy^{||}Consortium for Computational Molecular and Materials Sciences (CMS), Via Elce di Sotto 8, 06123 Perugia, Italy[⊥]Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Torino, Italy[#]Molecular Discovery Ltd., Centennial Park, WD6 3FG Borehamwood, Hertfordshire, U.K.

Supporting Information

ABSTRACT: The value of including protein flexibility in structure-based drug design (SBDD) is widely documented, and currently, molecular dynamics (MD) simulations represent a powerful tool to investigate protein dynamics. Yet, the inclusion of MD-derived information in pre-existing SBDD workflows is still far from trivial. We recently published an integrated MD-FLAP (Fingerprints for Ligands and Proteins) approach combining MD, clustering and Linear Discriminant Analysis (LDA) for enhancing accuracy, efficacy, and for protein conformational selection in virtual screening (VS) campaigns. Here we prospectively applied the MD-FLAP workflow to discover novel chemotypes inhibiting the Casein Kinase 1 delta (CSNK1D) enzyme. We first obtained a VS model able to separate active from inactive compounds, with a global AUC of 0.9 and a partial ROC enrichment at 0.5% of 0.18, and use it to mine the internal Pfizer screening database. Seven active molecules sharing a phenyl-indazole scaffold, not yet reported among CSNK1D inhibitors, were found. The most potent inhibitor showed an IC_{50} of 134 nM.

KEYWORDS: Casein kinase 1 delta, CSNK1D, kinase inhibitors, Alzheimer's disease, virtual screening, molecular dynamics, protein flexibility

Virtual Screening (VS) currently remains one of the main sources for the discovery of new chemical matter. In its different forms (ligand-, pharmacophore-, or structure-based), a VS campaign has been shown to give equal or higher hit rates with respect to High Throughput Screening (HTS).¹ Still, many aspects need to be improved for a more consistent system description, in particular when structural information need to be included in the VS campaign. Particularly, the inclusion of protein flexibility and the prediction of water in binding sites represent critical issues for the identification of new and reliable hits. The use of a single X-ray structure has been recognized as being biased toward the crystallized protein conformation, and new efforts are needed to improve protein sampling.^{2,3}

We recently developed a methodology for fast and efficient introduction of multiple target conformation/configuration in VS.⁴ This integrated approach combines molecular dynamics (MD) simulations to sample protein conformational space, clustering of the MD trajectory to find relevant states, and linear discriminant analysis (LDA) for model selection. In

brief, a MD simulation in the range of hundreds of ns is run on the protein target. The trajectory is then clustered by means of a k-medoids algorithm, and then the LDA methodology implemented in FLAP (Fingerprints for Ligands and Proteins)⁵ is used to find the top performing templates and scores, (i.e., combination of protein conformational states and statistical scores giving the best discrimination between active and inactive compounds). The best combination is then selected to build the final LDA model. Beside different conformations, also different target configurations can be used, that is, the target with and without ions, cofactors, and water molecules. This pipeline was applied in a retrospective way to different pharmacological targets (purine nucleoside phosphorylase, adenosine A_{2A} receptor, tyrosine-protein kinase ABL1,

Special Issue: Highlighting Medicinal Chemistry in Italy

Received: October 31, 2018

Accepted: December 13, 2018

Published: December 13, 2018

farnesyl pyrophosphate synthase, and acetylcholinesterase), proving it a valuable approach to improve VS performances in terms of enrichment and of chemical diversity of the top ranked candidates.^{4,6} Here, for the first time, we applied our integrated methodology in a prospective VS campaign, looking for novel inhibitors of casein kinase 1 delta (CSNK1D) among the internal Pfizer compound database.

