

# An overview of Pocket-based drug design

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## Abstract

De novo drug design has been receiving significant attention lately due to its ability to create new ligands. Pocket-based algorithms can explore the extensive chemical space to produce new compounds with strong affinity for a specific protein pocket, making them valuable for treating diseases.

This article presents a cutting-edge review of pocket-based algorithms. The assessed models are founded on well-known algorithms such as diffusion models, transformers, genetic algorithms, and graph neural networks. Furthermore, it introduces a candidate selection protocol to filter the generated ligands, ensuring optimal properties such as non-toxicity and high docking values. Finally, these compounds are analyzed using bioinformatics tools like Maestro, Schrödinger, and Glide. The findings demonstrate that these algorithms can generate potential lead compounds, ultimately concluding that pocket-based research is a promising field that can address the challenge of exploring the chemical space effectively.

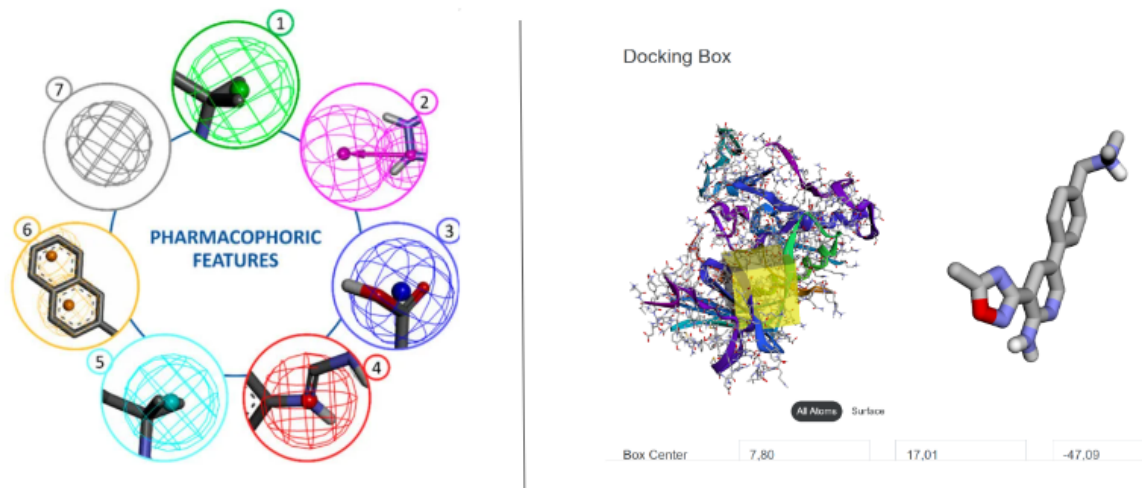
# INTRODUCTION

In recent years, advances in artificial intelligence (AI) techniques have greatly impacted the field of drug discovery.<sup>1</sup> The rise of deep learning has enabled computational drug design to increasingly become a reality. One of the main advantages of using these algorithms is the possibility of exploring relevant areas of a chemical space that is just immense.<sup>2</sup> Researchers have tried to estimate its size, which refers to the number of possible compounds that could be synthesized. However, there is no scientific consensus on the number, with some estimating it to be between  $10^{30}$  to  $10^{60}$ .<sup>3</sup> The discrepancy in the estimations is due to different criteria applied in the studies, such as the maximum size of the molecules, the types of atoms that compose them, or the presence of physicochemical restrictions like the Lipinski rules.<sup>4-6</sup>

Such a vast search space poses significant challenges when optimizing or searching for molecules with specific properties. As a result, the development of methods to navigate this space has gained considerable interest in recent years. Several techniques are now available to explore the huge chemical space and identify new potential drug candidates. The current methods can be classified into two families: ligand and pocket-based (Figure 1). Ligand-based methods use a set of high-affinity molecules to a target protein as input to create new ligands.<sup>7,8</sup> This is useful when the research focuses on an interesting part of the chemical space where the corresponding algorithms generate new potential drug candidates around a specific point that represents a molecule.

On the other hand, pocket-based methods, are used to generate appropriate ligands for protein pockets by utilizing detailed structural information of the target binding site.<sup>9</sup> For this reason, pocket-based algorithms are highly effective when the three-dimensional (3D) structure of a protein is available, allowing for precise molecular docking and virtual screening to design molecules that fit within the structural constraints of the binding site. Such approaches can accurately model interactions between the ligand and the protein, simplifying the design of molecules optimally configured to the target site and making them especially useful to create new drugs that inhibit certain proteins involved in the appearance of dis-

eases. One of the main problems with pocket-based algorithms is the lack of experimental validation.<sup>10</sup> Therefore, developing a protocol to select the best candidates for subsequent synthesis in the laboratory is essential.



**Figure 1.** Ligand *vs* Pocket-based drug design. Adapted from reference.<sup>11</sup>

A typical pipeline of pocket-based drug discovery is shown in Figure 2. Steps 1 and 2 correspond to getting the PDB file of the interest protein and its binding site respectively. The remaining steps complete the process of generating drug candidates with optimal properties. This article will ~~actually~~ focus on current generative models based on the pocket structure of proteins and the evaluation metrics of the molecules, corresponding to steps 3 and 4 of Figure 2. Its main contributions are \*,

