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Luciferase Advisor: High-Accuracy Model To Flag False Positive Hits in Luciferase HTS Assays

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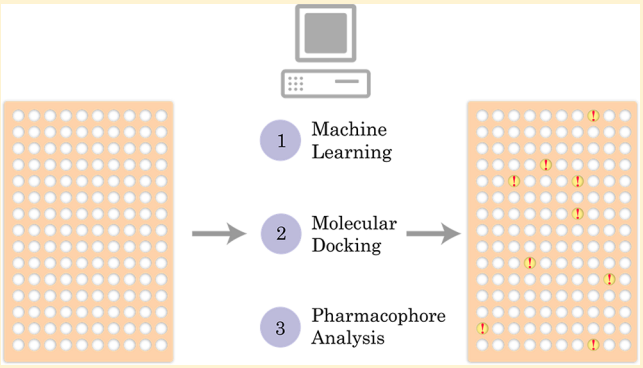
Supporting Information

ABSTRACT: Firefly luciferase is an enzyme that has found ubiquitous use in biological assays in high-throughput screening (HTS) campaigns. The inhibition of luciferase in such assays could lead to a false positive result. This issue has been known for a long time, and there have been significant efforts to identify luciferase inhibitors in order to enhance recognition of false positives in screening assays. However, although a large amount of publicly accessible luciferase counterscreen data is available, to date little effort has been devoted to building a chemoinformatic model that can identify such molecules in a given data set. In this study we developed models to identify these molecules using various methods, such as molecular docking, SMARTS screening, pharmacophores, and machine learning methods. Among the structure-based methods, the pharmacophore-based method showed promising results, with a balanced accuracy of 74.2%. However, machine-learning approaches using associative neural networks outperformed all of the other methods explored, producing a final model with a balanced accuracy of 89.7%. The high predictive accuracy of this model is expected to be useful for advising which compounds are potential luciferase inhibitors present in luciferase HTS assays. The models developed in this work are freely available at the OCHEM platform at http://ochem.eu.



INTRODUCTION

With advances in molecular biology and other areas such as engineering and computation, high-throughput assay formats have become routine and are widely used in early-stage drug discovery today.[1] For hit detection, a large fraction (~20%)[2] of these assays rely on bioluminescence, a technique that reduces background noise and benefits from an excellent signal-to-noise ratio. Such assays primarily rely on the luciferase enzyme, which is naturally found in various organisms across the animal kingdom, such as fireflies (*Photinus* sp.), larvae of certain beetles known as glow worms, and various marine organisms. Among these, the firefly luciferase (FLuc) obtained from fireflies (*Photinus pyralis*) is the most common and widely used variant. The natural substrate for luciferase is luciferin. The enzyme catalyzes the production of oxyluciferin and light via a luciferyl adenylate intermediate, which is detected and measured in the assay.

It has been known for a long time that ligand molecules tested in luciferase-based assays can inhibit the luciferase protein and thus affect the assay outcome.[3−5] For this reason, there has been significant interest in understanding and evaluating luciferase inhibition, especially in the context of high-throughput assays.[1] In 2008, Auld et al.[6] published the first comprehensive study, in which they tested ~72 000 compounds for luciferase inhibition. They also identified important scaffolds for FLuc inhibition.[6] In 2012, the same group published a follow-up study in which they tested a much larger set of compounds and identified a few additional scaffolds.[2] They also published a crystal structure of benzothiol, an inhibitor, bound to FLuc, establishing the binding mode and identifying key interactions.[2]

However, despite this significant interest and the public availability of large data sets, little to no reported effort has been devoted to building a computational model for luciferase inhibitors. Such models could potentially be used to identify and filter out these aberrant and false positive results from high-throughput screening (HTS) with good accuracy and relative ease.

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Table 1. Summary of the Data Used in This Study, Including PubChem Assay IDs

set	concentration used for testing (μM)	number of compounds tested	number of compounds after excluding inconclusives	% of actives	PubChem assay ID	year
1	50	72359	70658	2.17	411	2008
1	11.5	70231	70231	0.72		
2	10	195634	195634	1.52	1006	2010
3	50	364105	326367	6.91	588342	2012
3	11.5	323224	323224	3.25		

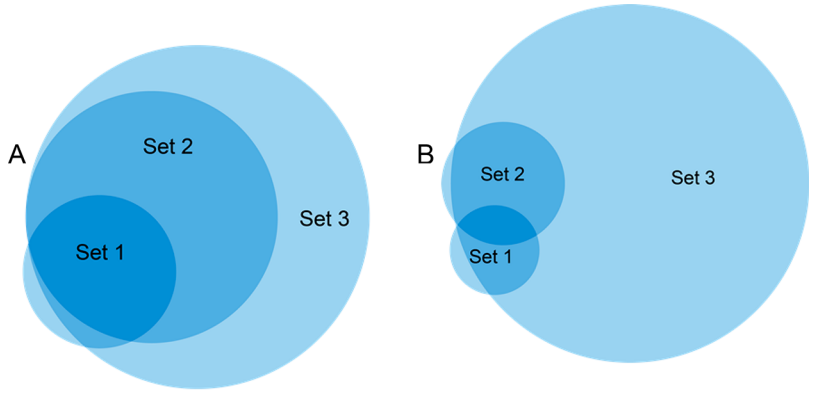

Figure 1. Venn diagram representation of the data sets used: (A) all molecules; (B) active molecules. The sizes of the circles reflect the relative sizes of the data sets.