CSNK1D belongs to the class of human casein kinases 1, comprising several Ser/Thr kinase isoforms, the most investigated being delta and epsilon isoforms.⁷ CSNK1D is involved in neurotransmitter release, dopamine signaling, and neurotransmitter receptor phosphorylation. It has proved to be highly expressed in Alzheimer's disease (AD) tissues and to be involved in neuronal cell death.⁸ Also, it is overexpressed in different tumors (breast, ovarian, and pancreatic adenocarcinoma) and involved in regulating cell growth, apoptosis, metabolism, and differentiation.^{9,10}

In the last years several CSNK1D inhibitors, belonging to different chemical classes and acting as ATP-competitors, have been developed and published. The most interesting and versatile scaffolds, having an isoform-specific inhibition profile, are the amino-anthraquinone, the imidazole, and the pyrazolo-pyrimidine moiety. More recently, also benzimidazole derivatives have been identified as new promising compounds (Table S1).^{11,12}

The possibility of finding a novel chemotype when performing structure-based virtual screening (SBVS) could be related to the use of different conformational states of a protein or receptor. On the basis of previous findings,⁴ we decided to exploit the intrinsic structural flexibility of the target, looking for new chemical scaffolds able to modulate CSNK1D as a potential therapeutic for Alzheimer's disease.

Although the intrinsic dynamics of kinases and their ability to host different inhibitor types is well-known,¹³ currently only type I inhibitors for CSNK1D are known and the DFG segment has been only observed in the "in" conformation. Structural evidence suggests the N-terminal loop and the binding site undergo a certain level of conformational fluctuation (Figure S1). The structure of CSNK1D in complex with the imidazol-pyrimidine derivative PF670462 was selected as starting point for all the modeling work (PDB code 3uzp).¹⁴ The selection was based on high structural resolution (1.94 Å) and absence of missing loops and tails. Aware of the importance of including conserved water molecules in drug design,¹⁵ we carefully analyzed all the available complexes and identified one/two waters often present in CSNK1D complexes (w1 and w2 in Figure S2). The network built by the two waters, being an integral part of the kinase binding site, could help ligands to interact with the protein. In the case of 3uzp, w1 bridges the ligand to Lys38 and Asp149, while w2 bridges w1 with Glu52 and Tyr56. According to the structure resolution and to the ligand nature, sometimes only one or none of them has been detected, making a dynamic prediction of their possible presence and role critical for the success of the VS campaign.

We run a MD trajectory of 200 ns of the solvated protein in the absence of the crystallized ligand and assigned the trajectory frames to 11 clusters using the k-medoids algorithm,¹⁶ based on the binding site RMSD metric. We selected a medoid without water for each cluster, for a total amount of 11. The possible presence of the two conserved waters w1 and w2 was checked by superposing each medoid to the original X-ray structure. When corresponding water

molecules were found, another copy of each template with w1 and/or w2 was added to the data set. The same was done for the reference X-ray structure, considered with and without waters w1 and w2. Eventually, 24 different target templates (T) were used for building the LDA virtual screening models.

The LDA approach is based on the assumption that combining different variables in a single model should do a better job of discriminating actives from inactives, compared to the use of a single template structure.⁴ In our experiments the variables are the 24 templates and the 19 FLAP scores (S). These are used to rank screened candidates according to the similarity of their Molecular Interaction Fields with that of the templates.⁵ The pipeline was first applied on a training set of 300 active and 400 inactive compounds. Different T/S combinations were tested, going from a minimum of 1T/2S, to a maximum of 3T/3S and 2T/4S. The best performance, in terms of AUC and partial ROC enrichments, was obtained for the 1T/3S combination. The chosen template, interestingly, was the medoid number 4, having only water w2 (H-bonding Glu52 and Tyr56) in the binding site. The three scores were DRY*N1, DRY*O, and Glob-Prod. These provide a complete description of the binding site, essentially in terms of hydrophobic (DRY), H-bond acceptor (N1), and H-bond donor (O) properties of the pocket. The Glob-Prod is produced by multiplying all the scores of the individual probes. Interestingly, the best enrichment was obtained not using the original X-ray structure but a single medoid produced by the MD simulation. Moreover, the training demonstrated that retaining only one water in the pocket gave better predictions. The following results would, likely, not be achieved if the SBVS had been performed based only on X-ray information. The final model was then used to screen a test set of 3157 compounds, made of 368 actives and 2789 inactives. The best enrichment was obtained ranking the compounds according to the LDA-group score (see [Experimental Procedures](#)). For comparison we also run a single receptor conformation virtual screening (SRC-VS) using the original X-ray structure as template and the best performing FLAP score (H*N1*H), which provides limited information about the shape and H-bond acceptor similarity.