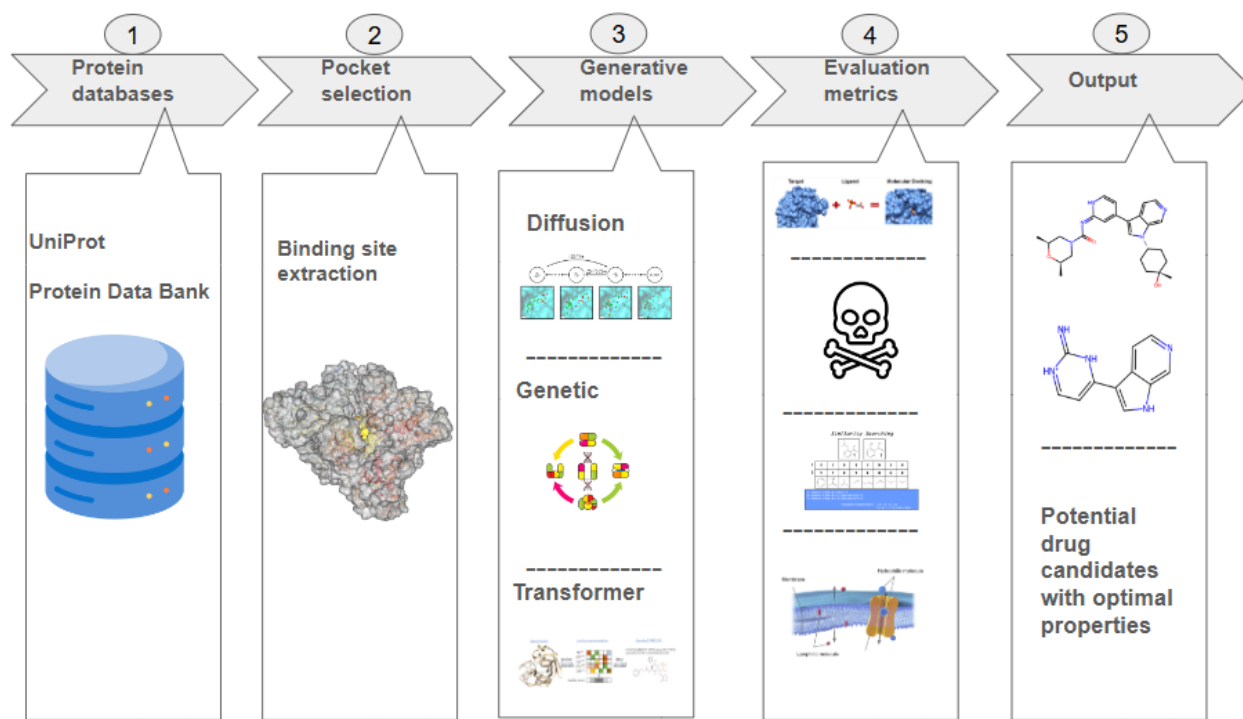
- To create a common framework of current pocket-based molecular generation methods.
- The benchmarking of these methods to assess which are the most promising in pocket-based drug design, creating a reference framework to evaluate additional algorithms.
- To develop a protocol to filter optimal compounds given a target protein.
- To validate experimentally pocket-based algorithms to generate potential drug candidates for DYRK1A protein, an enzyme directly implied in Alzheimer’s Disease (AD).

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\*For reproducibility reasons, all the code used in this article can be found in <https://github.com/pvaras8/pocketdrugdesign>.

Normalmente, se pone Section 1, Section 2,... No sé si la revista te pide ponerlo así, con los títulos de las secciones en mayúsculas, porque luego las secciones no están numeradas. Usas \section\*{} en lugar de \section{} en el .tex?

The manuscript is organized as follows. POCKET-BASED GENERATIVE MODELS section presents the current pocket-based generative models models. In MAIN EVALUATION METRICS section the characteristics for choosing a drug candidate are explained. CASE STUDY section presents a specific case study, where the generative models and the evaluation metrics are used to generate optimized molecules for the DYRK1A protein. Finally, in CONCLUSIONS we finished with the analysis extracted from the article and the future lines of pocket-based generative models.



**Figure 2.** Pipeline of pocket-based drug discovery.

Con qué has hecho la figura?  
Con draw.io puedes hacer que estas figuras queden bien cuadradas y con los textos alineados

## POCKET-BASED GENERATIVE MODELS

Protein pocket-based models are currently being used to generate new ligands and explore the vast chemical space. This section examines key technical features of major models, with the Supporting Information displaying key aspects of algorithms implementing them.

## Transformer based models

Transformer-based algorithms are gaining popularity in drug discovery because they can capture complex relations within training data through the celebrated *attention mechanism*, and generate high-affinity ligands for a given protein using a sequential-based approach,<sup>10</sup> creating new molecules by joining atoms or molecular substructures.

Qué es? A lo mejor una pequeña explicación de qué hace

These models typically require a tokeniser to convert the Simplified Molecular Input Line Entry System (SMILES) code of the molecule and the protein's amino acid chain into tokens. Its ligand and protein embeddings are then obtained and used to train the weights of the so-called transformer multi-attention layers. Once trained, the model can generate molecules related to a given amino acid sequence.

Following this approach, DrugGPT<sup>12</sup> employs the GPT-2 model and the Byte Pair Encoding (BPE) tokenizer, which can represent a huge number of compounds using a limited vocabulary, to explore the chemical space and discover new ligands for specific proteins. Lingo3DMol<sup>9</sup> proposes a new representation for SMILES called FSMILES, which fragments SMILES into molecular groups according to certain rules. The protein pocket is then encoded through an encoder, and, starting from the initially calculated growth point, the decoder builds the molecule by iteratively joining molecular groups produced.

TamGent<sup>13</sup> incorporates a variant of the Transformer encoder designed to process 3D geometric information of targets. Moreover, Ang et al.<sup>14</sup> employ an Encoder-Decoder Transformer combined with Reinforcement Learning through an Adaptive Monte Carlo Tree Search, emphasizing the generation of valid small molecules with desirable drug-like characteristics and binding affinities.

## Difussion based models

Diffusion models have attracted special interest in recent years due to their capacity to generate novel compounds. They create a Markov chain of progressive noising steps to add random Gaussian noise to real data until the original sample becomes unrecognizable.

Consequently, a model is trained to reverse this process. Once trained, this model can generate new molecules by sampling from a normal distribution and denoising this data until a new compound is created. In the field of pocket-based drug design, this denoising procedure is conditioned on the protein pocket for which the model will create new potential drug candidates.<sup>15</sup>

One example is DiffSBDD,<sup>16</sup> which has proved its capability to generate novel ligands with high predicted binding affinities to given protein pockets, highlighting its potential as a tool for molecules design in structure-based drug design. [Another example is TargetDiff, which introduces ...](#) ~~Moreover,~~ TargetDiff<sup>17</sup> introduces an advanced diffusion model to generate molecules in the 3D space. This ensures that molecular generation is sensitive to the spatial conformation of protein targets.

## Genetic algorithms

[to direct the compound search procedure.](#)

Genetic Algorithms (GA) implement heuristics inspired by natural evolutionary processes. They use mutation and/or crossover operations to explore the chemical space and maximize a target property, generally the docking value. Following this approach, after several generations, these algorithms can create novel ligands with high affinity given a target protein.<sup>18</sup>

One example of GA for pocket-based drug design is AutoGrow4,<sup>19</sup> which generates novel drug-like molecules by applying a series of mutations and crossovers to an initial population of seed molecules. This process is further refined through a fitness function that ranks compounds based on their predicted binding affinities, continuously selecting the top performers for subsequent generations. [No es así como funciona cualquier GA? Es una aplicación directa del paradigma?](#)

Another innovative approach, the Reinforced Genetic Algorithm (RGA),<sup>20</sup> proposes a technique that relies on reinforcement learning to choose mutation and crossover operations. The goal is to maximize the docking value, which serves as the fitness function to optimize. The process starts with the selection of approximately 100 drug candidates from an initial database. These compounds are then subjected to mutation and crossover conditioned to the protein pocket to create new ones that improve the docking score.