The goal of our study was to develop a model that can advise against possible luciferase inhibitors present in an HTS data set. In this study, we analyzed the publicly available data to build such a model using machine-learning methods that can identify luciferase inhibitors. We also analyzed the influence of molecular shape and geometry in luciferase inhibition.

DATA

All of the data used in this study are publicly available in PubChem, as summarized in Table 1. The activity data were downloaded in spreadsheet format and structures in SMILES format from PubChem following the Substance ID. The data were then uploaded to the OCHEM platform, which has established workflows for normalizing and managing the data. The data gathered were processed to look for overlap in the compounds tested, which should give an idea about the coverage and reproducibility of the results. We found significant overlaps between the data sets (Figure 1).

Set 2 is a complete subset of set 3, and set 1 has some unique compounds with respect to set 3. The union of all sets contains 375 001 compounds. This data set size is good for building models and performing analysis.

In sets 1 and 3 there were a few molecules with inconclusive properties. For these molecules, it was not possible to obtain a concentration−response curve, and therefore, the activity was uncertain. We excluded these molecules from our analysis, and because of this, set 2 was no longer a complete subset of set 3.

We also performed a similar analysis on the active compounds from the three assays. Here we noticed that set 3 has a much larger active compound pool compared with the others (Figure 1B). This is explained by the fact that there is a significant difference between the highest concentrations tested in the respective assays: set 2 was measured at a maximum concentration of 10 μM, whereas both set 1 and set 3 were tested at a maximum concentration of 50 μM. Because of the higher concentration, sets 1 and 3 contained larger percentages of active molecules compared with set 2. To compare data at the same concentration for all sets, we extracted and used the inhibition data at 11.5 μM for sets 1 and 3. For a few molecules, there were no data points available at 11.5 μM, so they were not considered.

We found that the more recent assays had a significantly larger percentage of active molecules when compared at the same concentration (Table 1). This could be due either to a difference in the chemical spaces or to greater sensitivity of more recently performed assays due to improvements in assay technology. To assess whether the chemical space plays a role, we analyzed the common molecules in all three assays (N = 61 224), and we found the same increasing trend (0.7%, 1.0%, and 2.4% for sets 1, 2, and 3 respectively). Because the chemical space is fixed, this result points to an increase in assay sensitivity. Indeed, to identify potential luciferase inhibition through counterscreening, calibration of the counterscreen assay with known inhibitors is recommended to determine the assay sensitivity.[2] Because of this problem, the different assays cannot be directly compared.

METHODS

Docking Studies. For molecular docking, Autodock Vina was used.[7] SMILES of the molecules were downloaded from PubChem, and their optimized three-dimensional (3D) structures were obtained using CORINA.[8] The molecules were prepared for docking using AutoDockTools[9] and were then docked into the luciferase enzyme with an optimal binding box enclosing the binding pocket. The binding box was chosen to be large enough to cover the intended docking site but not too large in order to minimize calculation time. Default settings were used for the preparation and docking processes.

The resulting docking poses were analyzed using PyMOL.[10] A plane was defined by choosing three points just outside the binding pocket. This plane denoted the beginning of the binding pocket, and for each atom of a ligand, a position vector was calculated with respect to this plane. From this, we calculated which atoms were inside and outside the binding pocket.

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This information was then averaged over all of the docking poses, resulting in the final score that determined how much of a ligand was inside the binding pocket.

Pharmacophore Analysis. Because the crystal structure of luciferase bound to an inhibitor was available, we investigated a 3D-structure-based pharmacophore approach to distinguish between the active molecules and the inactives. Pharmacophore development and screening were performed using LigandScout.[11] The detailed procedure for developing the pharmacophores is described in the Results.

Machine Learning Methods. Using the freely accessible platform Online Chemical and Modeling Environment (OCHEM),[12] we built more than 150 models for all three data sets. We used primarily associative neural network (ASNN)[13,14] and support vector machine (LIBSVM)[15] algorithms for training the models. ASNN is an ensemble-based method inspired by the function and structure of neural network correlations in the brain. The method operates by simulating the short- and long-term memory of neural networks and thalmocortical organization of brain.[16] These methods on average provided the highest predictive accuracy in comparison with other methods available on the OCHEM Web site. The methods were used with default parameters as specified on the OCHEM Web site.

Molecular Descriptors. A variety of descriptors available within the OCHEM environment were used to train the models.

Adriana.Code[17] comprises a unique combination of topological (2D), spatial (3D), and global molecular descriptors calculated on a sound geometric and physicochemical basis. Adriana offers simple molecular property descriptors such as molecular weight and molecular dipole moment as well as increasingly sophisticated geometric descriptors such as molecular radius of gyration.

