

## **New inhibitors of p38 mitogen-activated protein kinase: Repurposing of existing drugs with deep learning**

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### **Abstract**

The p38-alpha (MAPK14) is a protein kinase that is implicated in the pathological mechanisms of BAG3 P209L myofibrillar myopathy, cancers, Alzheimer's disease and other diseases like rheumatoid arthritis. Inhibition of p38 has shown promise as treatment for these diseases. Traditional drug discovery methods were unable to create both effective and safe small molecule inhibitors, so we used machine learning to elucidate potential p38 blockers from existing FDA-approved drugs. Using available bioactivity data, we determined the best existing p38 inhibitors and applied fingerprint clustering to isolate the compounds with similar structures. Descriptors were calculated for these clustered compounds and the most important of these descriptors were determined through a machine-learning based feature selection algorithm. This data served as the training set for a deep neural network that was fine-tuned to a 92% validation accuracy. The neural network model was applied to a database of FDA-approved drugs, revealing 149 potential p38 inhibitors, whose efficacy were confirmed by

docking simulations to be statistically significantly higher than random FDA drugs and slightly higher than known inhibitors. Our study not only reveals potential treatments for p38-mediated diseases but also demonstrates the capability of integrating various machine-learning techniques and computational algorithms to predict novel functions of existing pharmaceuticals.

## 1 | INTRODUCTION

The p38-alpha, or MAPK14, is a mitogen-associated protein kinase that is activated by dual phosphorylation of a tripeptide motif (Thr-Xaa-Tyr) located in its activation loop. The p38 kinase is regulated by stress-activated MAPK Kinase 3 (MKK3), MKK6, and MKK4. Its signaling cascade ultimately is responsible for the release of proinflammatory cytokines such as TNF, IL1 and IL6.<sup>1-3</sup> The p38 has been found to play a large role in the inflammatory mechanisms of rheumatoid arthritis, Alzheimer's disease, Parkinson's disease, Crohn's disease, and several types of myopathies and lung diseases. It has also had shown tumor promotion properties in different types of cancer, including breast, liver, and colorectal cancer.<sup>4,5</sup> A p38's contribution to the severe phenotype of so many degenerative diseases has made it an attractive target for treatment via molecular inhibitors.

Myofibrillar myopathy (MFM) is another disease in which p38 plays a significant role. This disease is caused by a mutation in the Bcl2-associated athanogene 3 (BAG3) protein. The modular domain composition of the BAG3 co-chaperone makes it incredibly versatile in its interactions with other molecules. It suppresses apoptosis, mediates selective autophagy, and maintains cellular homeostasis during stress, making it important in a variety of diseases, including cancers, myopathies, and neurodegeneration.<sup>6</sup> A BAG3 missense mutation of P209 into leucine and several mutations in the same site have been shown to result in a severe childhood MFM phenotype, characterized by progressive limb and axial muscle weakness, respiratory insufficiency, and cardiomyopathy. Three of 53 random MFM patients showed the P209 mutation.<sup>7,8</sup> Further investigation of the P209L mutation in zebrafish discovered that the mutation leads to a toxic aggregation of mutated BAG3, ultimately causing a deficiency of functional BAG3, triggering myofibrillar disintegration.<sup>9</sup> Research on BAG3 P209L mice found significantly increased activation of p38. The mechanism of p38 activation is similar to that seen in Alzheimer's and other tauopathies and neurodegenerative diseases, in which

oxidative cell stress activates the MAPK signaling pathway, causing an inflammatory response.<sup>8,10,11</sup> In these brain diseases, cellular stress is caused by misfolded amyloid-beta and tau proteins, while in BAG3<sup>P209L</sup> MFM, cellular stress results from a lack of functional selective autophagy.<sup>10,12</sup> Targeted activation of p38 in vivo has been shown to induce heart failure and a similar cardiac hypertrophy as that seen in the mutant mice, and the mutant mice also show increases in inflammatory infiltrates and activation of NF- $\kappa$ B, a prototypical proinflammatory signaling pathway, which is characteristic of p38 activation.<sup>8,11-13</sup> All these evidence points to targeted inhibition of p38 as an effective treatment of BAG3 mutation caused MFM. Our research is the first attempt we know of to discover p38 inhibitors as a treatment for a genetic myopathy.

Similar to other protein kinase inhibitors, MAPK14 inhibitors mostly function through competitive inhibition at the ATP-binding site. There are two types of these competitive inhibitors: Type 1, which bind in the active DGF-in conformation (e.g., compound SB 203580), and Type 2, which bind in the inactive DFG-out conformation (e.g., compound Birb 796).<sup>14</sup> The two binding modes differ in the orientation of the DFG motif within the ATP pocket.<sup>15</sup> Many different p38 inhibitors have been, or are currently being, investigated in phase I or II clinical trials, including Birb 796 and VX-745, but none have been recommended for use, mostly due to high toxicity or lack of significant efficacy. Since existing p38 inhibitors have been artificially synthesized, their side effects are unknown until human clinical trials, which is why even though they may work very well during in-vitro trials or even in-vivo animal models, they end up failing at the clinical trials. Development of new drugs is a very long and expensive process, so in this project we propose to repurpose existing FDA-approved drugs to find effective, non-toxic p38 inhibitors with already known side effects. FDA drug repurposing significantly expedites the drug discovery process, delivering safer yet effective treatments to patients in a much timelier manner.

There have been a few attempts to use computational techniques to find p38 kinase inhibitors, using structure-based virtual screening or structure-based design of novel inhibitors.<sup>16-19</sup> However, there has been no use of deep learning (DL) for the repurposing of FDA-approved drugs as p38 inhibitors that we are aware of. Deep learning is a form of machine learning that mimics the human brain and its networks of neurons. It has applications in a variety of fields like healthcare, cybersecurity and even video games, but it has recently

emerged as one of the most effective machine-learning techniques in all aspects of drug discovery, from target prediction to synthesis to identification of prognostic biomarkers.<sup>20</sup> Furthermore, data-filtration techniques like fingerprint clustering and feature selection have both been shown to significantly increase deep-learning accuracy.<sup>21,22</sup> That's why in this study we utilized these data preparation algorithms along with a deep neural network to discover which FDA approved drugs could also function as p38 inhibitors, revealing 149 candidates. These predictions were then ranked through ligand-docking, a structure-based virtual screening method that shows sufficient accuracy in determining how thermodynamically favorable the binding of a ligand to a protein is.<sup>23</sup> With corroboration from experimental trials, the most promising of these drugs could be used as treatment for BAG3<sup>P209L</sup>-MFM or other diseases with similar p38-mediated pathological mechanisms.

## 2 | METHODS

All research was completed *in silico*. The programs, tools, and websites used were the PubChem, DrugBank, CADD group Cheminformatics Tools and User Services (CACTUS), Molecular Operating Environment (MOE), Waikato Environment for Knowledge Analysis (Weka), PaDEL-Descriptor, TensorFlow Python Library, Netron, and Net2Vis.<sup>24-32</sup>

### 2.1 | Clustering

Activity values and structure files for 12 456 compounds tested with the p38 kinase was retrieved from PubChem.<sup>24</sup> To limit the tested compounds to the strongest inhibitors, only those with an activity value (IC<sub>50</sub>, K<sub>d</sub>, K<sub>i</sub>) less than 100 nM were considered. These compounds were then analyzed with the MOE<sup>27</sup> fingerprint clustering function, which uses Jarvis–Patrick Clustering, to determine compounds with similar structures and pharmacophores to improve specificity of the deep-learning model. Clustering parameters were set at 56% similarity and overlap, revealing 297 clusters. The largest of these clusters contained 1206 compounds and was what the deep-learning model would be trained on. The final predicted inhibitors also underwent fingerprint clustering with the same parameters to provide more information about the results.