## Graph Neural Networks based models

Graph neural networks have received special attention in recent years within the field of drug discovery. These models are very important for data processing based on structured graphs.<sup>21</sup> Molecules and proteins are represented as graphs, denoted  $G = (V, E)$ , whereby:

- $V$  represents the nodes of the graph, each corresponding to an atom in the molecule or an amino acid protein.
- $E$  represents the edges of the graph, signifying the bonds between atoms or the sequential proximity between amino acids in proteins.

Following this approach, Pocket2Mol<sup>22</sup> introduces an equivariant generative network aiming to efficiently sample molecular structures based on the 3D structure of protein pockets. Its innovation lies in its dual-module design: a novel graph neural network capturing spatial and bonding relationships, and an efficient algorithm for conditional molecular sampling.

## MAIN EVALUATION METRICS

To create a benchmark for evaluating the molecules generated by the different models, ~~the metrics considered are:~~ we will consider the following metrics:

- *Virtual Docking.* It predicts the interaction between a drug molecule and a target protein by simulating their binding affinity.<sup>23</sup> This helps to identify potential drug candidates by assessing how well they fit into the target site.
- *Pharmacological activity.* It measures the biological effects of a drug molecule on the body or specific cells or tissues, therefore assessing the action mechanism of a compound in producing a therapeutic effect, such as inhibiting a disease-related enzyme or activating a receptor. The continuous variable pChEMBL, defined as the  $-\log_{10}$  of molar concentration ( $IC_{50}$ ,  $XC_{50}$ ,  $EC_{50}$ ,  $AC_{50}$ ,  $Ki$ ,  $Kd$ , or Potency), is employed to assess the pharmacological activity of a compound.<sup>24</sup>

- *Quantitative estimation of drug likeliness (QED)*. It measures the likelihood of a chemical compound to be a successful drug candidate based on its physicochemical properties. It quantifies drug-like properties through a single score, considering factors such as solubility, permeability, and molecular weight. These factors are indicative of the ability of a compound to become an effective oral drug in humans.<sup>25</sup>
- *Lipophylicity (LogP)*. It assesses the tendency of a compound to dissolve in fats, oils, and lipids over aqueous (water-based) solutions, therefore indicating the compound’s ability to penetrate cell membranes, affecting its absorption, distribution, metabolism, and excretion properties. Often expressed as LogP, which is the logarithm of the partition coefficient between N-octanol and water.<sup>26</sup>
- *Molecular Diversity*. It assesses the structural variety among the A and B through the formula

$$\text{Tanimoto Similarity} = \frac{c}{a + b - c},$$

where  $a$  and  $b$  are the counts of features present in molecules A and B respectively, and  $c$  is the count of features common to both molecules. Specifically, the features usually employed are represented by the Morgan Fingerprints, converting the molecular structure into a binary vector representing the presence or absence of certain chemical substructures in the molecule. A higher Tanimoto score indicates greater similarity.<sup>27</sup>

- *Molecular Weight*. Its analysis is crucial for evaluating the molecule’s atomic composition and determining the drug pharmacokinetics.<sup>26</sup>
- *Synthetic Accessibility Score (SAS)*. It is calculated as the sum of the molecular fragment scores plus a complexity penalty based on the presence of certain molecular groups. This metric assesses the ease with which a chemical compound can be synthesized, providing crucial insights about the feasibility of its production on a larger scale.<sup>28</sup>



- *Toxicity.* In the evaluation of potential drug candidates, assessing the toxicity profile is crucial to determine their safety and viability of molecules to guarantee further development. These toxicity classes include interactions with various nuclear receptors (e.g., androgenic, estrogenic), responses to environmental and endogenous stress signals (e.g., oxidative stress response, heat shock response), and effects on key cellular processes (e.g., DNA damage response) as shown in Table 1. Each class represents a distinct mechanism through which a compound might exhibit toxicological effects, providing a comprehensive view of its safety profile.<sup>29</sup>

**Table 1. Toxicity classes.**

Nuclear Receptor Panel (biomolecular targets)	Stress Response Panel
ER-LBD: estrogen receptor, luciferase	ARE: nuclear factor (erythroid-derived 2)-like 2 antioxidant responsive element
ER: estrogen receptor alpha	HSE: heat shock factor response element
Aromatase	ATAD5: genotoxicity indicated by ATAD5
AhR: aryl hydrocarbon receptor	MMP: mitochondrial membrane potential
AR: androgen receptor	p53: DNA damage p53 pathway
AR-LBD: androgen receptor, luciferase	
PPAR: peroxisome proliferator-activated receptor gamma	

## CASE STUDY

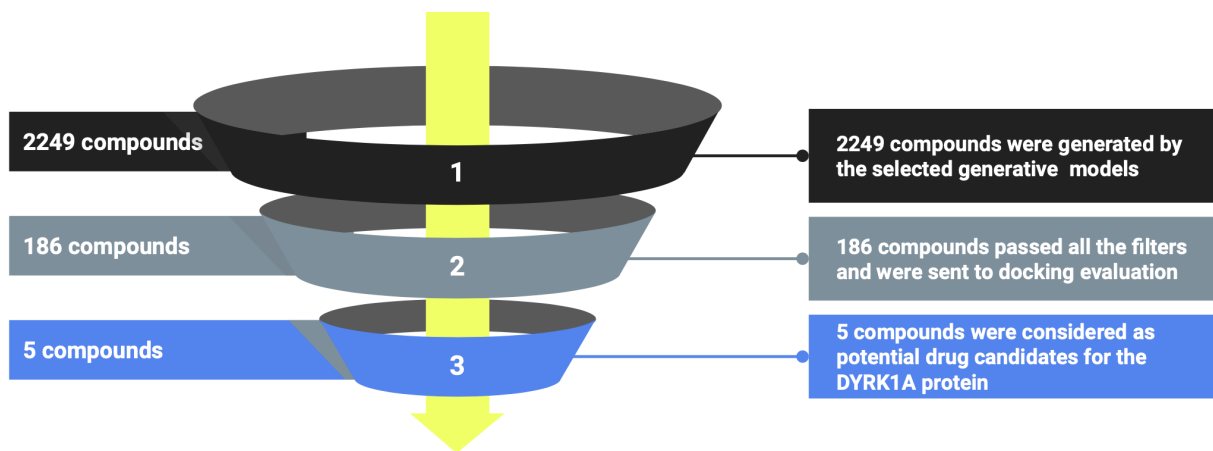
[ In this section we present a case study for evaluating the performance of different generative algorithms.] Ya lo has dicho en la intro, tal vez lo obviaría y empezaría directamente con el siguiente párrafo