We observed a significant improvement in the global AUC (from 0.77 to 0.90) and, most importantly, in the partial ROC enrichments (Figure 1). Almost 60% of true positives (5% ROC enrichment equal to 0.57) were found at 5% of screened false positives, and the selection of actives improved in the

Figure 1. Comparison of the enrichment given on the test set by the SRC-VS approach in cyan and the LDA-VS model in blue. (a) ROC curves. (b) AUC and partial ROC enrichments.

early stage screening. The final LDA model was applied prospectively to mine the internal Pfizer database for CSNK1D inhibitors. As described in the [Experimental Procedures](#) and in [Figure S3](#), the database was screened and ranked according to the LDA-group and LDA-R scores produced by the LDA-VS model. Virtual hits were clustered, filtered for duplicates, low LDA-R score, predicted kinome selectivity, physical–chemical properties, and similarity to known inhibitors. Based on sample availability 42 molecules were submitted to the enzymatic inhibition assays. Seven virtual hits passed our percent inhibition threshold and were tested in the IC₅₀ assay to get a complete dose–response curve. However, only compounds **1**, **3**, **4**, and **6** had enough material to be tested for IC₅₀. The percent inhibition data and corresponding IC₅₀ are reported in [Table 1](#).

Table 1. Inhibition Values for the Most Representatives CSNK1D Virtual Screening Hits^a

Cpd	Structure	% Inhibition	IC ₅₀ (nM)
1		99 (0.71)	134 (2.1)
2		92 (0.71)	-
3		80 (1.41)	354 (2.72)
4		66 (0.71)	1030 (10.73)
5		57 (3.54)	-
6		53 (0.71)	848 (9.49)
7		25 (0.71)	-

^aCompounds were tested in duplicates, and the value reported is the average plus standard deviation. Single-point percent inhibition determined at 1 μ M ligand concentration.

All molecules share a 3-phenyl-1H-indazole core. Amino-indazole derivatives have been previously disclosed as potential CSNK1D inhibitors,¹⁷ and phenyl-indazole compounds with a triazole group in position 5 of the indazole core were found to be potent inhibitors of JNK kinase.¹⁸ To our knowledge, this is the first disclosure of a series of CSNK1D inhibitors sharing the 3-phenyl-1H-indazole scaffold. The most active compound

1 showed an IC₅₀ is in the high nanomolar range (134 nM), one order of magnitude higher with respect to the most recently published imidazole-based derivatives.^{19,20}

Having no experimental insight about the possible binding mode of the hits, we decided to perform a dynamic docking for the most active compound **1**. We used a recently published adaptive electrostatic bias to guide the ligand toward the protein binding site, allowing full flexibility for the ligand and protein structures (see the experimental section).¹⁶ We performed 20 MD replicas and reported here the most represented and reliable pose ([Figure 2](#)). In this pose the

Figure 2. Docking pose for compound **1**. **a.** right side. **b.** left side. The protein is shown as cartoon, the ligand, the residues lining the pocket and the bridging waters in capped sticks. Hydrogen bonds are shown as dash lines. Relevant waters on the two sides are labeled red.

ligand forms H-bonds with the indazole hydrogen to the backbone carbonyl of Leu85 and Gly86 and to Ile15 by means of the triazole hydrogen. The contact with Leu85 is well-known and conserved in all CSNK1D-ligand X-ray structures. The hydrophobic fluoro-phenyl group is buried in the hydrophobic region of the binding site, lined by Tyr56, Ile68, Met80 and Met82. On the opposite side the basic dimethyl-amine center points toward the solvent. In general, the ligand orientation reflects that of benzimidazol derivatives cocrystallized with CSNK1D (PDB codes 4tw9¹⁹ and 4twc²⁰). Quite interestingly, waters play a fundamental role in stabilizing the protein–ligand interactions. The water molecule originally present in the reference template moves from the original position of water w2 to that of water w1, bridging the amide ligand interaction with Asn149 ([Figure 2a](#)). On the other side an organized network of three water molecules mediates the contact with Glu83, Leu84, Leu85 and Gly86 (w3–5 in [Figure 2b](#)). No structure released up to now involves such an active role of waters, but water w3 and w4 were observed in an apo structure of CSNK1D (PDB code 5ih4²¹). Thus, waters could be retained, displaced, or reorganized, according to the ligand nature, further increasing the intrinsic plasticity of kinase binding site.