ALogPS calculates two descriptors provided by the ALOGPS[18] program, which determine the water/octanol partition coefficient ($\log P_{calc}$) and the water solubility coefficient ($\log S_{calc}$).[19]

CDK (3D) or the Chemistry Development Kit is an open-source chemoinformatics project.[20] There are several types of descriptors available from the package that are integrated into the OCHEM environment. Descriptors calculated with the recently released version 2.0 of CDK were used in this study.[21]

ChemAxon Descriptors (3D) are a set of descriptors developed and implemented by the ChemAxon company.[22] The available descriptors are subdivided into seven categories, namely, elemental analysis, charge, geometry, partitioning, protonation, isomers, and others. Descriptors that return a Boolean or numerical value were implemented into OCHEM.

Dragon[23] *(3D)* is a well-known software package for the calculation of molecular descriptors that was developed by the Milano Chemometrics and QSAR Research Group of Prof. R. Todeschini. It comprises perhaps one of the largest and most comprehensive molecular descriptor libraries available, with a total of 5270 descriptors available. The descriptors are divided into 30 discrete blocks, such as topological, constitutional, drug-like indices, etc. Dragon is built into OCHEM, and for this study, Dragon version 6 (hereafter denoted as Dragon6) was used.

GSFRAG[24] belongs to the category of 2D fragment descriptors. It calculates the occurrence numbers of certain special fragments from $k = 2$ to 10 vertices in a molecular graph G, which can be used as molecular descriptors in quantitative structure−property/activity studies.

ISIDA descriptors are part of the In-Silico Design and Data Analysis (ISIDA) Project.[25] These fragmentlike 2D descriptors are calculated from molecular graphs using three different methods, namely, paths, trees, and neighbors. The descriptors are generated from the fragments using different atom and bond labeling methods.[26]

Mera and *Mersy*[27] *(3D)* are two related groups of descriptors. Mera provides a group of descriptors that deal with molecular area and surface. Mersy is an abbreviation of Mera Symmetry, and the descriptors are calculated using 3D representations of molecules in the framework of the MERA algorithm.

Spectrophores are 1D descriptors that encode the property fields surrounding the molecules. This provides chemical-class-independent descriptors that can be used to build models.

Quantitative Name−Property Relationship (QNPR) descriptors are 1D descriptors that are directly based on the IUPAC names or SMILES representations of the molecules. The descriptors are calculated by splitting the respective string into all possible continuous substrings.[28]

ToxAlert's[29] *Extended Functional Group (EFG)*[30] category is a descriptor based on classification initially provided by the CheckMol software.[31] The coverage was extended to include new groups, particularly heterocycles.[30] ToxAlert covers a total of 583 functional groups.

Statistical Coefficients. For internal validation of the generated models, we used 5-fold stratified cross-validation. Accuracy (ACC) is defined as the percentage of correctly classified samples, given by the formula

$$\mathrm{ACC} = (\mathrm{TP} + \mathrm{TN})/(\mathrm{TP} + \mathrm{FP} + \mathrm{TN} + \mathrm{FN}) \quad (1)$$

where TP and TN stand for true positive and true negative, respectively, and FP and FN stand for false positive and false negative, respectively. Because of the large size difference between the active and inactive populations, balanced accuracy (BA) was used to determine the quality of the models. It is defined as

$$\mathrm{BA} = 0.5\cdot(\mathrm{TP}/\mathrm{P} + \mathrm{TN}/\mathrm{N}) \quad (2)$$

where P = TP + FN and N = TN + FP are numbers of positive and negative samples, respectively.

RESULTS

Molecular Docking. In an effort to directly visualize the interaction of the ligands with luciferase, we performed high-throughput molecular docking using Autodock Vina. Interestingly, through visual inspection we found that there was a positional difference between the docked populations of the inhibitory and noninhibitory molecules (Figure 2). However, the docking scores reported by Vina did not show significant differences between the two sets. The optimal score to separate active and inactive compounds (−7.1) using Vina provided a BA of 65.8%. In order to quantify the difference in binding, we calculated the percentage of the ligand that was inside the binding pocket on an atom-by-atom basis and then averaged over all the ligand poses (Figure 2). Doing this allowed us to quantify the positional difference, which can be seen in Figure 2C, together with a measure of compatibility between the binding pocket and the ligand. From the distribution, one can see that the inhibitory ligands are docked inside the active site significantly more than the noninhibitory molecules. We applied a threshold of 0.4 and were able to obtain a balanced accuracy of 67.2% in classifying the two groups. Therefore, by calculating the fraction of the ligand inside the active site, one can differentiate between the inhibitors and noninhibitors with an even better accuracy than using the Vina docking score.