In recent years, the DYRK1A enzyme has been identified as a promising target for therapeutic intervention in AD, as it is involved in multiple biological functions. Several studies have shown that DYRK1A undergoes alterations linked to the progression of AD, such as the

phosphorylation of proteins like TAU<sup>30</sup> and APP.<sup>31</sup> Therefore, DYRK1A is a highly promising enzyme as a therapeutic target for designing new drugs that could be used potentially to treat AD. [For this reason, we will evaluate the performance of different generative algorithms by searching for compounds that target the DYRK1A. \(O algo similar. Queda mejor introducir tu objetivo aquí que al principio de la sección en la página anterior\)](#)

The protocol that we have followed to obtain potential lead compounds is shown in Figure

3



**Figure 3.** Candidates selection protocol.

## Experimental section

### Binding site definition

[First,](#)  
~~To begin with,~~ we obtained the DYRK1A 3D structure from the Protein Data Bank (PDB code: 6EIF<sup>32</sup>). We extracted the mass centre of the crystallographic ligand B5T of chain A by taking the average of the  $x$ ,  $y$  and  $z$  coordinates, which were 7.80, 17.01, and -47.09 respectively. Molecules were then generated around a box of 15 Å from this point.

### Selected generative models

The models used to develop new ligands against the DYRK1A protein were DiffSBDD, Drug-GPT, Lingo3DMol, Pocket2Mol and RGA. These models were chosen for their open-source nature, effectiveness and accessibility. As illustrated in Table 2, these models were trained using different datasets, each with its unique size and composition of training compounds.

The DrugGPT model utilizes a substantial dataset from ‘jglaser/binding affinity’<sup>33</sup> and ZINC 20,<sup>34</sup> totalling 1.9 million and 2 billion compounds, respectively. In contrast, RGA, DiffSBDD and Pocket2Mol are trained on the CrossDocked dataset,<sup>35</sup> with DiffSBDD further supplemented by Binding MOAD<sup>36</sup> with 41 thousand protein-ligand pairs for training and RGA with 250 thousand compounds of the ZINC 15<sup>37</sup> database. In turn, Lingo3DMol is trained with 20 million commercially available compounds, and in its fine-tuning phase, uses a smaller subset of CrossDocked and DUD-E,<sup>38</sup> focusing on precise adjustments with 11.8 thousand and 6.5 thousand compounds, respectively.

**Table 2. Summary of models and databases used for training.**

Model	Databases	Training Compounds
DiffSBDD	CrossDocked, Binding MOAD	100 K, 41 K
DrugGPT	jglaser/binding affinity, ZINC 20	1.9 M, 2 B
Lingo3DMol (fine-tuning phase)	CrossDocked, DUD-E	11.8 K, 6.5 K
Pocket2Mol	CrossDocked	22.5 M
RGA	CrossDocked, ZINC 15	22.5 M, 250 K

These models were run to generate 2249 new compounds for the given pocket. For DrugGPT and Lingo3DMol, the number of molecules generated can be chosen as a hyperparameter. For the other three algorithms, the models were run 5 times to obtain a significant number of molecules for comparison, as shown in Table 3.

La tabla 3 está en Pamplona después de los experimentos, no la menciones tan pronto. Con decir que los has lanzado 5 veces basta.

## Filters

The parameters used to filter the molecules are explained in the Supporting Information). For toxicity prediction, the Chemprop algorithm<sup>39</sup> was used, which employs a directed message-passing neural network to predict molecular properties. The remaining properties were calculated using the RDKit package.<sup>40</sup>

The simulated docking value for all the molecules was obtained using Smina, provided by PyScreener,<sup>41</sup> allowing efficient and flexible computation of docking scores. Pharmacological activity was calculated using Morgan fingerprints to process the initial SMILES and a

Falta un (



Gaussian process to obtain the pChEMBL value. [These two metrics were used to compared the models performance in Table 3 In the end, 186 molecules passed all the filters as shown in Figure 3 ]

Sigo pensando que es algo pronto para introducir los resultados directamente

## Docking studies

The following steps were taken to validate the 186 resulting molecules experimentally:

- *Ligand Preparation.* The conversion from SMILES to SD format was carried out using the structconvert tool available in the Schrödinger suite.<sup>42</sup> Ligand preparation was performed utilizing the LigPrep tool included in the Maestro package.<sup>43,44</sup> Progressive levels were generated, encompassing possible ionization states at physiological pH and potential tautomers. Final energy minimization was implemented using the OPLS4 force field, with default settings applied for stereoisomers.
- *Protein Preparation.* Human DYRK1A (PDB code 6EIF<sup>32</sup>) was prepared for subsequent computational analyses using the Protein Preparation Wizard<sup>45,46</sup> integrated within Maestro.<sup>44</sup> The preparation protocol included preprocessing steps such as bond order assignment and structural adjustments carried out using Prime.<sup>47–49</sup> Protonation and metal charge states for cofactors and metals at pH  $7 \pm 2$  were generated using Epik.<sup>50,51</sup> The hydrogen-bonding network was optimized, and residue protonation states at pH 7 were calculated using PROPKA.<sup>52</sup> Water molecules beyond a 5 Å radius from protein residues were excluded, and a final restrained minimization was performed using the OPLS4 force field.
- *Ligand Docking.* The centroid of the crystallized ligand in the catalytic pocket was used as the grid center. During grid generation, a van der Waals radius scaling factor of 1.0 and a partial charge cutoff of 0.25 were applied. Docking was performed using the Glide extra precision (XP) mode available in the Schrödinger software suite,<sup>53–57</sup> without applying any constraints. Default parameters were employed for ligand setting,

including flexible ligand sampling and the incorporation of Epik state penalties into the docking score. The final step involved post-docking minimization using default settings.

- *Docking Validation Protocol.* To validate the docking protocol for DYRK1A using the Glide program, we redocked the ligand B5T (XMD7-117) into the binding site of the crystal structure 6EIF.