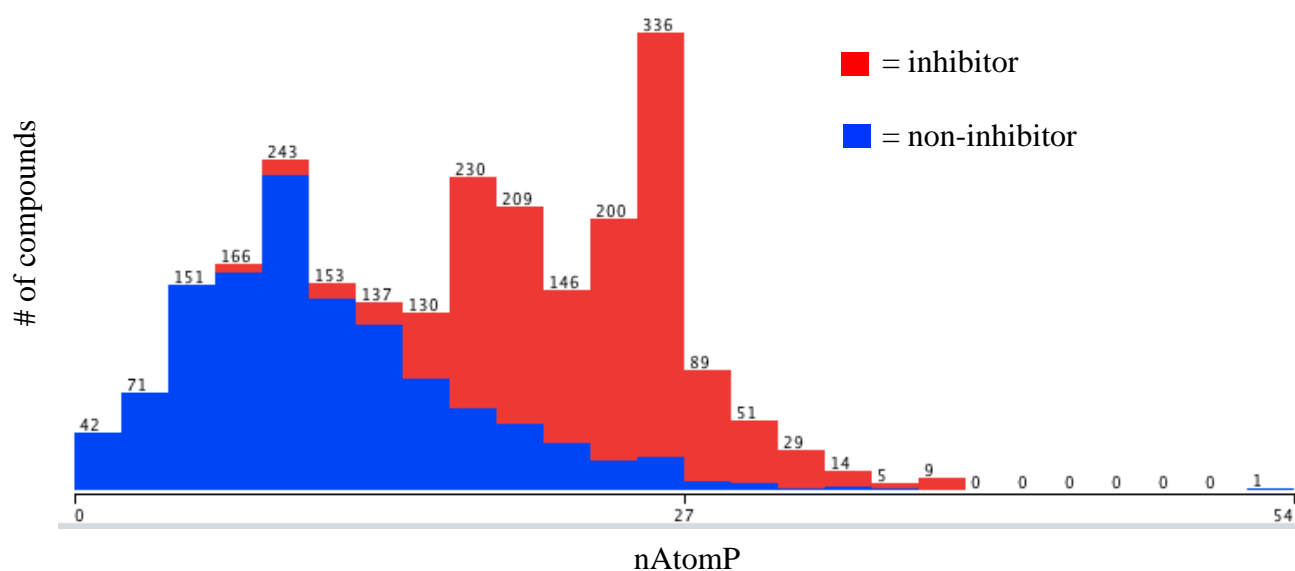
Since clustering is based on the molecule's 3D structure, the structure files inputted to the software had to include 3D coordinates. So, we converted SMILES, which are a structural representation consisting of one-dimensional ASCII strings, into a 3D Spatial Data File (SDF) format using the CACTUS Structure Files Generator.<sup>26</sup> The SDF files were then imported to the MOE database.

## 2.2 | Descriptor Calculation

PaDEL-Descriptor<sup>29</sup> is a graphical or command line interface that mostly utilizes the Chemistry Development Kit and a few additional descriptor categories. It was employed to calculate 1875 one-, two-, and three-dimensional descriptors. These descriptors—the data that the deep-learning model analyzes to train and make predictions—were calculated for three dimensional SDFs of the largest cluster of known inhibitors, random molecules (control) pulled from PubChem, and for FDA-approved drugs retrieved from DrugBank.<sup>25</sup>

## 2.3 | Feature Selection

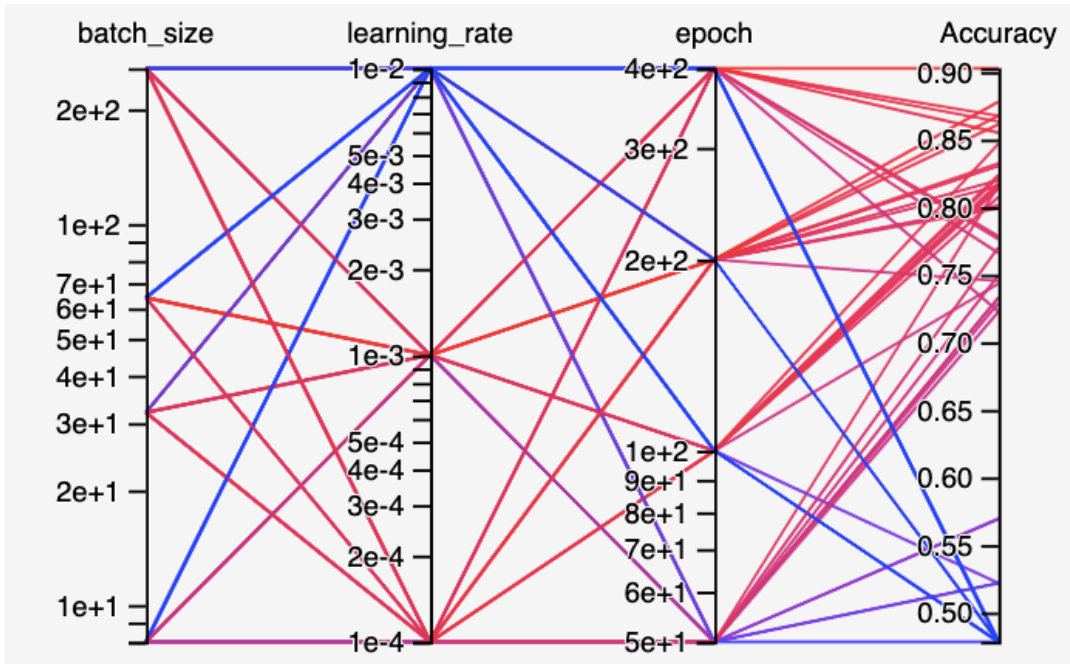
To narrow down the calculated descriptors to only the most significant ones, we employed attribute selection from WEKA, an open-source machine learning software.<sup>28</sup> The descriptors were ranked by the Information Gain Attribute Evaluation (InfoGain) function, an unsupervised machine-learning algorithm, that measures how important each descriptor is in determining whether a given molecule is an inhibitor or not. Only the most significant descriptors were selected to be used by the machine-learning algorithm in order to reduce noise. Figure 1 is a histogram that illustrates the most informative descriptor, nAtomP. The vertical bars represent the number of molecules for each value of nAtomP, the length of the longest pi chain in the molecule. Inhibitors (red) tend to have larger values for nAtomP than non-inhibitors (blue). This clear distinction between the two is what makes this descriptor useful for deep-learning classification.



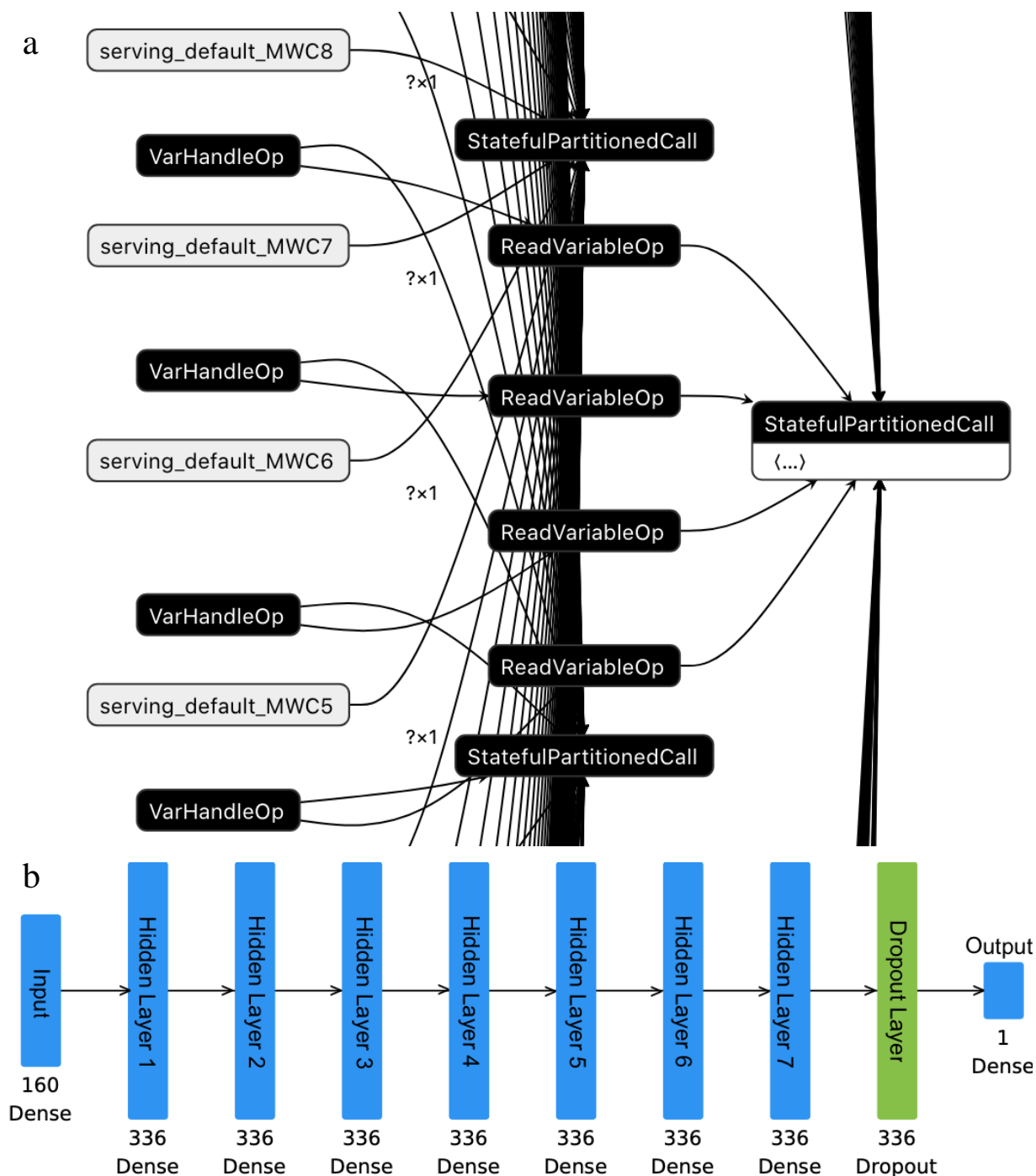
**FIGURE 1** Histogram of the length of the largest pi chain in the molecule for all molecules in the training set. This descriptor, nAtomP, was ranked as most informative by an information gain algorithm.