Scaffold Analysis. We were also interested in the chemical nature of the active compounds, so we performed a scaffold tree analysis using Scaffold Hunter.[32,33] This allowed us to directly

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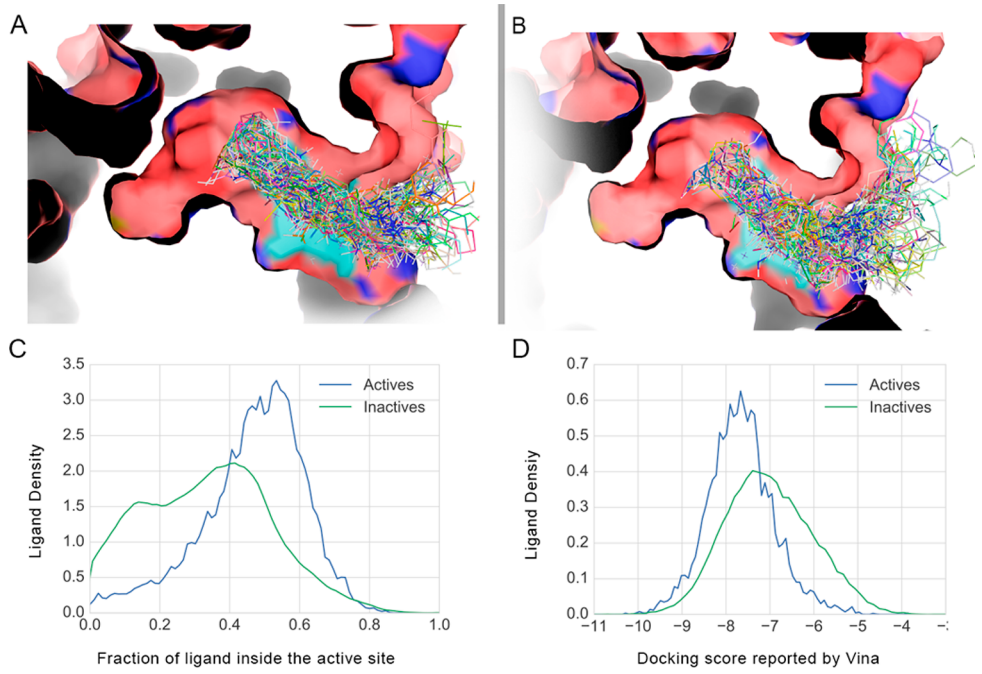

Figure 2. (A, B) Graphical representations (A) luciferase inhibitors and (B) luciferase noninhibitors docked onto luciferase. (C) Density plots of ligands vs fraction of ligand inside the active site. (D) Density plots of ligands vs docking score reported by Vina. It should be noted that the Vina score is not able to distinguish between the inhibitors and the noninhibitors as effectively.

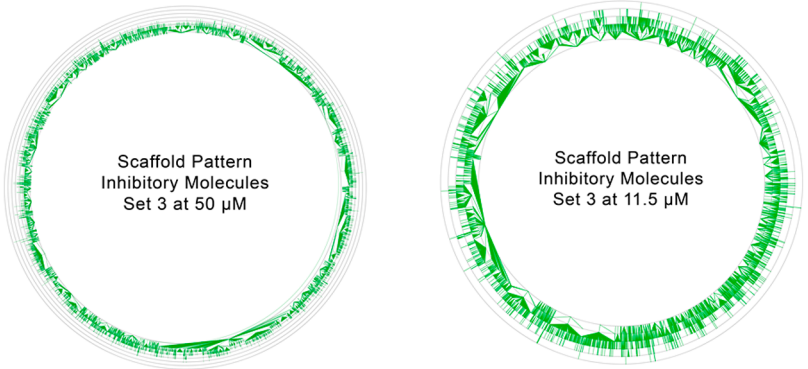

Figure 3. Scaffold tree of set 3 at two different concentrations. The larger size and much higher variability in the chemical space can be clearly seen.

visualize the structural hierarchy of the active compounds. It was immediately clear that there is a great deal of variability in the chemical motifs involved; they are not specific to a chemical subtype (Figure 3). We compared the scaffold structures of set 3 at 50 and 11.5 μM and found that at the lower concentration, the scaffold hierarchy gets simplified considerably as a result of the reduced number of active molecules (reduction of about 50%; see Table 1). We also noticed that some prominent scaffolds emerged.

Upon closer examination, it became apparent that a clear majority of the scaffolds involved, although they belong to different chemical families, have a very flat structure with multiple aromatic rings. Using the SetCompare utility of OCHEM,[34] we quantified this observation and found that such scaffolds are enriched several times in the inhibitor population compared with the noninhibitors (Table 2). This implies that the presence of particular functional groups is less important than the overall 3D shape and structure of the molecule when considered from the perspective of luciferase inhibition. This was also corroborated by reported literature,[2] where the addition of a nonplanar element, such as cyclohexane or a branched motif, to a preestablished motif drastically reduced the inhibition. It should also be noted that all of the scaffolds have a very limited coverage, therefore indicating a high variability in the chemical space.

In order to take the idea of prominent scaffolds one step further, we decided to build a filter using SMARTS to screen active molecules from inactive ones on the basis of the scaffold structure. All of the SMARTS were uploaded to ToxAlerts[29] on the OCHEM platform and can be accessed there online. As can be seen from Table 3, even with a general scaffold such as benzoimidazole, only ca. 21% of the actives were captured, along with 13% of the inactive molecules. The addition of further groups increases the selectivity but reduces the coverage significantly. Because of this, the SMARTS query suffers from exclusivity between selectivity or specificity, and creating an effective filter with this approach proved to be very difficult because of the large chemical space and variability of the set. Although the scope of such a filter is limited, we gained an understanding of the governing scaffold structure behind the inhibition process. This was useful in designing and refining the pharmacophore during our pharmacophore analysis.