## RESULTS

This section provides an in-depth analysis of the performance of the molecular generation models. The evaluation includes the metrics described in Section 3, designed to provide a comprehensive view of each model’s capabilities. Taken together, these metrics provide insights about the ability of the models to generate structurally innovative and diverse molecules that may be potential therapeutic agents. The results are critical to understanding the current state of the art in molecule generation algorithms and highlight the strengths and weaknesses of each model.

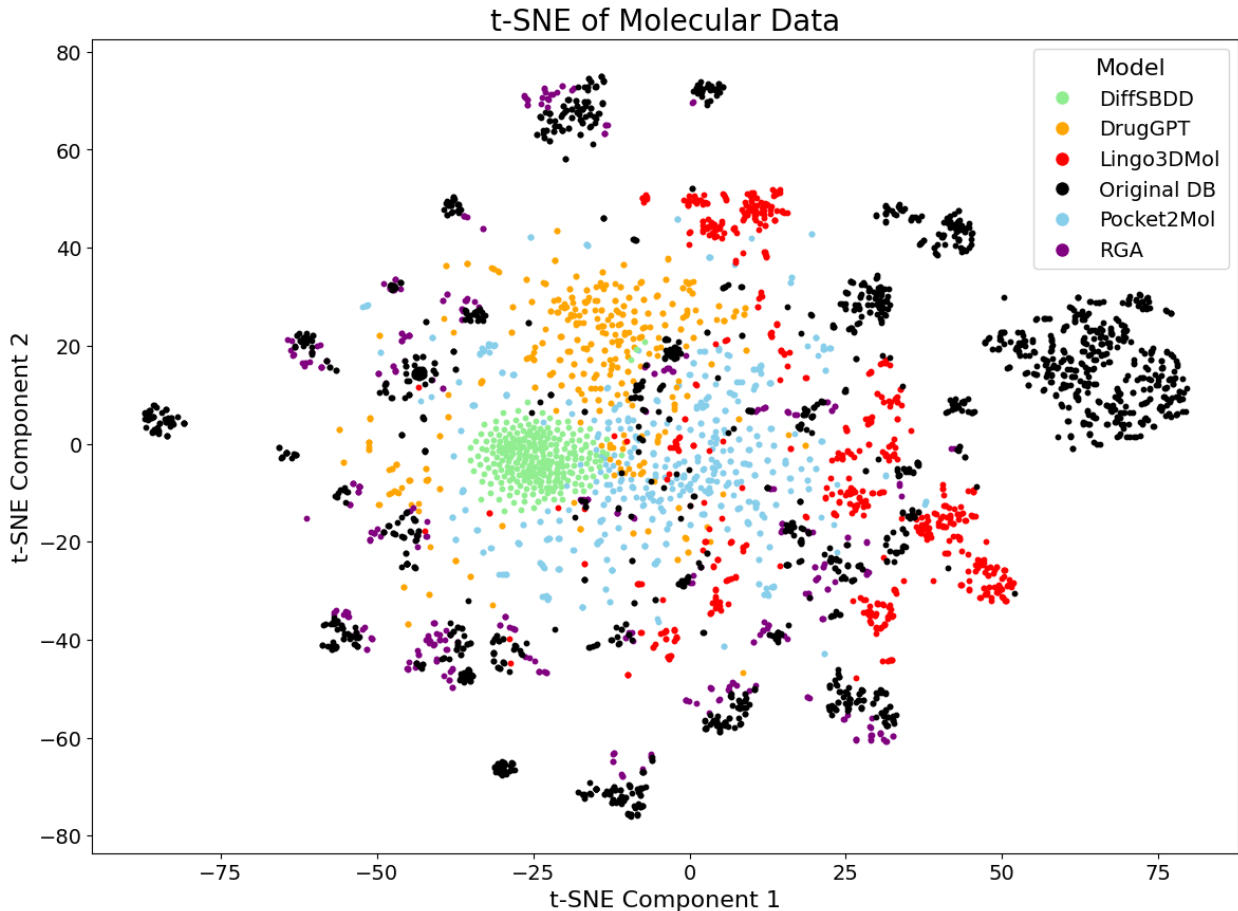
First, it is important to understand whether these models are exploring different parts of the chemical space, or whether they are focusing on a narrow part. Figure 4 displays a t-distributed Stochastic Neighbour Embedding (t-SNE)<sup>58</sup> plot to illustrate the molecular diversity generated by different computational models to help answer this question. Each point in the plot represents a molecule, and the proximity of the points indicates their structural similarity.

The distinct clustering of points suggests that each model has a unique signature in terms of molecular generation, with some models, as indicated by the concentrated clusters, producing molecules with higher structural homogeneity. In contrast, the more dispersed clusters suggest models that generate a more structurally diverse set of molecules. The presence of distinct and well-defined clusters also implies that certain models may specialize in particular regions of the chemical space, potentially aligning with specialized drug discovery

objectives. The original molecules are marked separately, serving as a baseline reference for the diversity introduced by each model. Overall, this figure highlights the importance of diversity in molecular design and the capacity of different models to explore the vast chemical space.

Thus, the RGA model produces molecules grouped in a molecular space quite similar to the original database. This is expected since the genetic algorithm forms molecules by joining common molecular substructures given an initial population of molecules.

Similarly, the DIFSBDD molecules are grouped in a very specific chemical space, similar to those of DrugGPT. On the other hand, both Pocket2Mol and Lingo3DMol molecules achieve greater molecular diversity by exploring different areas of the chemical space that are distant from the molecules in the original database.



**Figure 4.** Diversity comparison of proposed generative models.

Table 3 showcases the algorithmic performance through different evaluation metrics. The results underscore the efficacy of Pocket2Mol in docking score averages and top-ranking molecules, suggesting a superior fit for potential drug candidates within the target protein’s binding site. Meanwhile, RGA distinguishes itself in predicting binding affinities, hinting at its utility in identifying potential drug candidates. In addition, all the models except DiffSBDD and DrugGPT create molecules with a median logP between 1.5-3.5, which is essential in our case study, where the compounds have to penetrate the blood-brain barrier (BBB).<sup>59</sup> By its way, DrugGPT, Lingo3DMol, Pocket2Mol, and RGA design molecules with a QED higher than 0.5, so the *de-novo* design molecules are highly likely to become a drug candidate.<sup>60</sup> Looking at the SAS, DrugGPT excels among the others, so the molecules created by this algorithm are easier to synthesise.<sup>61</sup> Finally, all the models create molecules with a reasonable molecular weight.