## 2.4 | Neural Network Construction

The deep-learning algorithm was created in Python and works by first converting the training data of known inhibitors' and random molecules' most important descriptors into a shuffled TensorFlow<sup>30</sup> dataset with an 80–20 train–validation split. The inhibitor label is removed and then compared to the neural network's predictions to determine training and validation accuracy. The neural network (Figure 3) used was a Keras sequential model, a type of multilayer perceptron. To determine the best parameters that maximized accuracy, we applied hyperparameter tuning with TensorBoard (TensorFlow's visualization kit), which essentially looped over the training algorithm changing a single parameter one at a time (Figure 1). The hyperparameter tuning results indicated that the optimal algorithm trains with 7 hidden layers with 336 nodes each, 160 epochs (how many times the dataset passes through), 1 batch per epoch (maximum batch size), and a learning rate of 0.001 with the Adam optimizer.



**FIGURE 2** A visual representation of the hyperparameter tuning process. The parameters being considered here are batch size, learning rate, and number of epochs. The red lines represent effective combinations of parameters, while the blue lines are ineffective.



**FIGURE 3** Neural network visualizations. (a) A snippet of a complex representation of the deep neural network showing how the input data of molecular descriptors connects to the different nodes of the first two hidden layers.<sup>31</sup> (b) This more simplified representation shows how the input data with the 160 descriptors travels through 7 different hidden layers with 336 nodes, a dropout layer (reduces overfitting to the training data by randomly setting input units to 0), and then a final output layer.<sup>32</sup>

## 2.5 | Conformational Search and Docking



A preparation of the conformers sets was performed on all the molecules. MOE's Conformations/ Import function uses a stochastic search and energy minimization algorithm that finds the possible conformations of a molecule given limits on strain energy. We set the strain energy limit to 1 kcal/mol and restricted it to a maximum of 100 conformations per molecule.

The conformers were docked in MOE, using Dock module, with the PDB structure 1A9U, a MAPK14 protein bound to the compound SB 203580, a strong known inhibitor, at the ATP-binding site. The protein structure was prepared by removal of the inhibiting ligand, 3D protonation (determines the lowest energy configurations of titratable groups in the protein), and MOE's Structure Preparation module, which corrects any errors in the crystal structure. The conformers prepared through MOE's Conformations/Import module were then docked using MOE's Dock module, with the protein structure, determining five poses for each compound with the Triangle Matcher placement method and London dG scoring method. Then, the poses were refined with the Induced Fit mode (flexible docking) and scored for a final time with the GBVI/WSA dG method. The competitive binding site was determined through MOE's pocket function.

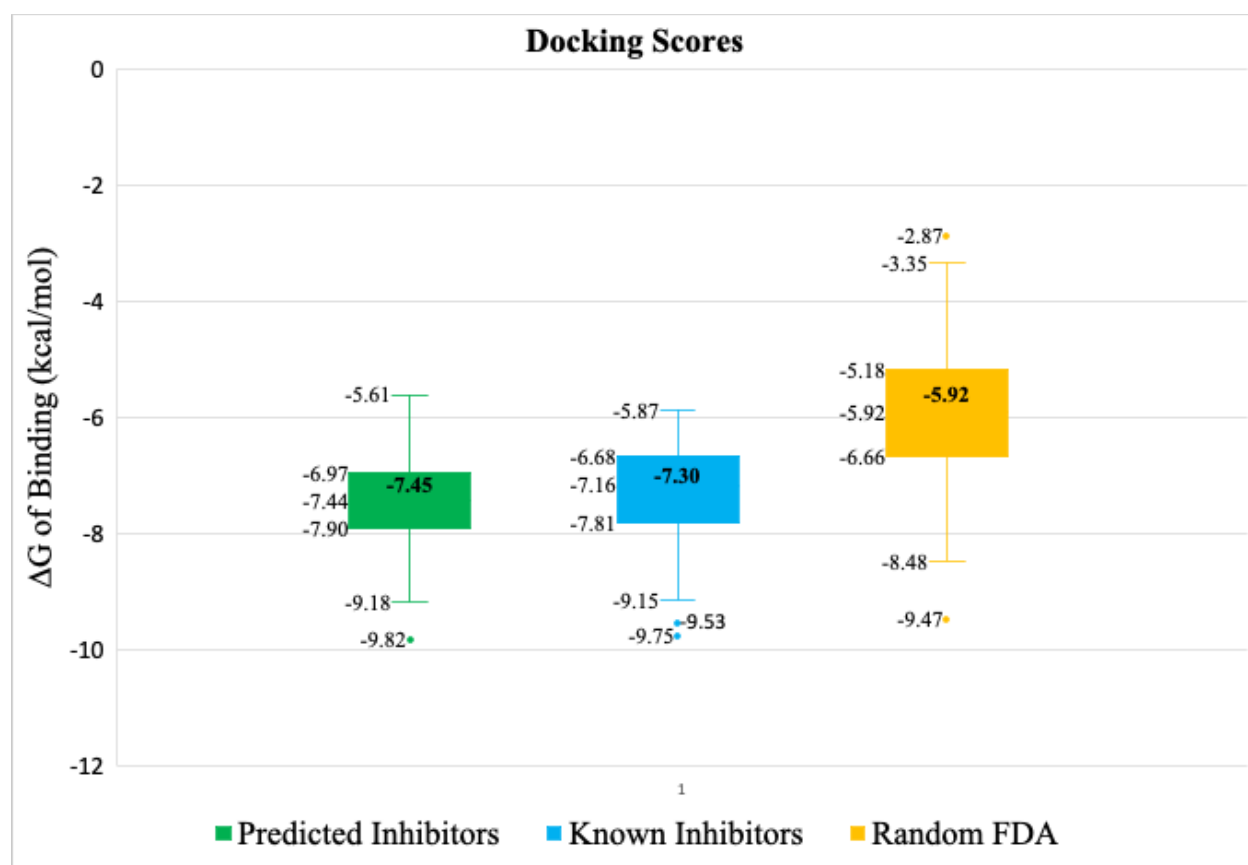
### **3 | RESULTS**

To present a comprehensive picture of all important compounds for repurposing we engaged two scaling measures: first, by the docking scores of compounds to p38 (Table 2), second, by the likelihood that the compounds inhibit p38 according to the deep-learning model (Table 5). We also combined the two scaling techniques in Table 6 to provide a more comprehensive ranking system. However, the first method, by docking score, can be most reliable because it ranks the compounds directly by their simulated interactions with p38, which is most important in determining effective inhibitors.

#### **3.1 | Docking**

Our conformational search resulted in 8890 conformers of p38 inhibitors predicted by our neural network, and 6616 conformers of already known p38 inhibitors along with 5473 conformations of random FDA drugs added as negative fits. These conformers were then docked with p38 and given