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J. Chem. Inf. Model. 2018, 58, 933−942

Table 2. Scaffold Analysis Using OCHEM

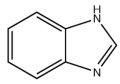
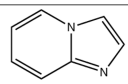
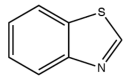
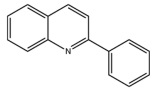
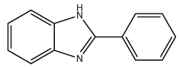
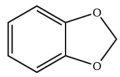
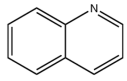
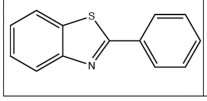
Scaffold Structure	Inhibitors	Non-inhibitors	Enrichment Factor
	6.5	1.8	3.6
	2.2	0.4	5.5
	4.8	1.1	4.3
	1.4	0.2	7.0
	0.8	0.1	8.0
	4.3	2.0	2.2
	3.5	1.6	2.2
	0.9	0.1	9.0

Table 3. Filtering Active Compounds Using SMARTS

scaffolds encoded in SMARTS[a]	actives	inactives	enrichment factor
benzoimidazole scaffold	21.66	12.93	1.7
benzylimidazole scaffold	4.46	1.06	4.2
biphenyl system with nonaromatic linker	8.85	6.21	1.4
2-(2-(1*H*-pyrrol-2-yl)ethyl)-1*H*-benzoimidazole scaffold	0.71	0.07	10.1
6-phenylnaphthyl scaffold	2.87	0.92	3.1
biphenyl system with nonring linker	6.83	4.11	1.7
2-phenylbenzoimidazole scaffold	5.97	0.78	7.7
2-(2-(naphthalen-2-yl)ethyl)-1*H*-pyrrole scaffold	0.25	0.13	1.9

[a]For representation purposes, scaffolds that the SMARTS query represents have been used. All of the SMARTS queries can be found in the TOXALERTS section of the OCHEM platform.

Pharmacophore Analysis. From the scaffold analysis, we saw that the inhibitors are not scaffold-specific but depend on the overall 3D structure of the molecule. Therefore, we investigated a 3D-structure-based pharmacophore approach to distinguish between the active molecules and the inactives. We started with a crystal structure of luciferase bound to a benzothiol inhibitor (PDB ID 4e5d), and using LigandScout[11] we identified the key interactions between the ligand and the enzyme (Figure 4). This provided the basis of our pharmacophore, which lacks selectivity but is moderately specific (Table 4). The initial pharmacophore was defined as a combination of three hydrophobic groups and two hydrogen-bond acceptors, as can be seen in Table 4. We added aromatic rings to the pharmacophore to increase the selectivity and further made optional both the hydrogen-bond donor to water interactions and the hydrophobic interactions of the pharmacophore. This significantly increased the coverage but had a negative impact on the specificity (Table 4). We then looked at various scaffolds identified in our earlier analysis (Table 2) and found that there are several active compounds in which two aromatic systems are bound to a linker group.

To cover this possibility during searching, we allowed for one feature to be omitted. This made the pharmacophore much more flexible, as it could accommodate a biphenyl, benzyl, or benzoimidazole, and many other scaffolds, as long as the aromatic groups satisfy the geometry criteria. This is the crucial difference between the pharmacophore and the SMARTS query. For example, in the case of the SMARTS filter that was designed to capture biphenyl systems with a nonaromatic linker, the shape information is irrelevant. If because of the nature of linker the structure of the ligand becomes nonplanar, the SMARTS would still pick it up. On the other hand, in a pharmacophore query, we do not specify the motifs involved; as long as there are two aromatic groups present at the specified 3D position and orientation, it will be picked up. For this reason, we were able to get a balanced accuracy of 74.2% with our designed pharmacophore with our current data set. This resulting accuracy is higher than that for any approach based on SMARTS analysis and molecular docking that we have explored thus far.

Machine Learning Models. We built models with various different descriptors that were discussed in Methods. Across

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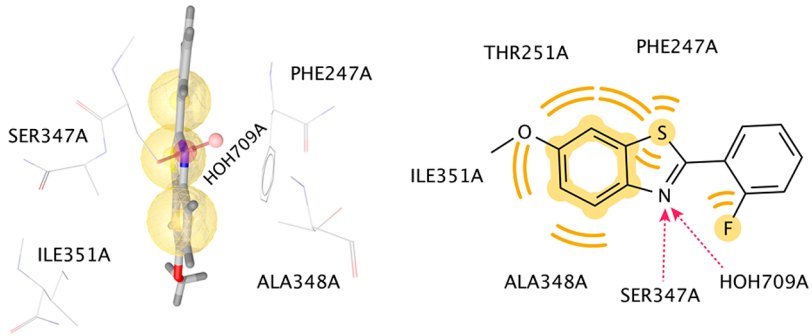
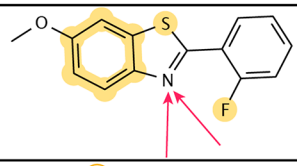
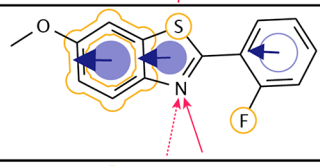
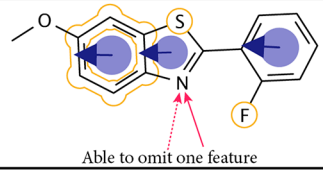


Figure 4. 3D and 2D representations of the interactions of luciferase and benzothiol, its inhibitor (PDB ID 4e5d). The yellow spheres represent hydrophobic interactions, and red arrows show hydrogen-bond donor interactions.