We then performed a standard rigid receptor docking for the other compounds, using the protein structure returned by molecular dynamics. Rigid docking was performed with and without simulation waters. In addition, the docking was run with the option to choose whether to retain waters or not (toggle option in GOLD). The best pose for each compound is shown in [Figure 3](#). All the compounds, directly or indirectly, maintain the interaction with Leu85. Compound **2** exploits the presence of water w4 to contact the Leu85 amino group ([Figure 3a](#)). Both waters w3 and w4 create a H-bond network between the ligand, Glu83 and Leu85. Water w5 is still conserved but interacts only with Leu85. The ligand amide carbonyl forms a H-bond with Lys38, while the amino-

Figure 3. Docking pose for compounds 2 (a), 3 (b), 4 (c), 5 (d), 6 (e), and 7 (f). The protein is shown as cartoon; the ligand, the residues lining the pocket, and the bridging waters are capped sticks. Hydrogen bonds are shown as dashed lines. Relevant waters on the two sides are labeled. Only relevant residues are shown. Pictures were prepared with Pymol v2.x.

pyrimidine moiety is solvent exposed and stabilized by an additional H-bond with Asp91 and by hydrophobic interactions with Pro87. Interestingly, the orientation of the indazole group in the binding site is the opposite with respect to that of compound 1. This is likely given to the outside orientation of the amino-pyrimidine moiety. Compound 3, 4, and 5 have similar orientation to 1, interacting with Leu85 and placing the aromatic moiety in the hydrophobic region lined by Tyr56, Met80, Met82, and Ile148. Again, 3 and 5 maintain the interaction with the three waters on the left side further stabilizing the contact with Glu83 and Leu85. The amide moiety of compound 3 also weakly interacts with Asp91 (Figure 3b). Compound 4 uses water w4 to reach Leu85 carbonyl. No other water molecule is retained in the binding site. The sulfonamide group is oriented toward the solvent and interacts with both Pro87 and Asp91 (Figure 3c). As mentioned, compound 5 assumes the same orientation of compound 3 but is not able to make any additional H-bond rather than that with Leu85 (Figure 3d). Compounds 6 and 7 show a different orientation, placing the triazole in front of Leu85. Compound 6 shows the conserved hydrogen bond network between Glu83, Leu85, and the three water molecules w3–5, previously observed for 2. The fluoro-phenyl moiety is located in the hydrophobic pocket, while the opposite pyrrolidine group slightly contacts Pro87. The indazole group is oriented toward the right side of the pocket and H-bonds to both Lys38 and Asp149 (Figure 3e). In the case of 7,

no water was detected in the binding site and the docking pose resulted quite unstable. In the orientation reported in Figure 3f, the triazole contacts Leu85 and the pyrrolidinone carbonyl interacts with Arg13. As in compound 6, the indazole core H-bonds both Lys38 and Asp149. The different orientation observed for compounds 6 and 7, and the poor pose conservation, could be responsible for the lower target inhibition.

We have shown how waters can increase the plasticity of CSNK1D binding site, complementing protein–ligand interactions around the hinge region as well as in the catalytic pocket. Crystallographic analysis will be critical to better investigate the role played by waters, define their structural stability, and help in the optimization of the new identified compounds. The capability of inhibiting the target by means of a specific network of waters could be, in fact, exploited for the design of more specific kinase inhibitors.

The experimental validation of the seven virtual screening compounds further supports the potential of an integrated MD-VS workflow as a more relevant way to screen large libraries of compounds including protein dynamics and water information. This led to the identification of a novel chemical series previously known to modulate JNK kinase activity, but never reported to be active against CSNK1D. This finding, along with the low nanomolar potency for compound 1, will likely allow the development of new candidates to be used to test the hypothesis of inhibiting CSNK1D as potential therapeutics against Alzheimer's disease.