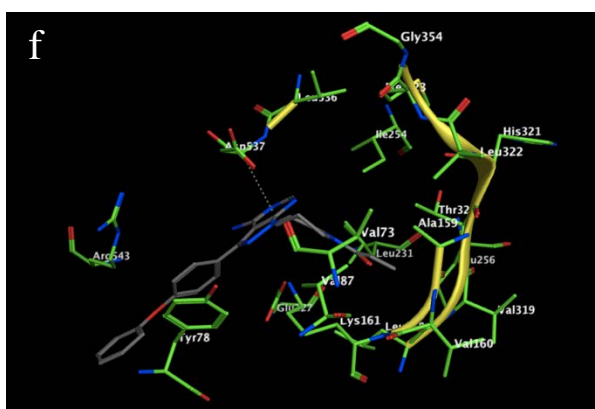
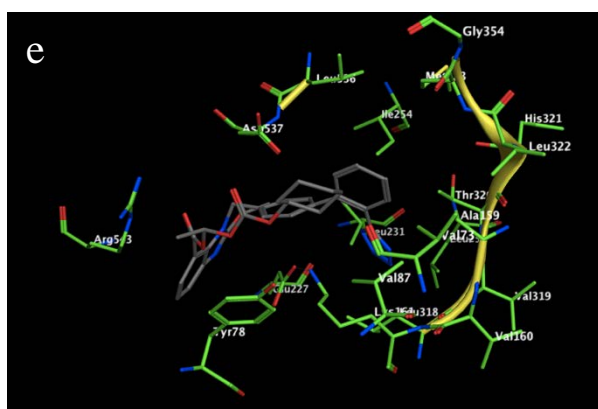
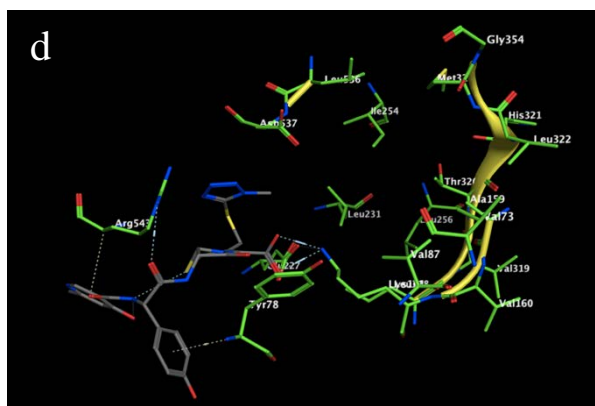
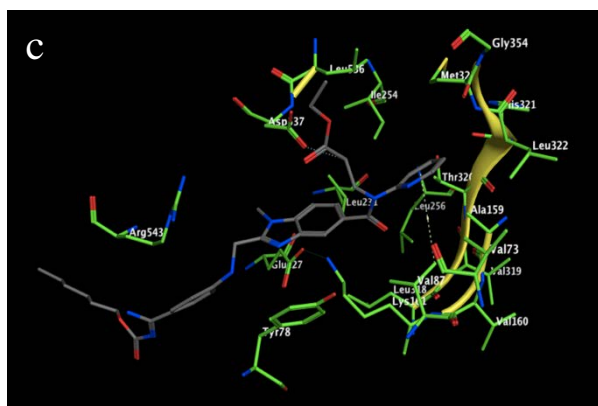
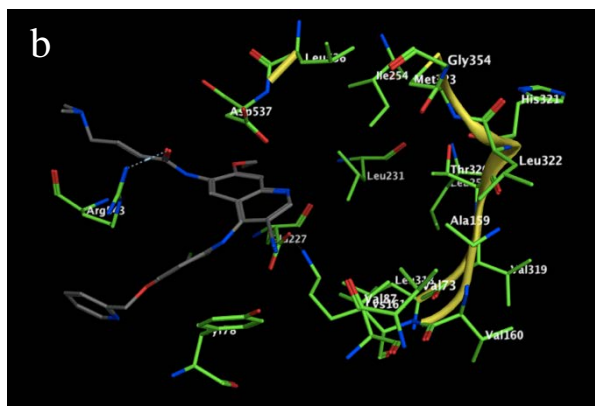
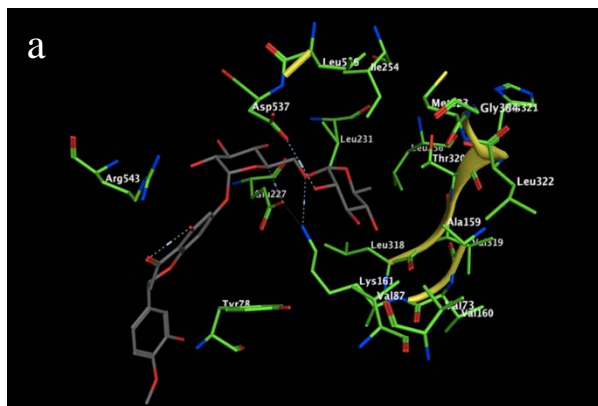
a final GBVI/WSA score. The random control compounds had an average docking score of  $-5.92$  kcal/mol, the known inhibitors had a score of  $-7.30$  and the predicted inhibitors had a score of  $-7.45$  (Figure 4). A t-Test (Table 1) confirmed that the predicted inhibitors were statistically significantly better at binding to p38 at the ATP-binding site—therefore inhibiting p38's function—than random FDA-approved drugs (Since the docking results measure binding energy, a more negative value means stronger binding). The t-Test also shows that the performance of the predicted inhibitors is much more consistent, with a variance less than half that of the random control group. The compounds exhibiting the best docking energies are listed in Table 3 and displayed with the p38 binding pocket in Figure 5.

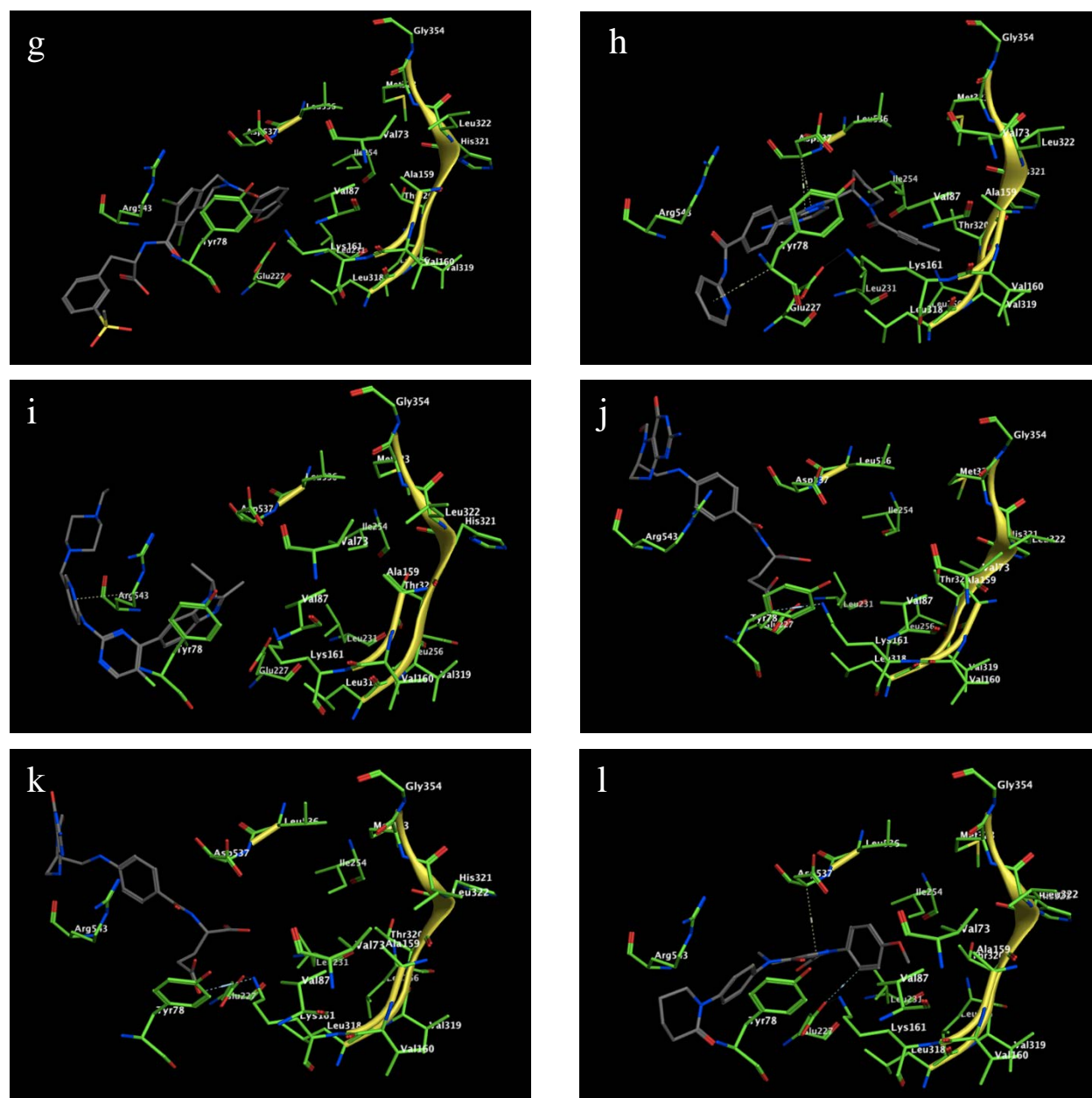


**FIGURE 4** Docking scores (kcal/mol) for 149 predicted inhibitors, 149 selected known inhibitors, and 149 random FDA-approved drugs.