Table 4. Filtering Luciferase Inhibitors Using Pharmacophores

Pharmacophore Representation[a]	Actives (%)	Inactives (%)
	8.2	3.5
	33.5	15.6
	74.2	24.7

[a]Hydrophobic interactions are shown in yellow, aromatic groups in purple, and hydrogen bonds in red. An outlined shape indicates that the feature was marked as optional.

all three data sets, we found that Dragon descriptors, along with CDK and Adriana, provided the highest performance. Dragon6 comprises a total of 5270 descriptors. Many of them capture the shape attributes of the molecules well. The same is true for the ChemAxon, CDK, and Adriana sets, which also have similar types of descriptors in the package. Thus, 3D-based descriptors provided the highest accuracy for prediction of inhibitors of luciferase, which indicates the importance of including 3D structural information when modeling luciferase inhibition.

On the other hand, descriptors based on functional groups, such as Structural Alerts,[29] performed poorly throughout. The best results were calculated with the ISIDA descriptors, which provide a comprehensive coverage of different molecular types with automatically generated descriptors. The 2D E-state indices resulted in the second-best models, which had performance that was not statistically different from the performance of models based on ISIDA descriptors.

Consensus Models. Consensus models were built for each data set. This was done by averaging the results of the four best-performing models, selected on the basis of the balanced accuracy. As shown in Table 5, the consensus models had an accuracy ca. 1−3% better than the individual models. All further analysis was performed using these consensus models.

Analysis across Data Sets. To observe the effects of the increasing volume of data in the training sets of the models, as well as to determine the performance of the models against new compounds, we used the other two sets as test sets against each trained model.

Since set 1 was the smallest and also had the least sensitivity among the three data sets, models from this set would not be able to effectively predict molecules from set 2 and set 3. As one can see from Table 6, set 1 models showed lower accuracy against set 2 or set 3 in comparison with itself. In the case of set 2, the sensitivity was higher and the training set size was larger than those of set 1, and therefore, the model could effectively predict molecules from set 1. However, against set 3 the same model did not perform well, and this can be explained by the same argument as in case of set 1. The model built from set 3 provided the best results, as the training set was the largest and also had the highest sensitivity, providing the largest number of active molecules in the training set. This made set 3 the main data set from which to build our final model.

Analysis of Incorrect Predictions. In order to gain a better understanding of the inaccuracy of the models, we analyzed the compounds that were predicted incorrectly. First, we selected molecules that were predicted incorrectly in at least two consensus

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J. Chem. Inf. Model. 2018, 58, 933−942

Table 5. Associative Neural Network Analysis

descriptor	balanced accuracy (%)[a]		
	set 1	set 2	set 3
Dragon6 (3D)	83.7 ± 0.8[b]	83.6 ± 0.3[b]	88.1 ± 0.1[b]
CDK (3D)	83.5 ± 0.9[b]	84.3 ± 0.3[b]	88.0 ± 0.1[b]
ISIDA fragments	81.3 ± 0.8	82.7 ± 0.4[b]	87.7 ± 0.1[b]
Adriana (3D)	85.1 ± 0.8[b]	83.4 ± 0.3[b]	86.7 ± 0.2[b]
ALogPS, OEstate	81.3 ± 0.9	81.5 ± 0.3	86.6 ± 0.2
GSFrag	79.5 ± 0.9	80.7 ± 0.4	85.8 ± 0.2
QNPR	79.3 ± 0.9	80.2 ± 0.4	85.4 ± 0.2
ChemAxon descriptors (3D)	81.2 ± 0.8	81.8 ± 0.3	85.3 ± 0.2
SIRMS	78.1 ± 0.9	81.1 ± 0.4	85.3 ± 0.2
Mera, Mersy (3D)	82.1 ± 0.8	81.8 ± 0.4	84.3 ± 0.2
Inductive Descriptors (3D)	78.1 ± 0.9	78.8 ± 0.4	80.7 ± 0.2
Structural Alerts	73.0 ± 1.0	72.7 ± 0.4	79.1 ± 0.2
Spectrophores (3D)	78.1 ± 0.9	77.4 ± 0.4	78.4 ± 0.2
consensus model	86.2 ± 0.7	86.4 ± 0.3	89.3 ± 0.1

[a]Balanced accuracy for all three data sets obtained using various descriptors and the associative neural network algorithm sorted by accuracy of models for set 3. [b]This model was used to create the consensus model.