EXPERIMENTAL PROCEDURES

Molecular Dynamics. The structure of CSNK1D was retrieved from the Protein Data Bank (PDB code 3uzp¹⁴) and submitted to MD simulations upon removing of the cocrystallized ligand. The protein was parametrized by Amber99SB-ILDN force field,²² and Gromacs 5.1.4²³ was used to run MD Simulations. TIP3P was used as water model. The solvated system was minimized by 5000 steps of steepest descent. The Verlet cutoff scheme, the Bussi–Parrinello thermostat Lincs for the constraints (all bonds), and the particle mesh Ewald for electrostatics, with a short-range cutoff of 11 Å, were applied. The system was equilibrated in four subsequent steps: 500 ps in NVT ensemble at 100, 200, and 300 K, and 1 ns in NPT ensemble, to reach the pressure equilibrium condition. In the two first equilibration steps harmonic positional restraints were applied on the backbone of the protein with a spring constant of 1000 kJ/(mol·Å²). The integration step was set to 2 fs. The production run was carried out in the NVT ensemble at 300 K without any restraint for 200 ns.

Trajectory Clustering. The trajectory was then automatically clustered according to the binding site RMSD, using a k-medoids algorithm. Eleven clusters were obtained, and each of them was represented by a centroid reference structure, that is, a medoid. The 11 medoids and the original X-ray structure were used in the following VS campaign with and without relevant waters in the binding site. For relevant waters, we intended one/two waters possibly bridging the protein–ligand recognition (Figure S2). A total amount of 24 possible templates were used to train the LDA model. Of these, 22 were used desolvated, four contained only one water (w1 or w2), and eight contained both w1 and w2. Both MD setup and clustering were performed using the BiKi LifeScience environment.^{16,24}

Data Sets. The three different data sets provided by Pfizer are listed below. The training and test sets were extracted from a pool of 8275 compounds, for which experimental data against CSNK1D was available. Active and inactive compounds were defined as having IC₅₀ < 30 nM and IC₅₀ > 3 μM, respectively. Actives and inactives were clustered, separately, using CDK fingerprints and Ward's hierarchical

clustering. The obtained clusters were sampled using the in-house MaxDiversity and Even Sampling (19%) algorithms to create the training and test sets. Training set: 700 compounds, 300 actives, and 400 inactives. Test set: 3157 compounds, 368 actives, and 2789 inactives. Prospective set: around 4 M compounds.

Virtual Screening. All SBVS simulations were performed with FLAP^{5,25,26} developed and licensed by Molecular Discovery Ltd., based on Molecular Interaction Fields. The complementarity among candidates (data set ligands) and templates (medoids and X-ray structures) is scored by means of 19 different scores, encoding MIF similarity. In all the screenings, the binding site was defined as the volume occupied by the original reference ligand, extended by 3 Å. The LDA implemented within FLAP was used to identify the templates, and the FLAP scores were better able to discriminate actives from inactives in the training set. Different combinations considering a number of templates and scores between 1 and 4 were tested. The best performing model (1T/3S) was obtained by combining one template (medoid 4) and three scores (DRY*N1, DRY*O, Glob-Prod), and was used to screen the test set (see SI for further details). When the inclusion of multiple templates/scores improves the VS prediction, the highest enrichment is obtained by ranking the molecules according to the LDA-R or LDA-group scores. The LDA-R provides a numerical value: the higher the value, the higher the probability of the molecule to be active. The LDA-group simply predicts the candidate activity/inactivity. In this case the highest AUC was provided by the LDA-group-based ranking. The performance of the model was compared to that of the original X-ray structure in a standard SBVS, where the molecules were ranked according to the H*N1*H score, combining the similarity of the shape and of the H-bond acceptor MIF (SRC-VS in Figure 1). Finally, the 1T/3S model was used to screen the entire internal Pfizer database. Molecules were first ranked according to the LDA-group score and filtered to remove duplicate and inactive compounds (having LDA-group of 2). Compounds were reranked based on the LDA-R score, and a cutoff lower than 1.2 was used to filter out unwanted compounds. Post-processing of this list with additional physchem properties (Lipinsky's RO5, CNS MPO) and *in silico* kinase selectivity models reduced the list of candidates to 1000. For CNS MPO, compounds with a score >3 passed the filter, while for the RO5, only compounds with no violations were retained. We then applied Ward's hierarchical clustering using CDK fingerprints and a distance cutoff = 0.3 to group the remaining compounds in 132 clusters. Subset selection was performed within each cluster to maximize diversity and coverage, leaving at the end of the complete virtual screening funnel 200 compounds. Based on sample availability only 42 were able to be tested in the single point inhibition assay. Only molecules giving an inhibition percentage higher than 20 were considered for further analysis.