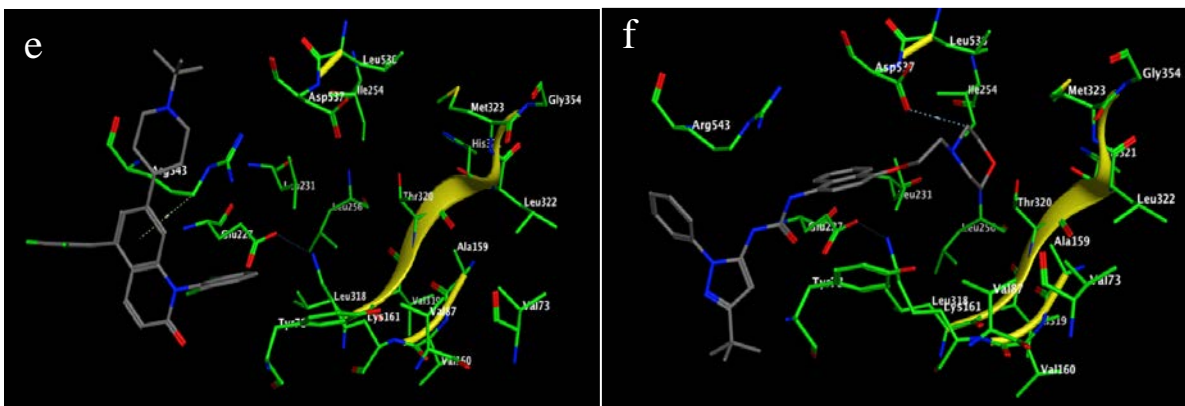
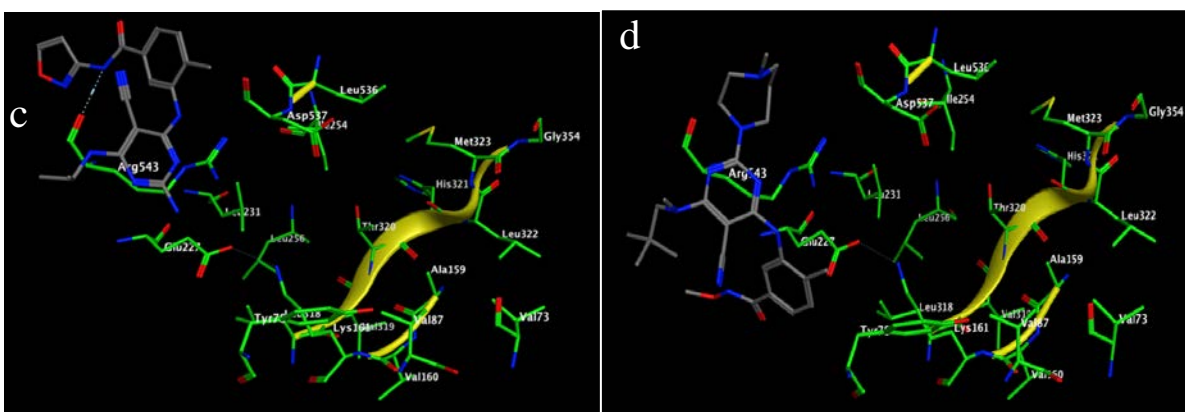
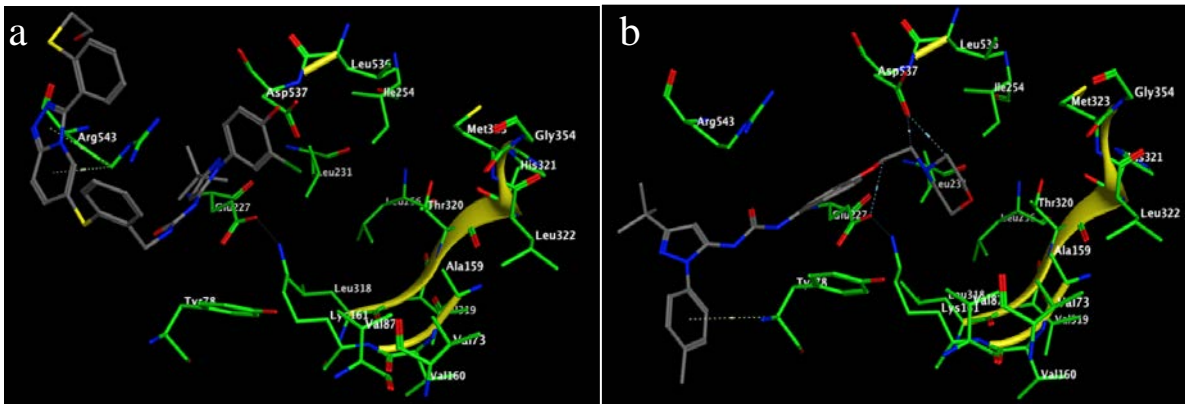
**TABLE 1** t-Test: Two-Sample Assuming Unequal Variances. Since  $t \text{ Stat} < -(t \text{ Critical two-tail})$ ,  $-13.89 < -1.97$ , the difference between the predicted inhibitors' and random FDA-approved drugs' docking scores is statistically significant

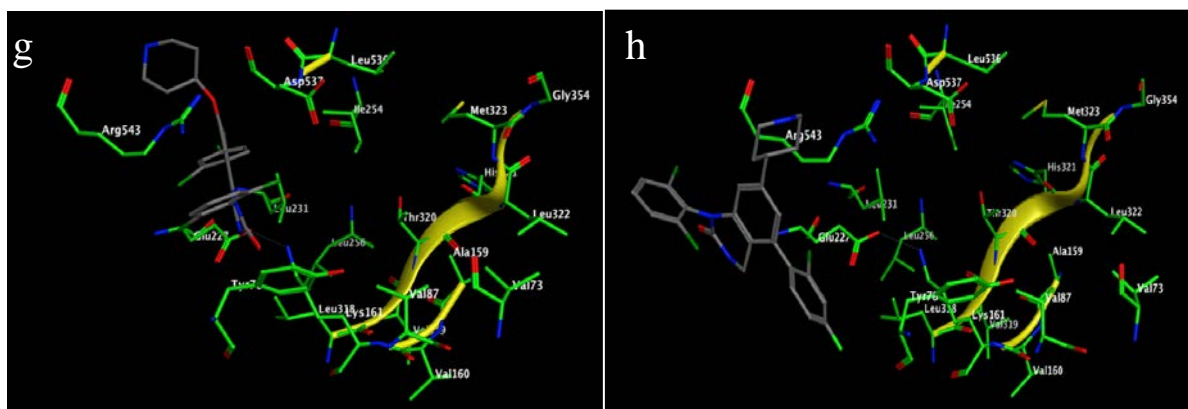
<b>Parameter</b>	<b>Predicted Inhibitors</b>	<b>Random FDA-approved drugs</b>
Mean	-7.45	-5.92
Variance	0.55	1.27
Observations	149	149
Hypothesized Mean Difference	0	
df	256	
t Stat	-13.89	
P(T<=t) one-tail	2.30E-33	
t Critical one-tail	1.65	
P(T<=t) two-tail	4.61E-33	
t Critical two-tail	1.97	





**FIGURE 5** Docking of the top 12 predicted inhibitors by docking score with the p38 ATP-binding site: **(a)** hesperidin; **(b)** neratinib; **(c)** dabigatran etexilate; **(d)** cefpiramide; **(e)** candesartan cilexetil; **(f)** ibrutinib; **(g)** lifitegrast; **(h)** acalabrutinib; **(i)** abemaciclib; **(j)** leucovorin; **(k)** levoleucovorin; **(l)** apixaban.





**FIGURE 6** Docking of the top 8 selected known inhibitors (based on activity values) with the p38 ATP-binding site: (a) PF-03715455; (b) doramapimod (Birb 796); (c) CHEMBL197206; (d) CHEMBL196741; (e) CHEMBL199679; (f) CHEMBL318810; (g) CHEMBL327959; (h) CHEMBL328614.

Table 2 also includes the original uses and protein interactions of the top 40 predicted inhibitors according to docking score, as well as their clusters (see section 3.2). Of these 40, 34 already act as some kind of protein inhibitor and 14 (35%) act on protein kinases specifically, which share structural similarities with p38. By comparison, 52 of the 2151 FDA-approved drugs (only 2.4%) used in this project are protein kinases. This means that the model and docking simulations are successfully identifying drugs that are already clinically proven to be inhibitors of proteins structurally similar to p38.