Table 6. Cross-Correlation of Models between the Datasets Used in the Study[a]

		test set		
		set 1	set 2	set 3
training set	set 1	86.2 ± 0.7 (70,231)	81.2% ± 0.3 (195,546)	81.0% ± 0.2 (323,224)
	set 2	89.8% ± 0.7 (70,231)	86.4 ± 0.3 (195,546)	85.5% ± 0.2 (323,224)
	set 3	90.8 ± 0.5 (70,231)	87.7 ± 0.2 (195,546)	89.3 ± 0.1 (323,224)

[a]Numbers inside parentheses denote the numbers of tested molecules in the respective sets.

models. There were 130 FN molecules (actives predicted as inactives) and 13 594 FP molecules (inactives predicted as actives). We attempted to understand the nature of the false positives by docking them against luciferase and performing the analysis described in the Docking Studies. This revealed that FP molecules have the propensity to dock inside the active site of luciferase more than regular inactives (Figure 5) but less than regular actives. This means that these molecules have some structural features that are capable of fitting inside the active site of luciferase but that the interactions are not favorable. This is well-corroborated by the docking score reported by Vina, where the binding energy of the false positives is more favorable compared with the inactives but less favorable compared with the actives. The structural features are recognized by the machine-learning algorithms, and because the machine-learning methods do not consider the interactions, they mark the molecules as inhibitors when in fact they do not inhibit luciferase because of the unfavorable interactions.

Since aggregation is known to play a role in inhibition,[35] we decided to investigate whether the activity of some compounds could be due to aggregation. As a property, aggregation is dependent on many variables, and therefore, it is very difficult to predict. There has been significant effort in developing this area, and an aggregation advisor (http://advisor.bkslab.org)[36] has been established to address this problem. This online server checks new molecules against a database of known aggregators; the database contains compounds that are known to aggregate at concentrations of 10 μM or lower. Because at elevated concentration aggregation is promoted further, this test will identify such molecules in our data sets that were screened at 10 and 50 μM.

We found that 3.2% of the active compounds are known to aggregate, compared with 2.1% among the inactive molecules. It is also worth mentioning that in set 1 and set 3 assays, 0.01% Tween-20 was used as a detergent, presumably to prevent aggregation. In the case of set 2, compounds were dissolved in DMSO. Therefore, one might expect that in set 2 more aggregators would be present in the active pool. However, because of the small number of aggregator molecules, we observed no appreciable

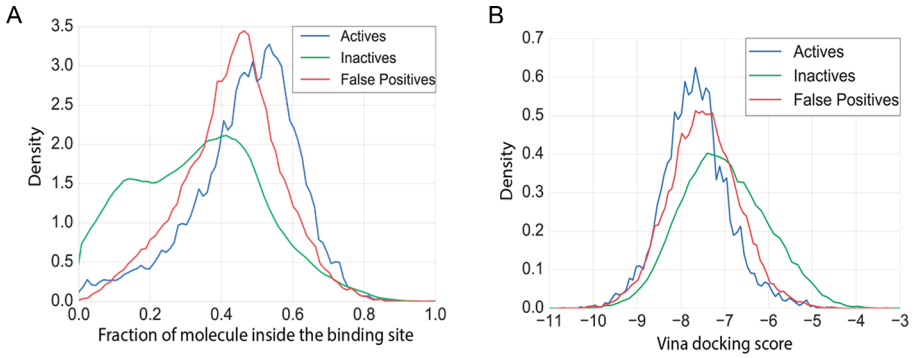

Figure 5. (A) Plot of density vs the fraction of ligand present inside the active site for the false positive predictions. The majority of the population lies between the regular active and inactive molecules. (B) Plot of density vs docking score reported by Vina.

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J. Chem. Inf. Model. 2018, 58, 933−942

difference in percentages of aggregation for actives and inactives in set 1 or set 3 vs set 2. The use of detergent could decrease the percentage of aggregators amid active molecules. Still, the fraction of aggregators amid active molecules is 50% larger than that amid nonactive ones. Thus, aggregation plays a significant role in making the molecules change class across experiments and may have played some role in inhibiting luciferase.

Effect of Concentration. As mentioned previously, there is a concentration difference in the data sets taken for this study, and the models built are dependent on this concentration because the activities of molecules change with concentration. We noted that at higher concentrations the models became less accurate (Table 7).

Table 7. Effect of Concentration on Balanced Accuracy in Consensus Models for Set 1 and Set 3

set	50 μM	11.5 μM
set 1	85.3 ± 0.4	86.2 ± 0.8
set 3	87.2 ± 0.1	89.3 ± 0.1

To better understand this, we counted the number of molecules (N = 2666) that were incorrectly predicted as inactives by the model developed using 50 μM data. We found that 81% of these molecules became inactive upon lowering the concentration. Contrary to that, only 54% (N = 22 303) of the correctly predicted active molecules (corresponding to an average 50% decrease of actives when the concentration was lowered from 50 to 11.5 μM) became inactive. Therefore, at higher concentration, such molecules introduce noise into the data, leading to inaccuracy. The models reported here were built using activity data at 10 or 11.5 μM. This must be taken into consideration when applying the model.