The compounds ranking and filtering scheme is reported in Figure S3. More details about the LDA-based screening and the MD-FLAP integrated pipeline can be found in ref 4.

Molecular Docking. Prior to any docking simulation, the tautomeric and protonation state was checked with Moka.²⁷ The most promising compound 1 was submitted to MD docking simulations (flexible target/flexible ligand) to get insight about the possible binding pose in CSNK1D binding site. The procedure, developed by BiKi Technologies,¹⁶ consists of several brief replicas of generally 20 ns each, in which an electrostatic bias is applied to the system, to decrease the distance between subset A (ligand) and subset B (CSNK1D binding site). When subset A reaches subset B, the bias is switched off and the simulation continues until the end of the time as a plain MD. Subset A was defined as the ligand and subset B as residues Glu52, Tyr56, Met80, Met82, and Leu85. Twenty replicas of 20 ns each were run, reassigning each time the initial atom velocities. The obtained protein conformation was then used to perform a rigid target/flexible ligand docking protocol on the other compounds (2–7). Rigid molecular docking was performed using GOLD suite version 5.5.²⁸ The region of interest for the docking studies was defined to contain all residues within 10 Å from the reference residue. The GOLD default parameters were used, and the compounds subjected

to 10 genetic algorithm runs using the CHEMPLP fitness function. Docking was performed without any water in the binding site, with water (water toggle option, thus retaining a fixed number of waters in the pocket) or allowing the program to choose which waters should be retained or removed.

Inhibition Assays. Enzymatic inhibition assays were performed with the ThermoFisher Z'-LYTE screening protocol. The assay employs a fluorescence-based, couple-enzyme format and is based on the differential sensitivity of phosphorylated and nonphosphorylated FRET peptides to proteolytic cleavage. Only nonphosphorylated peptides are cleaved by a site-specific protease (development reagent), with a consequent FRET disruption. The ratio of donor (coumarin) to acceptor (fluorescein) emission after excitation at 400 nm of the donor fluorophore is used to quantify the reaction progress. The extent of phosphorylation of the FRET peptide can be obtained from the emission ration. The CSNK1D/Ser/Thr 11 peptide mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, and 0.02% NaN₃. The final 10 μL kinase reaction consists of 9.97–72.9 ng of CSNK1D and 2 μM peptide in 50 mM Tris/HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, and 0.01% NaN₃. After 1 h kinase reaction incubation, 5 μL of a 1:16 dilution of development reagent B is added and the emission ratio is measured.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00523.

Structure of known CSNK1D inhibitors, superposition of the available CSNK1D X-ray structures, figure representing the waters in the reference 3uzp structure, VS compound selection pipeline, and a brief description of the FLAP score origin and meaning (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: gabri@chemiome.chm.unipg.it. Ph: +39 075 5855629.

*E-mail: francesca.spyrakis@unito.it. Ph: +39 011 6707185.

ORCID

Paolo Benedetti: 0000-0001-7985-1753

Gabriele Cruciani: 0000-0002-4162-8692

Francesca Spyraakis: 0000-0002-4016-227X

Author Contributions

The manuscript was written through contributions of all authors, and all authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We kindly acknowledge BiKi technologies for providing the BiKi Life Sciences suite.

■ ABBREVIATIONS

AD, Alzheimer's disease; CSNK1D, Casein Kinase 1d; CNS MPO, Central Nervous System Multiparameter Optimization; FLAP, Fingerprints for Ligands and Proteins; KSS, Kinase Selectivity Screening Panel; LDA, linear discriminant analysis; MD, molecular dynamics; PDB, Protein Data Bank; RO5, rule of Five; SBVS, structure-based virtual screening; SRC, single receptor conformation