**TABLE 2** Top 40 predicted inhibitors ranked by docking score

Docking Score (kcal/mol)	Name	Cluster	Uses	Protein interactions
-9.82	Hesperidin	1	Treats blood vessel conditions	Inhibits lipase Inhibits human epidermal growth factor receptor 2 (Her2) and epidermal growth factor receptor (EGFR) tyrosine kinases
-9.18	Neratinib	25	Treats certain types of breast cancer	Inhibits thrombin
-9.13	Dabigatran etexilate	4	Prevents stroke and harmful blood clots	Inhibits penicillin-binding proteins (PBPs)
-9.12	Cefpiramide	22	Antibacterial agent	Inhibits the type-1 angiotensin II receptor (AT1)
-8.98	Candesartan cilexetil	53	Treats hypertension	

-8.97	Ibrutinib	4	Treats certain cancers	Inhibits Bruton's tyrosine kinase (BTK) and other non-specific tyrosine kinases
-8.96	Lifitegrast	31	Treats dry eye disease	Inhibits the interaction between lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1)
-8.89	Acalabrutinib	4	Treats mantle cell lymphoma	Inhibits Bruton's tyrosine kinase (BTK)
-8.66	Abemaciclib	4	Treats certain types of breast cancer	Inhibits cyclin-dependent kinase (CDK) 4 and 6
-8.63	Leucovorin	133	Prevents harmful effects of methotrexate	N/A
-8.63	Levoleucovorin	133	Treats or prevents toxic effects of methotrexate	N/A
-8.62	Apixaban	4	Prevents strokes or blood clots	Inhibits coagulation factor Xa
-8.50	Zafirlukast	11	Prevents asthma symptoms	Inhibits cysteinyl leukotriene 1 receptor (CYSLTR1)
-8.43	Imatinib	4	Treats certain types of leukemia	Inhibits the BCR-ABL, platelet-derived growth factor (PDGF), and stem cell factor (SCF)/c-kit tyrosine kinases
-8.42	Edoxaban	4	Prevents strokes or blood clots	Inhibits coagulation factor Xa
-8.37	Lusutrombopag	95	Treats thrombocytopenia	Activates the thrombopoietin (TPO) receptor
-8.34	Argatroban	83	Treats or prevents blood clots	Inhibits fibrin-bound thrombin
-8.31	Lapatinib	58	Treats certain types of breast cancer	Inhibits human epidermal growth factor receptor 2 (Her2) and epidermal growth factor receptor (EGFR) tyrosine kinases
-8.30	Piperacillin	22	Antibiotic	Inhibits penicillin-binding proteins (PBPs)
-8.25	Chloramphenicol palmitate	109	Antibiotic	Inhibits bacterial peptidyl transferase
-8.22	Ximelagatran	97	Treats and prevents blood clots	Inhibits thrombin
-8.20	Ubrogepant	4	Treats migraines	Inhibits the calcitonin gene-related peptide (CGRP) receptor
-8.07	Bosutinib	4	Treats certain types of blood cancer	Inhibits the BCR-ABL and Src tyrosine kinases
-8.06	Telotristat ethyl	122	Treats carcinoid syndrome diarrhea	Inhibits tryptophan hydroxylase
-8.06	Fedratinib	31	Treats myelofibrosis	Inhibits Janus-associated kinase 2 (JAK2) and FMS-like tyrosine kinase 3
-8.06	Ponatinib	4	Treats a certain type of leukemia	Inhibits several tyrosine kinases including the BCR-ABL tyrosine kinase
-8.05	Doxazosin	4	Treats hypertension and enlarged prostate	Inhibits alpha-1 adrenergic receptors
-8.02	Carindacillin	8	Antibiotic	Inhibits penicillin-binding proteins (PBPs)



-7.99	Netarsudil	89	Treats glaucoma	Inhibits the Rho kinase (ROCK) and norepinephrine transport
-7.99	Azlocillin	22	Antibiotic	Inhibits penicillin-binding proteins (PBPs)
-7.98	Levomefolic acid	133	Active metabolite of folic acid used as a dietary supplement	N/A
-7.98	Entrectinib	4	Treats a certain type of non-small cell lung cancer	Inhibits tropomyosin receptor kinases (Trk) A, B and C, C-ros oncogene 1 (ROS1) and anaplastic lymphoma kinase (ALK)
-7.98	Cabozantinib	2	Treats advanced renal cell carcinoma	Inhibits several different receptor tyrosine kinases (RTKs), such as hepatocyte growth factor receptors (METs) and vascular endothelial growth factor receptors (VEGFRs)
-7.91	Encorafenib	31	Treats certain types of cancer	Inhibits the serine/threonine Raf kinase
-7.91	Doxorubicin	1	Treats certain types of cancer	Inhibits topoisomerase II
-7.90	Arzoxifene	19	Maintains bone density to prevent fractures	Selectively inhibits and activates estrogen receptors as a selected estrogen receptor modulator (SERM)
-7.90	Riociguat	4	Treats pulmonary arterial hypertension	Stimulates guanylate cyclase activity
-7.90	Erlotinib	30	Treats certain types of non-small cell lung cancer	Inhibits the epidermal growth factor receptor (EGFR) tyrosine kinase
-7.90	Ticagrelor	81	Prevents heart attack or stroke	Blocks P2Y12 adenosine diphosphate (ADP) receptors
-7.89	Eltrombopag	10	Increases platelet count	Activates the platelet thrombopoietin receptor (TPO-R)

Table 3 shows the 10 residues, which interacted most frequently with the predicted compounds. Lys161 appears to be the most reactive residue, interacting through hydrogen bonds with about 80% of the ligands.

**TABLE 3** Residues most frequently contacting with inhibitors.

<b>Residue</b>	<b>Number of Interactions (%)</b>	<b>Average Distance</b>	<b>Most Frequently Interacting Residue Atom</b>	<b>Most Frequent Type of Interaction</b>
Lys161	80	3.02	NZ	H-Bond
Arg543	70	3.23	CD	Hydrophobic
Glu227	50	3.30	OE2	H-Bond
Leu231	50	3.71	CD1	Hydrophobic
Tyr78	50	3.54	CA	Hydrophobic
Asp537	40	3.21	OD1	H-Bond

Ala159	30	3.75	CB	Hydrophobic
Leu318	30	3.84	CB	Hydrophobic
Thr320	30	3.57	CB	Hydrophobic
Val87	30	4.03	CG2	Hydrophobic

### 3.2 | Deep-Learning Model

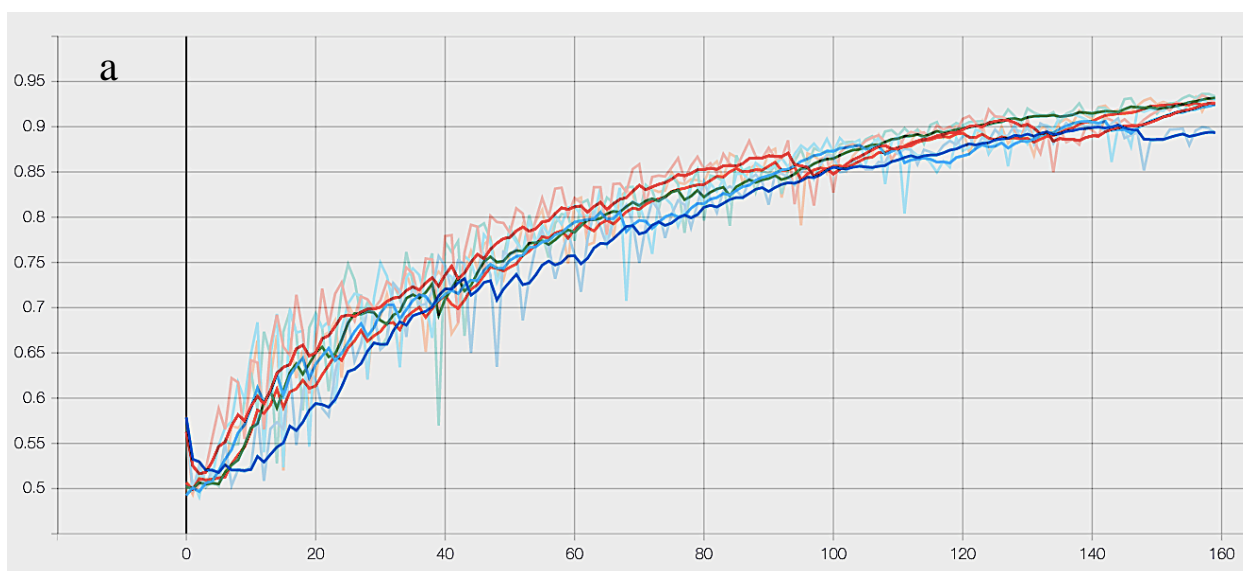
After the deep-learning algorithm was made and fine-tuned, it was run 5 times on a dataset of 2151 FDA-approved molecules with the same 160 descriptors as the training set. Figure 7 shows a graph of the training metrics of the model while training for each of the 5 trials. The fact that the accuracy and AUC start at 0.5, or random chance, but increase consistently with the epoch number, shows that the model is effectively learning and improving over time. The 5 trials applied had an average validation accuracy of 91.6% and an area under the receiver-operator characteristic (ROC) curve (AUC) of 0.969 (Table 3).