Merging Data Sets To Create the Final Model. To create the final model, we chose set 3 to be our primary set, as reasoned above. We then added to it only the unique active molecules from sets 1 and 2, reasoning that since these molecules are active in an assay with lower sensitivity, they have a higher probability to be active and not false positives. We decided not to merge the inactives from three data sets together, as doing so would lead to the inclusion of inactive molecules that come from experiments with lower sensitivity, which may bring false negatives. This gave us a merged data set with N = 323 443 and 3.3% active molecules. Using the same procedure as previously discussed, we obtained the consensus model, which has a balanced accuracy of 89.7%. It can be accessed at http://ochem.eu/article/104546.

Sensitivity of Existing Filters. As we explored the inhibition of luciferase and the nature of the inhibitors in this study, we wondered where these identified inhibitors lie in the context of existing frequent hitter and pan-assay interference substance (PAINS[37]) filters. These filters are implemented on OCHEM as part of the ToxAlerts platform,[29] and we ran them against our data set (Table 8). We found that PAINS filters flagged approximately twice as many active compounds as inactive compounds;

Table 8. Luciferase Inhibitors Tested against a Variety of Other Filters

compound filter[a]	actives (%)	inactives (%)	enrichment factor
PAINS (480)	9.8	4.9	2.0
promiscuity (178)	4.7	3.8	1.2
AlphaScreen FH filters (25)	1.7	0.6	2.8
reactive, unstable, toxic (340)	66.9	62.3	1.1

[a]The numbers in parentheses represent the numbers of alerts in the respective filters.

the AlphaScreen filters to detect promiscuous compounds also provided an approximate 3-fold enrichment of flagged actives over inactives. However, the promiscuity filter that was designed to identify compounds likely to hit multiple assays[38] provided a much smaller enrichment. The highest enrichment was calculated for the AlphaScreen filter, but this filter had the lowest coverage. The most prominent alert among the AlphaScreen filter that picked up luciferase inhibitors was the Aminal alert (aminal on a pyridine-based system; see Figure S1). This alert picked up several compounds with a planar structure (Figure S2) and provided an enrichment factor of 6.2. It should also be noted that the number of alerts involved in this case is very small, which gets reflected in the poor coverage of this filter. The difference in the number of alerts in each filter contributes to the specificity/selectivity trade-off.

We also noted that most of the compounds were flagged as being reactive, unstable, or toxic. This is expected, as the responsible filter is known to pick up drug-like molecules. It is worth mentioning here that the presence of such alerts by itself does not make a molecule toxic in the context of medicinal applications because of dosage and clearance from the body.

DISCUSSION

The developed chemoinformatic model is suitable for providing an early warning against potential inhibitors of luciferase that may interfere with HTS experiments. Since the model does not have 100% accuracy, some compounds can be predicted as luciferase inhibitors when in reality they are not. On the other hand, even if the molecule is indeed a luciferase inhibitor, that does not mean that it cannot be a potential lead. Hence, we strongly advise that flagged molecules not be discarded as false leads but rather be considered further to better interpret the experimental results.

Thus, the model described here should be used to identify *potential* interference in luciferase-based assay systems. The identified molecules should be retested using other assay protocols that do not rely on luciferase. The merit of this study is that one can find potential interference in very large data sets, and only the flagged molecules then need be tested by orthogonal assays. This reduces cost, time, and effort in counterscreening.

CONCLUSIONS

In this study, we explored various methods of filtering and detecting luciferase inhibitors in a luciferase-based HTS assay. We designed computational models using machine-learning methods on publicly available data from PubChem. We also used molecular docking to understand how inhibitors bind to luciferase and performed a scaffold analysis to gain a better understanding of the chemical nature of such inhibitors. The machine-learning models outperformed other methods of filtering luciferase inhibitors, such as SMARTS- or pharmacophore-based filters. We were able to obtain a prediction accuracy of 89.7%, which makes the final model a good tool for filtering potential luciferase inhibitors. Still, the predictions of the model should be considered as advice, and the flagged compounds can be retested in orthogonal assays. All of the models and data reported here are publicly accessible at http://ochem.eu/article/104546.

REPRODUCIBILITY OF OCHEM MODELS

All OCHEM models are developed using standardized workflows, which can be used at the OCHEM Web page to produce another model or reproduce previous results. The full specification of details of the workflow are stored in an XML file,

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J. Chem. Inf. Model. 2018, 58, 933−942

which can be exported, imported, or used as a template for model development. This feature provides the reproducibility of OCHEM models. Moreover, the OCHEM platform can be installed locally at the commercial or academic premises and be used to apply or reproduce models on local computers of the users. The majority of models available in OCHEM can also be exported and used as standalone versions. For both of these applications, commercial or academic licenses for some tools, such as descriptors calculation, 3D structure generation, standardization of chemical structures, etc., can also be required. Contrary to that, the predictions of models available in OCHEM can be used under the CC-BY-NC license, while the data can be downloaded under the CC-BY license. These features makes OCHEM a powerful public portal for the development and sharing of reliable and reproducible chemical information and models on the Web.[39,40]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jcim.7b00574.

The Aminal alert and the compounds filtered by the alert (PDF)

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Notes

The authors declare the following competing financial interest(s): I.V.T. is CEO of BIGCHEM GmbH, which develops the OCHEM platform (http://ochem.eu) used in this study. The other authors declare no conflicts of interest.

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