REFERENCES

- (1) Zhu, T.; Cao, S.; Su, P. C.; Patel, R.; Shah, D.; Chokshi, H. B.; Szukala, R.; Johnson, M. E.; Hevener, K. E. Hit Identification and Optimization in Virtual Screening: Practical Recommendations Based on a Critical Literature Analysis. *J. Med. Chem.* **2013**, *56* (17), 6560–6572.
- (2) Spyraakis, F.; Cavasotto, C. N. Open Challenges in Structure-Based Virtual Screening: Receptor Modeling, Target Flexibility Consideration and Active Site Water Molecules Description. *Arch. Biochem. Biophys.* **2015**, *583*, 105–119.
- (3) Cozzini, P.; Kellogg, G. E.; Spyraakis, F.; Abraham, D. J.; Costantino, G.; Emerson, A.; Fanelli, F.; Gohlke, H.; Kuhn, L. A.; Morris, G. M.; et al. Target Flexibility: An Emerging Consideration in Drug Discovery and Design. *J. Med. Chem.* **2008**, *51* (20), 6237–6255.
- (4) Spyraakis, F.; Benedetti, P.; Decherchi, S.; Rocchia, W.; Cavalli, A.; Alcaro, S.; Ortuso, F.; Baroni, M.; Cruciani, G. A Pipeline to Enhance Ligand Virtual Screening: Integrating Molecular Dynamics and Fingerprints for Ligand and Proteins. *J. Chem. Inf. Model.* **2015**, *55* (10), 2256–2274.
- (5) Baroni, M.; Cruciani, G.; Sciabola, S.; Perruccio, F.; Mason, J. S. A Common Reference Framework for Analyzing/Comparing Proteins and Ligands. Fingerprints for Ligands and Proteins (FLAP): Theory and Application. *J. Chem. Inf. Model.* **2007**, *47* (2), 279–294.
- (6) Spyraakis, F.; Benedetti, P.; Decherchi, S.; Rocchia, W.; Cavalli, A.; Baroni, M. Integrating Molecular Dynamics and Molecular Interaction Fields: A Way to Enhance Structure-Based Virtual Screening. Presented at 21st EuroQSAR, Verona, Italy, 2016.
- (7) Knippschild, U.; Krüger, M.; Richter, J.; Xu, P.; García-Reyes, B.; Peifer, C.; Halekotte, J.; Bakulev, V.; Bischof, J. The CK1 Family: Contribution to Cellular Stress Response and Its Role in Carcinogenesis. *Front. Oncol.* **2014**, *4*, 96.
- (8) Flajolet, M.; He, G.; Heiman, M.; Lin, A.; Nairn, A. C.; Greengard, P. Regulation of Alzheimer's Disease Amyloid-Beta Formation by Casein Kinase I. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (10), 4159–4164.
- (9) Li, G.; Yin, H.; Kuret, J. Casein Kinase 1 Delta Phosphorylates Tau and Disrupts Its Binding to Microtubules. *J. Biol. Chem.* **2004**, *279* (16), 15938–15945.
- (10) Knippschild, U.; Wolff, S.; Giamas, G.; Brockschmidt, C.; Wittau, M.; Würl, P. U.; Eismann, T.; Stöter, M. The Role of the Casein Kinase 1 (CK1) Family in Different Signaling Pathways Linked to Cancer Development. *Oncol. Res. Treat.* **2005**, *28* (10), 508–514.
- (11) Perez, D. I.; Gil, C.; Martinez, A. Protein Kinases CK1 and CK2 as New Targets for Neurodegenerative Diseases. *Med. Res. Rev.* **2011**, *31* (6), 924–954.
- (12) Cozza, G.; Pinna, L. A. Casein Kinases as Potential Therapeutic Targets. *Expert Opin. Ther. Targets* **2016**, *20* (3), 319–340.
- (13) Tong, M.; Seeliger, M. A. Targeting Conformational Plasticity of Protein Kinases. *ACS Chem. Biol.* **2015**, *10* (1), 190–200.
- (14) Long, A.; Zhao, H.; Huang, X. Structural Basis for the Interaction between Casein Kinase 1 Delta and a Potent and Selective Inhibitor. *J. Med. Chem.* **2012**, *55* (2), 956–960.