**Table 3. Model performance evaluation**

	DiffSBDD	DrugGPT	Lingo3DMol	Pocket2Mol	RGA
Docking (Mean ↓) <sup>a</sup>	-6.77	-8.72	-8.71	<b>-9.21</b>	-8.53
Docking Top1 (↓)	-9.2	-10.80	-11.30	<b>-12.51</b>	-11.2
Docking Top10 (↓)	-8.77	-10.47	-10.64	<b>-12.08</b>	-10.61
pChEMBL (Mean ↑) <sup>b</sup>	5.87	5.99	6.08	5.96	<b>6.18</b>
pChEMBL Top1 (↑)	6.83	7.08	8.03	7.13	<b>9.01</b>
pChEMBL Top10 (↑)	6.59	6.86	7.69	6.89	<b>8.22</b>
logP (Mean 1.5-3.5) <sup>c</sup>	0.43	3.98	<b>2.46</b>	<b>2.68</b>	<b>2.80</b>
QED (Mean ↑) <sup>b</sup>	0.48	0.53	0.65	<b>0.67</b>	0.62
SAS (Mean ↓) <sup>a</sup>	4.76	<b>2.57</b>	2.83	3.25	3.52
Validity (↑) <sup>b</sup>	<b>1</b>	<b>1</b>	0.99	<b>1</b>	0.85
Molecular Weight (180-480) <sup>c</sup>	<b>268.67</b>	<b>418.80</b>	<b>335.16</b>	<b>309.73</b>	<b>314.41</b>
Molecules evaluated	271	400	600	562	416

<sup>a</sup> Indicates better performance with lower values.

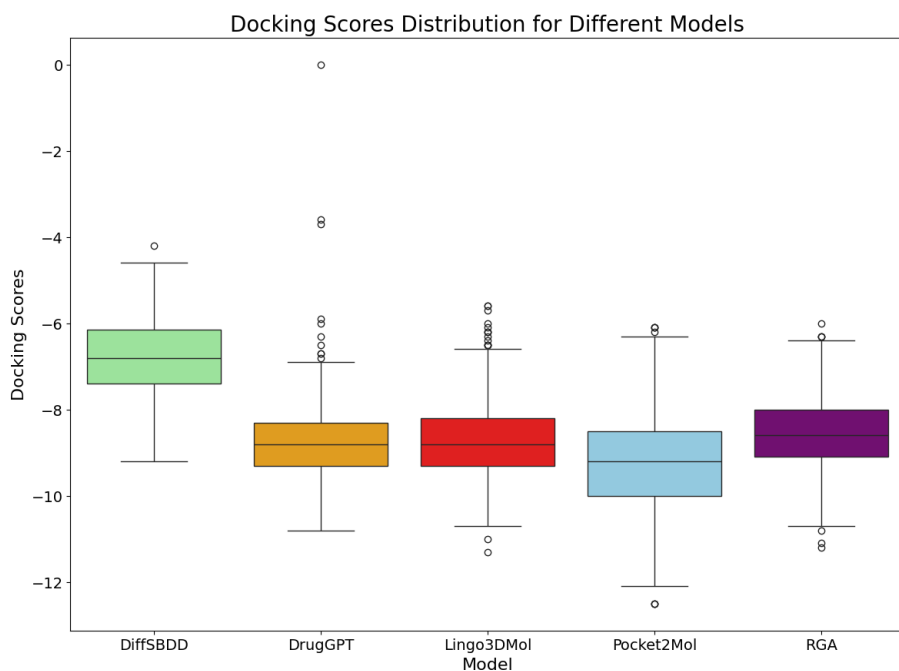
<sup>b</sup> Indicates better performance with higher values.

<sup>c</sup> Optimal range specified.

Figure 5 presents a visual comparison of the docking score distributions for the five molecular generation models. The median docking score, indicated by the horizontal line within each boxplot, serves as a robust indicator of the central tendency among the scores achieved by each model. To determine whether there were significant differences in docking scores

El anova asume normalidad en tus datos, lo cual no compruebas y es muy probable que no se cumpla. El Dunn se ejecuta tras el anova, y ese ya no asume una distribución de los datos, pero partes de resultados del propio anova. No sé si los revisores se fijarán en eso o no, pero si tras la revisión te dicen algo, el test de Kruskal Wallis es no paramétrico y no asume distribuciones. También existen tests en grupo en lugar de hacer múltiples anova por pares si te piden algo así.

among the five models, we conducted a one-way ANOVA test,<sup>62</sup> which indicated a significant difference ( $p < 0.001$ ). Given this, we performed Dunn’s post-hoc test with Bonferroni correction.<sup>63</sup> The results showed significant differences in docking scores between most pairs of models, except between DrugGPT and Lingo3DMol. Notably, Pocket2Mol achieves the most favourable median docking score, <sup>Queda rara esta frase</sup> suggestive of its adeptness in generating stable for <sup>which indicates its adequacy in generating stable compounds for the target protein pocket (o algo así?)</sup> the target protein pocket. The spread and range of scores, as denoted by the boxes and whiskers, also offer insights into the consistency and reliability of each model’s predictions. For instance, while RGA shows a comparatively tight distribution signalling consistency <sup>la mayor varianza no la tiene Pocket2Mol?</sup> in scoring, Lingo3DMol demonstrates a broader range, indicating greater variability in its docking score predictions. Outliers, represented by individual dots, highlight exceptional cases where molecules exhibit either particularly high or low docking scores compared to the typical range for the model output. Collectively, this graphical representation underscores the performance landscape of these models, guiding researchers to prudent selections.



**Figure 5.** <sup>Box-plot comparing the docking score of each model for the DYRK1A protein.</sup> Docking score comparison by Model.