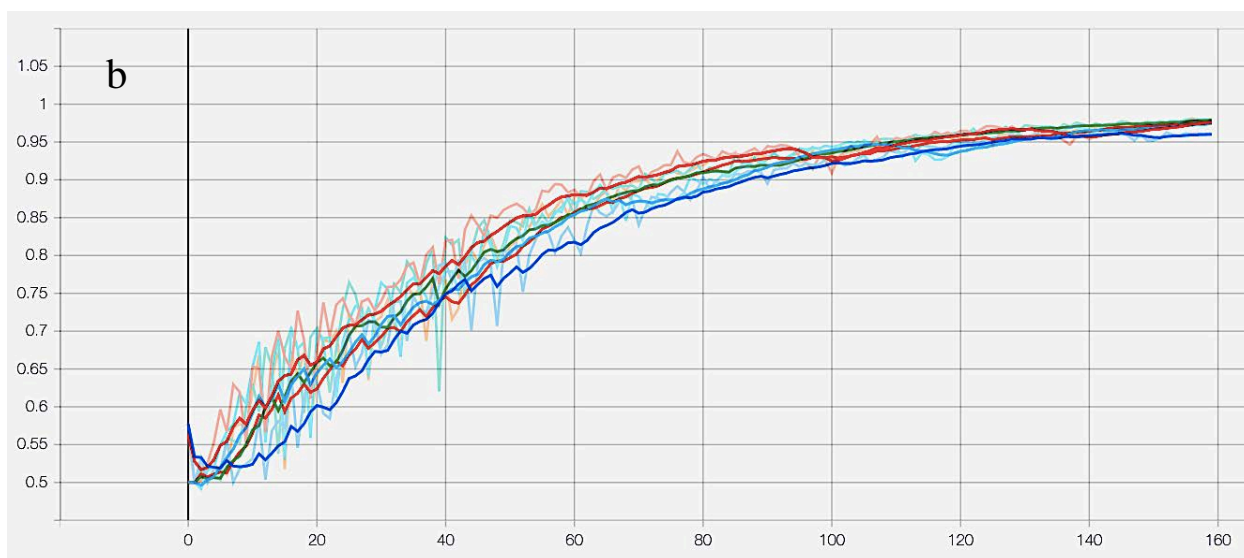
Table 4 contains the metrics for the deep-learning model, depicting accuracy, area under the ROC curve (AUC) and binary cross-entropy loss. Accuracy measures the number of correct predictions as a percentage of the total predictions. AUC is a slightly different but nonetheless important metric that demonstrates the model's ability to discriminate between two cases, which in this study are being an inhibitor or being a non-inhibitor. Our model's average AUC of 0.969 can be interpreted as meaning that 96.9% of the time, the model will correctly output a higher probability of inhibition for a randomly selected inhibitor than a randomly selected non-inhibitor, even if the overall classification is incorrect.

**Table 4** Metrics of deep-learning model predictions on validation set for all 5 trials

Trial	Validation Accuracy	Validation AUC
1	0.913043499	0.971671164
2	0.910973072	0.964130163
3	0.919254661	0.973077834
4	0.917184293	0.96619153
5	0.917184293	0.970728457
<b>Average</b>	<b>0.915527964</b>	<b>0.969159830</b>

It's important to note that the Figure 7 and Table 4 are not showing the same metrics, since Figure 7 shows the metrics while training, while Table 4 shows the metrics after training when the model is applied to the validation data, a data set randomly set aside from the training data. The validation metrics are a more accurate way to measure a deep-learning model because they better simulate the model's performance on a separate dataset and confirm that a model is not just overfitting to the training data. So, our model can be expected to perform on FDA-approved drugs with the same accuracy that it does on the validation data. After synthesizing the results from all 5 trials, the deep-learning model had classified 149 FDA-approved molecules as being p38 inhibitors, with 27 of them having a greater than 90% chance.





**FIGURE 7** (a) Accuracy (Y-axis) vs. epoch (X-axis) for training data for all 5 trials. (b) AUC (Y-axis) vs. epoch (X-axis) for training data for all 5 trials

Table 5 shows the top drug candidates ranked by the deep neural network score. For each given compound, the algorithm outputs a number between 0 and 1. The closer the value is to 0 the more likely it is a non-inhibitor, and the closer the value is to 1 the more likely it is a p38 inhibitor. Thus, the output value can be interpreted as probability of inhibition according to the deep-learning model. The compound with the highest probability of inhibition (0.99) is doxorubicin, which also has a strong docking score of  $-7.91$ , making it a promising candidate. Table 6 lists the top 100 drug candidates ranked by a combination of their docking score rank and deep-learning-predicted rank. Although ranking by docking scores (Table 2) is the most reliable, combining these two ranking methods can present a more comprehensive picture of the best candidates. For example, ibrutinib, acalabrutinib, and hesperidin, are the top two candidates by this join scaling measure, with combined ranks of 10, 13, and 15, respectively. The fact that these compounds' incredibly high-performing docking scores are backed by the deep-learning model makes them some of the most promising candidates.

**TABLE 5** Top 40 compounds ranked by deep-learning-predicted probability of inhibiting p38

DL rank	Probability of Inhibition	Name	Cluster	Docking Score kcal/mole
1	0.99	Doxorubicin	1	-7.91
2	0.98	Cabozantinib	2	-7.98
3	0.98	Diacerein	3	-7.28
4	0.98	Ibrutinib	4	-8.97
5	0.97	Acalabrutinib	4	-8.89
6	0.97	Entrectinib	4	-7.98
7	0.97	Lenvatinib	7	-7.52
8	0.96	Carindacillin	8	-8.02
9	0.96	Deferasirox	9	-6.95
10	0.96	Eltrombopag	10	-7.89
11	0.95	Zafirlukast	11	-8.50
12	0.94	Ponatinib	4	-8.06
13	0.94	Daunorubicin	1	-6.65
14	0.94	Hesperidin	1	-9.82
15	0.94	Diosmin	1	-7.83
16	0.94	Imatinib	4	-8.43
17	0.93	Delafloxacin	17	-6.95
18	0.93	Selpercatinib	18	-7.69
19	0.93	Arzoxifene	19	-7.90
20	0.92	Idarubicin	1	-7.61
21	0.92	Trovafloxacin	21	-6.93
22	0.92	Piperacillin	22	-8.30
23	0.91	Perampanel	23	-6.56
24	0.90	Tucatinib	24	-7.67
25	0.90	Neratinib	25	-9.18
26	0.90	Avanafil	4	-7.61
27	0.90	Raloxifene	19	-7.21
28	0.89	Pralatrexate	28	-6.75
29	0.88	Dabigatran etexilate	4	-9.13
30	0.88	Erlotinib	30	-7.90
31	0.88	Bendroflumethiazide	31	-6.85
32	0.88	Dacomitinib	4	-7.00
33	0.87	Etravirine	33	-6.78
34	0.87	Amsacrine	31	-6.93
35	0.86	Nilotinib	35	-7.68
36	0.86	Cromoglicic Acid	1	-6.74

37	0.86	Azilsartan medoxomil	37	-7.88
38	0.86	Duvelisib	35	-7.19
39	0.85	Fedratinib	31	-8.06
40	0.85	Isoxaflutole	40	-5.61