- (15) Spyraakis, F.; Ahmed, M. H.; Bayden, A. S.; Cozzini, P.; Mozzarelli, A.; Kellogg, G. E. The Roles of Water in the Protein Matrix: A Largely Untapped Resource for Drug Discovery. *J. Med. Chem.* **2017**, *60* (16), 6781–6827.
- (16) Spitaleri, A.; Decherchi, S.; Cavalli, A.; Rocchia, W. Fast Dynamic Docking Guided by Adaptive Electrostatic Bias: The MD-Binding Approach. *J. Chem. Theory Comput.* **2018**, *14* (3), 1727–1736.
- (17) Sherdan, J. M.; Heal, J. R.; Hamilton, W. D. O.; Pike, I. Casein Kinase 1 Delta (CK1delta) Inhibitors. WO 2012/080727 A2, 2012.
- (18) Evans, T. C.; Gavrilovich, E.; Mihai, R. C.; Isbănescu, I. (12) Patent Application Publication (10) Pub. No.: US 2006/0222585 A1, Figure 1, 2015.
- (19) Richter, J.; Bischof, J.; Zaja, M.; Kohlhof, H.; Othersen, O.; Vitt, D.; Alscher, V.; Pospiech, I.; García-Reyes, B.; Berg, S.; et al. Difluoro-Dioxolo-Benzimidazol-Benzamides as Potent Inhibitors of CK1δ and ε with Nanomolar Inhibitory Activity on Cancer Cell Proliferation. *J. Med. Chem.* **2014**, *57* (19), 7933–7946.
- (20) Bischof, J.; Leban, J.; Zaja, M.; Grothey, A.; Radunsky, B.; Othersen, O.; Strobl, S.; Vitt, D.; Knippschild, U. 2-Benzamido-N-(1H-Benzo[d]imidazol-2-yl)thiazole-4-Carboxamide Derivatives as Potent Inhibitors of CK1δ/ε. *Amino Acids* **2012**, *43* (4), 1577–1591.
- (21) Ursu, A.; Illich, D. J.; Takemoto, Y.; Porfetye, A. T.; Zhang, M.; Brockmeyer, A.; Janning, P.; Watanabe, N.; Osada, H.; Vetter, I. R.; et al. Epiblastin A Induces Reprogramming of Epiblast Stem Cells into Embryonic Stem Cells by Inhibition of Casein Kinase 1. *Cell Chem. Biol.* **2016**, *23* (4), 494–507.
- (22) Case, D. A.; Darden, T. A.; Cheatham, T. E.; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Walker, R. C.; Zhang, W.; Merz, K. M.; et al. AMBER11; University of California, 2010.
- (23) Hess, B.; Kutzner, C.; Van Der Spoel, D.; Lindahl, E. GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. *J. Chem. Theory Comput.* **2008**, *4* (3), 435–447.
- (24) Decherchi, S.; Bottegoni, G.; Spitaleri, A.; Rocchia, W.; Cavalli, A. BiKi Life Sciences: A New Suite for Molecular Dynamics and Related Methods in Drug Discovery. *J. Chem. Inf. Model.* **2018**, *58* (2), 219–224.
- (25) Spyraakis, F.; Singh, R.; Cozzini, P.; Campanini, B.; Salsi, E.; Felici, P.; Raboni, S.; Benedetti, P.; Cruciani, G.; Kellogg, G. E.; et al. Isozyme-Specific Ligands for O-Acetylserine Sulphydrylase, a Novel Antibiotic Target. *PLoS One* **2013**, *8* (10), No. e77558.
- (26) Spyraakis, F.; Cellini, B.; Bruno, S.; Benedetti, P.; Carosati, E.; Cruciani, G.; Micheli, F.; Felici, A.; Cozzini, P.; Kellogg, G. E.; et al. Targeting Cystatysin, a Virulence Factor of Treponema Denticola-Supported Periodontitis. *ChemMedChem* **2014**, *9* (7), 1501–1511.
- (27) Milletti, F.; Storch, L.; Sforna, G.; Cruciani, G. New and Original PK(a) Prediction Method Using Grid Molecular Interaction Fields. *J. Chem. Inf. Model.* **2007**, *47* (6), 2172–2181.
- (28) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and Validation of a Genetic Algorithm for Flexible Docking. *J. Mol. Biol.* **1997**, *267* (3), 727–748.