The distribution of ring sizes across the generated molecules through the five models, as presented in Table 4, offers a glimpse about the structural diversity of each algorithm. The

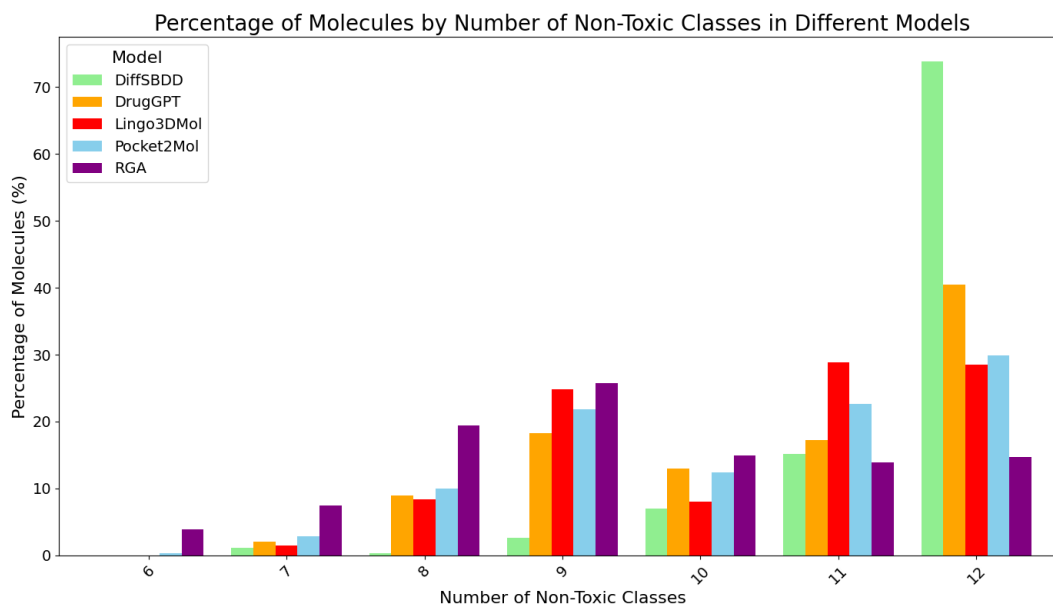


models display a varied propensity towards certain ring sizes, which could be indicative of their bias towards or against specific molecular frameworks. This diversity in ring sizes is essential in the exploration of the vast chemical space. The most common ring sizes are 5 and 6, with DiffSBDD exploring a wider range of ring sizes compared with the other models.

**Table 4. Ring size distribution**

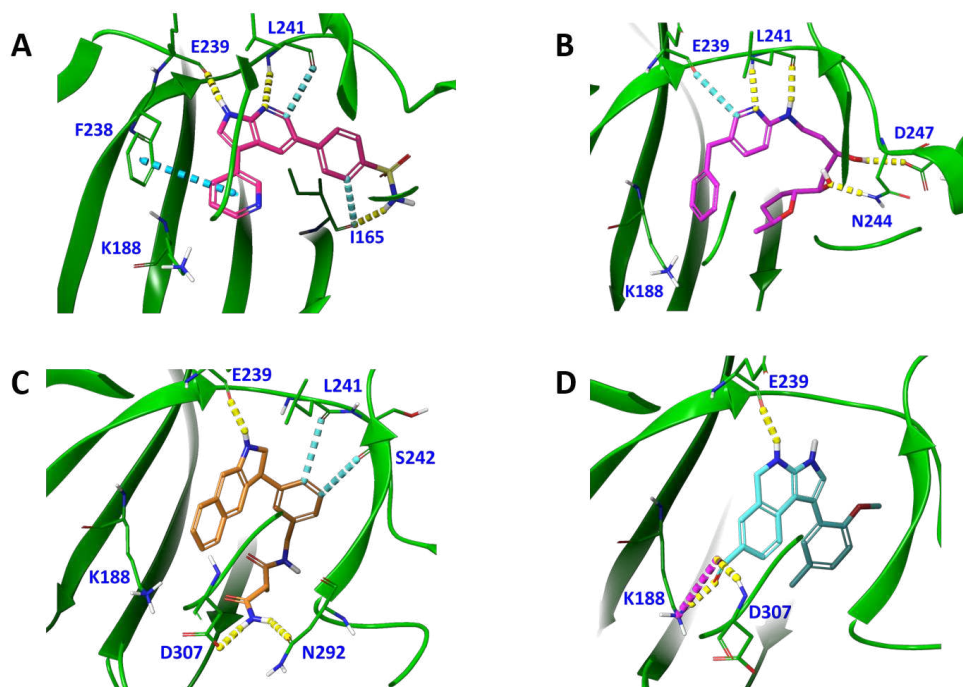
Ring Size	DiffSBDD	DrugGPT	Lingo3DMol	Pocket2Mol	RGA
3	36.71%	0.27%	0%	0.05%	1.36%
4	5.27%	0.27%	0.28%	0%	0.41%
5	21.94%	25.72%	36.44%	23.96%	31.45%
6	30.38%	72.61%	63.14%	72.21%	66.78%
7	4.22%	0.94%	0.14%	2.37%	0%
8	0.84%	0.07%	0%	0.33%	0%
9	0.42%	0%	0%	0.09%	0%
$\geq 10$	1.91%	0.20%	0%	2.15%	0%

Figure 6 illustrates the percentage of molecules across non-toxic classes for different models. Models like DiffSBDD and DrugGPT demonstrate a substantial percentage of molecules falling into non-toxic classes. These models are not directly trained to generate non-toxic medicines, but as they create molecules around a narrow part of the chemical space, the results suggest that these models have examined a region of the chemical space where less toxic molecules exist.



**Figure 6.** Toxicity comparison by Model.

After validation of docking protocol with the reference compounds B5T (XMD7-117) (RMSD value of 1.21 Å), <sup>a</sup> A virtual screening was made with the 186 molecules that had passed the filters mentioned above and presented good autodocking values. Of these 186 molecules, 5 of them presented higher docking scores than the crystallized ligand B5T (used as reference). The inhibitor B5T (XMD7-117) forms hydrogen bonds with Ile165, Glu239, Phe238, and Leu241 (Table 5, Panel A Figure 7).



**Figure 7.** Interactions of residues in the ATP binding site of DYRK1A structure (PDB 6EIF) with novel inhibitors. Panel A: B5T (reference), Panel B: Compound I, Panel C: Compound II, Panel D: Compound III

Remarkably, interactions with catalytic residues Glu239 and Leu241 are also consistently observed in the top five molecules according to the best docking scores, suggesting their crucial role in ligand binding typical for the other inhibitors.<sup>32</sup> Compounds I, IV and V exhibited a pose very similar. On the other hand, compounds B57, I, II, IV and V do not interact with the catalytic lysine Lys188 (Figure 7, Panels A, B and C). However, they possess a terminal arene oriented towards this residue. In contrast, compound III forms a hydrogen bond and a salt bridge with Lys188 (Figure 7, Panel D).