**TABLE 6** Top 100 predicted inhibitors, ordered by the sum of docking rank and deep-learning rank

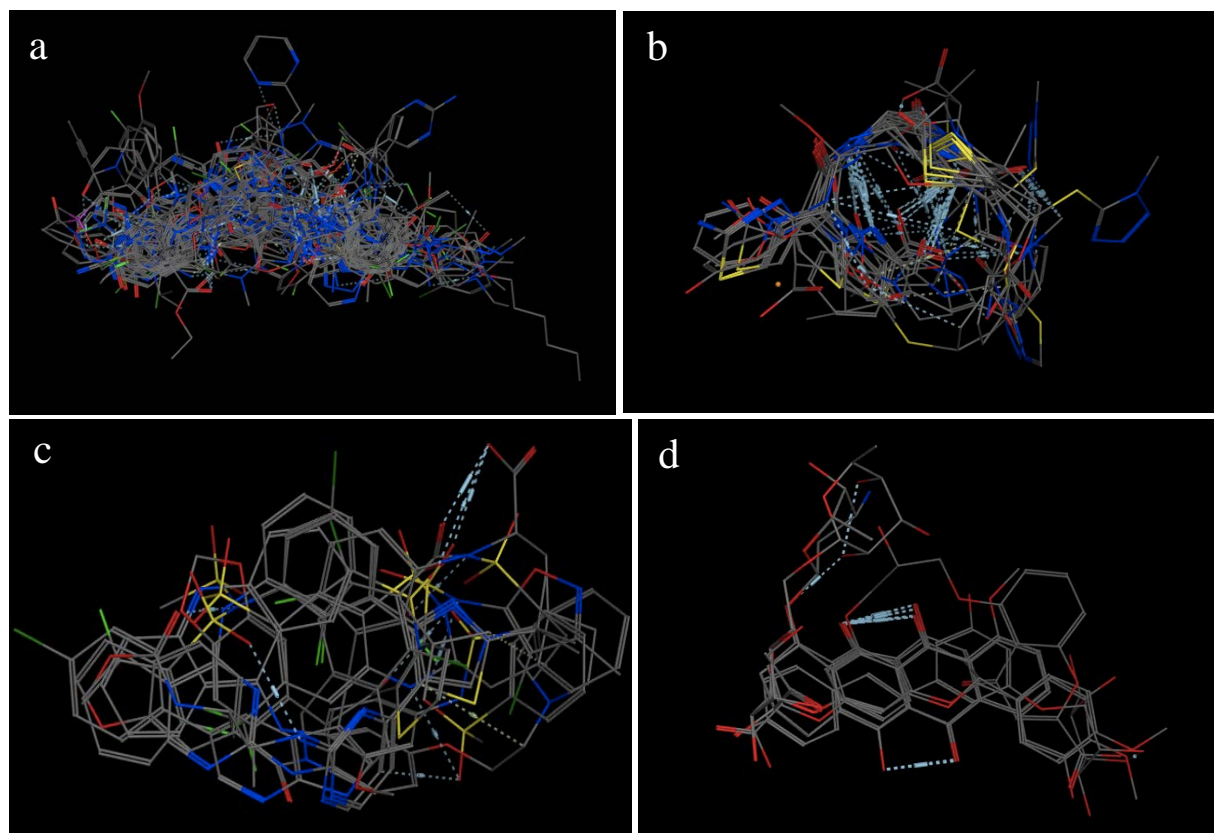
<b>DL Rank + Docking Rank</b>	<b>Name</b>	<b>Cluster</b>	<b>DL Rank + Docking Rank</b>	<b>Name</b>	<b>Cluster</b>
10	Ibrutinib	4	129	Chloramphenicol palmitate	109
13	Acalabrutinib	4	129	Duvelisib	35
15	Hesperidin	1	130	Ketoconazole	4
24	Zafirlukast	11	131	Vemurafenib	31
27	Neratinib	25	131	Delafloxacin	17
30	Imatinib	4	132	Cefepime	22
32	Dabigatran etexilate	4	136	Copanlisib	4
35	Cabozantinib	2	137	Cefuroxime	22
36	Carindacillin	8	138	Afatinib	4
36	Doxorubicin	1	138	Pemetrexed	28
38	Ponatinib	4	138	Trovafloxacin	21
38	Entrectinib	4	139	Ubrogapant	4
41	Piperacillin	22	142	Methotrexate	70
50	Eltrombopag	10	143	Tadalafil	42
55	Arzoxifene	19	143	Dacomitinib	4
58	Candesartan cilexetil	53	144	Levoleucovorin	133
62	Diosmin	1	144	Betrixaban	79
64	Fedratinib	31	144	Daunorubicin	1
68	Erlotinib	30	146	Telotristat ethyl	122
71	Selpercatinib	18	148	Aclidinium	102
72	Abemaciclib	4	151	Bendroflumethiazide	31
74	Azlocillin	22	152	Amsacrine	31
76	Lapatinib	58	153	Larotrectinib	4
76	Lenvatinib	7	154	Ribociclib	4
80	Azilsartan medoxomil	37	154	Lumacaftor	71
80	Tucatinib	24	155	Nefazodone	4
81	Idarubicin	1	155	Sulfinpyrazone	67

84	Lifitegrast	31	155	Pralatrexate	28
86	Riociguat	4	156	Olaparib	56
89	Nilotinib	35	157	Leucovorin	133
89	Avanafil	4	157	Etravirine	33
89	Diacerein	3	157	Perampanel	23
90	Edoxaban	4	159	Gefitinib	4
91	Antrafenine	4	160	Cefoxitin	22
96	Linagliptin	4	161	Apixaban	4
97	Udenafil	47	164	Cromoglicic Acid	1
98	Alatrofloxacin	4	166	Ripretinib	62
100	Argatroban	83	170	Ceftazidime	22
103	Erdafitinib	45	173	Lumefantrine	98
106	Encorafenib	31	173	Apalutamide	66
107	Cefpiramide	22	173	Isavuconazonium	61
108	Montelukast	57	174	Suvorexant	4
111	Lusutrombopag	95	174	Acemetacin	96
111	Bosutinib	4	175	Topotecan	50
117	Raloxifene	19	176	Dabrafenib	31
118	Ximelagatran	97	177	Levomefolic acid	133
118	Netarsudil	89	179	Macimorelin	80
120	Ticagrelor	81	181	Cefditoren	22
124	Deferasirox	9	184	Ezetimibe	43
128	Doxazosin	4	189	Cefotaxime	22

### 3.2 | Clustering

Fingerprint clustering of the predicted inhibitors resulted in 78 clusters. The flexible alignments of the four largest clusters created by MOE's Database Viewer/Compute/Fingerprints/Clusters submodule are shown Figure 8. The clusters are groups of predicted inhibitors with similar pharmacophore features and can be used to further classify the compounds and determine the best candidates. For example, certain clusters tend to outperform the average predicted inhibitor, making members of that group more promising drug candidates due to shared structural features with other strong candidates. One such cluster is number 4, which, as seen in Tables 2, 5, and 6, contains many of the top candidates like dabigatran etexilate, Ibrutinib, acalabrutinib, and abemaciclib. Furthermore, despite making up only 23.5% of all 149 predicted inhibitors, cluster 4

compounds make up 32.5% of the top 40 compounds by docking score, making members of this cluster more promising candidates.



**FIGURE 8** The four largest clusters of predicted compounds: (a) Cluster 4, 35 molecules; (b) Cluster 11, 13 molecules; (c) Cluster 98, 8 molecules; (d) Cluster 1, 7 molecules

#### 4 | Discussion

We elucidated 149 potential p38 inhibitors that can be tested through in vitro and in vivo experimental trials. With an average validation accuracy of 92% and area under the ROC curve of 0.97, the deep-learning model has shown significant efficacy in predicting the ability of a compound to inhibit p38. Furthermore, protein docking scores indicate that our predicted inhibitors statistically significantly better (Table 1) than random FDA-approved molecules, and even slightly better than the top known p38-inhibitors. Following experimental trials, these compounds could be used as treatments for various p38-mediated diseases, including not just MFN, but also cancers and inflammatory diseases like rheumatoid arthritis and Alzheimer's.



Furthermore, the procedure described in this study can be applied to repurpose existing drugs as protein inhibiting or activating treatments for a wide variety of other diseases.

One significant difference between our project and other deep-learning drug discovery projects is its application on FDA-approved drugs. The repurposing of FDA-approved drugs has many practical benefits, especially for p38 inhibitors. Many of the most common and potent p38 inhibitors do well in *in vitro* experiments but fall short during *in vivo* or human trials due to unexpected side effects or issues with toxicity (Hammaker & Firestein, 2010). When repurposing approved drugs, however, the safety of the compound has been studied extensively by the FDA and often other organizations like the European Medicines Agency (EMA).<sup>33</sup> This not only ensures that a drug is safe and its side effects are well documented, but it also significantly cheapens and shortens the researcher to patient pipeline, which normally takes an average of 10–15 years, because doctors can provide off-label prescriptions before official approval.<sup>34</sup> Thus, if clinical trials bode well, these inhibitors could very soon be available to patients suffering from potentially life-threatening p38-mediated diseases.

Traditional drug research and development is a long and tedious process that has become incredibly inefficient relative to the amount of money put in. In fact, “the number of new drugs approved per billion US dollars spent on R&D has halved roughly every 9 years since 1950.”<sup>35</sup> Although *in silico* research cannot replace empirical trials, it allows us to efficiently and cheaply elucidate promising drug candidates to expedite the creation of new treatments, especially when multiple computational procedures are used in tandem. As new technologies emerge and the amount of accessible drug data continues to grow, machine learning and other computational algorithms will continue to improve in efficacy and allow for unprecedented advancements in the pharmaceutical field.

## **ACKNOWLEDGMENTS**

We thank CCG (Montreal) for their support.

## **AUTHORS CONTRIBUTION**

IT and VK proposed the research described in the article, outlined the possible tools and discussed the strategies, results, and conclusions. AV developed a program for computation, trained the machine learning model and investigated the results of machine learning selection.

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