**Table 5. Interactions of the top five molecules and control with the protein DYRK1A (PDB: 6EIF)**

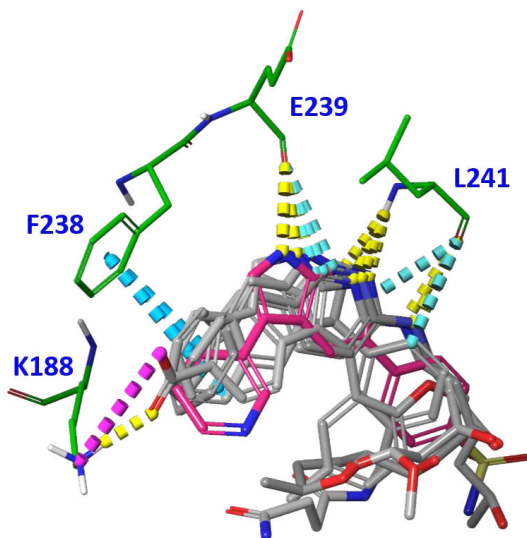
	Ile165	Lys188	Phe238	Glu239	Leu241	Ser242	Asn244	Asp247	Asn292	Asp307	Docking Score (kcal/mol)
<b>B5T</b>	AHB <sup>a</sup> HB <sup>b</sup>		PP <sup>c</sup>	HB	AHB HB						-11.413
<b>I</b>				AHB	2xHB		HB	HB			-13.229
<b>II</b>				HB	AHB	AHB			HB	HB	-12.108
<b>III</b>		HB SB <sup>d</sup>		HB						HB	-12.093
<b>IV</b>			PP	AHB	2xHB			2xHB			-11.792
<b>V</b>				AHB	2xHB	PP		HB			-11.520

<sup>a</sup> AHB: Aromatic Hydrogen Bond; <sup>b</sup> HB: Hydrogen Bond; <sup>c</sup> PP: Pi-Pi stacking; <sup>d</sup> SB: Salt Bridge.

The conserved hinge motif found in protein kinases, characterized by two solvent-exposed carbonyl groups and one exposed backbone amide, is widely recognized for its role in forming traditional hydrogen bonds with inhibitors.<sup>64</sup> The hinge region consists of three amino acids, identified according to their positions in the sequence downstream of the "gatekeeper" residue as gk+1, gk+2, and gk+3. The adenine moiety of ATP interacts with this hinge region through specific hydrogen bonds. The N10 amino group of adenine establishes a hydrogen bond with the main chain carbonyl group of gk+1, where the carbonyl group acts as the hydrogen acceptor. Concurrently, the N1 nitrogen of adenine forms a hydrogen bond with the main chain amide of gk+3, with the amide group serving as the hydrogen donor. Additionally, there is a third, noncanonical hydrogen bond in this interaction network, where the C2-H group of adenine donates a proton to the main chain carbonyl of gk+3, acting as the hydrogen acceptor. Similarly, the crystallized inhibitor B5T and the novel inhibitors I, IV, and V exhibit interactions with the hinge backbone (Figure 8). The hydrogen bonds with residues forming the hinge motif in these compounds involve the same interactions as those in the adenine moiety of ATP within the ATP-binding pocket, particularly with gk+1 (Glu239) and gk+3 (Leu241) (Figure 8). The identification of both canonical and noncanonical hydrogen interactions aligns with the way inhibitor scaffolds mimic adenine's interaction with the hinge. These scaffolds incorporate hydrogen bond donors and acceptors that engage

with the carbonyl groups of gk+1 and gk+3.<sup>65,66</sup>

Our pipeline has identified that hinge backbone interactions are relevant in the design of hinge-binding scaffolds for novel kinase inhibitors intended for therapeutic use. By incorporating these interactions, our model enhances the potential efficacy and specificity of these inhibitors, ensuring they effectively target the desired kinase while minimizing off-target effects.



**Figure 8.** Superposition of the control molecule (magenta) and the top five candidates (grey) showing hydrogen bonding interactions with the hinge backbone residues gk+1 (E239) and gk+3 (L241), which are crucial for protein function

The application of generative algorithms has successfully produced a valid set of DYRK1A inhibitors exhibiting desirable drug-like properties. Notably, among these generated compounds, five demonstrate score docking values superior to the reference compound, indicating potentially enhanced efficacy. These five compounds were generated by the Pocket2Mol algorithm, which according to our analysis, excels among the others in generating novel ligands given a protein pocket. Moreover, these top-performing inhibitors display key interaction characteristics, suggesting their robust binding affinity and therapeutic potential against DYRK1A. This advancement underscores the capability of generative algorithms to expedite and refine the drug discovery process, yielding promising candidates for further

development.

## CONCLUSIONS

Al está introducido al principio, pero no usas el acrónimo

The use of artificial intelligence tools like diffusion models, genetic algorithms, or transformers in designing molecules has gained significant attention in recent years due to their ability to generate new molecules related to a given protein. This article has provided an overview of primary pocket drug design models. It also evaluates the performance of five models in generating molecules against the DYRK1A protein, an enzyme associated with AD. The models evaluated for their ability to generate protein-related molecules included DiffSBDD, DrugGPT, Lingo3DMol, Pocket2Mol and RGA. However, there are many more models under development. These algorithms proved to be highly effective in developing new molecules and exploring new areas of the vast chemical space.

In addition, a metrics framework has been proposed to assess the performance of protein pocket-based drug design models. This framework is particularly useful as the lack of experimental evaluation is one of the major challenges in the field. It is therefore important to have a filter-based framework to ensure that molecules are screened for suitability before chemists evaluate them in the laboratory. The code used to build this evaluation framework has been made available. Furthermore, as running these models can be complicated, a simple code is supplied so that anyone can generate new molecules given the PDB of a protein and the coordinates of its protein pocket.

In summary, the algorithms generated new molecules that had a strong attraction to the DYRK1A protein. These virtual docking results were later confirmed using traditional docking tools, which showed that five molecules had higher docking values than the crystallographic ligand B5T of chain A of the DYRK1A protein. Among the five algorithms discussed in the article, Pocket2Mol appears to be the most promising, outperforming the others in most of the tasks.

## ASSOCIATED CONTENT

### Data Availability Statement

The DYRK1A crystal structure is retrievable from the Protein Data Bank

<https://www.rcsb.org/structure/6eif>. The GitHub repository containing the source code and tools used in this article can be accessed at <https://github.com/pvaras8/pocketdrugdesign>.

### Supporting Information


The Supporting Information is available free of charge at .

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### Notes

The authors declare no competing financial interest(s)

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