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Expanding the Boundaries of the Chemical Space of DNA Methyltransferase 1 Modulators

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Abstract

DNA methyltransferase 1 (DNMT1), one of the main epigenetic targets, is involved in the duplication of the DNA methylation pattern during replication, and it is essential for proper mammalian development. Small molecule DNMT1 modulators are attractive for biochemical epigenetic studies and have the potential to become drugs. So far, more than five hundred small molecules have been reported as DNMT1 inhibitors. However, only a limited number of DNMT1 activators have been disclosed because, at least in part, DNMT1 activators are typically regarded as negative data in virtual screening campaigns or optimization projects. This manuscript aims to report the chemical structures and biological activity of small molecules that increase the enzymatic activity of DNMT1. Results of the biochemical experimental assays are discussed. It was found that small molecule activators have a large variety of chemical scaffolds but share pharmacophore features. Visual analysis of the chemical space and multiverse based on molecular fingertips supported that activators are structurally diverse. This is the first report of eight small molecules that increase the enzymatic activity of DNMT1 by more than 400% in an enzymatic-based assay. The outcome warrants further investigation of the epigenetic activity of the compounds in a counter-screen assay, e.g., cell-based and *in vivo* context.

Keywords

Chemical Multiverse, Chemoinformatics, Epigenetics, Pharmacophore Hypothesis, Small Molecules

1. Introduction

Epigenetic drug discovery is a promising strategy for treating cancer and other

complex diseases. Over the past twenty years, several small molecules with novel chemical scaffolds have been investigated with high affinity and selectivity against specific epigenetic targets [1]. DNA methyltransferases (DNMTs) are amongst the clinically validated epigenetic targets. The DNMT enzyme family includes two de novo methyltransferases, DNMT3A and DNMT3B, and the maintenance methyltransferase, DNMT1, the most abundant. DNMT1 duplicates the pattern of DNA methylation during replication, which is essential for proper mammalian development. Since DNA methylation is a key epigenetic mechanism for gene regulation, developing inhibitors of DNMTs represents promising perspectives for new therapies. DNMT inhibitors are well-known, and there are more than seven hundred compounds with reported activity as inhibitors of DNMT1 in enzymatic inhibition assays as reported in ChEMBL, version 33 [2]. Among the DNMT1 inhibitors (representative compounds are shown in **Figure S1** in the Supplementary Material [3]), azacitidine and 5-aza-decitabine (**Figure S1**) stand out as they are nucleoside inhibitors, approved for clinical use for the treatment of myelodysplastic syndrome [4]. However, DNMT1 activators in enzymatic assays are poorly investigated, and few compounds are known [5]. Just recently, Rodriguez-Mejia *et al.* disclosed two activators that are hybrids of N-phthalyl-L-tryptophan (RG108), a well-known inhibitor of DNMT1 [5], and a tetrapeptide and heptapeptide (**Figure 1(a)**). DNMT1 activators have the potential to serve as pharmacological agents for the amelioration of conditions characterized by DNA hypomethylation [6]. Conversely, DNMT1 activators also offer a means to discern the intricate physiological consequences induced by DNMT1 enzyme activation. To illustrate, instances arise, such as in carcinogenesis, wherein a comprehensive reduction in DNA methylation accompanies the cellular transformation process. In essence, the significance of hypomethylation lies in its propensity to instigate genomic instability, thereby potentially contributing to the evolutionary progression of malignancies [6].

This communication discusses small molecules that activate the enzymatic activity of DNMT1 and highlight common pharmacophoric features. We also analyzed differences between potent nucleoside DNMT1 inhibitors and activators using chemoinformatics approaches. The outcome of this study paves the way for further investigation and development of activators of the enzymatic activity of DNMT1.

2. Methods

2.1. Compound Selection and Similarity Searching

In a previous virtual screening campaign searching for DNMT1 inhibitors, eleven molecules that activate the catalytic activity were found and reported in a thesis dissertation [7]. Such results were not disclosed before in a peer-reviewed publication because they were initially regarded as negative data of a virtual screening effort. However, there is an increasing awareness of the significance of disclosing initially non-interesting compounds, aka negative data [7].

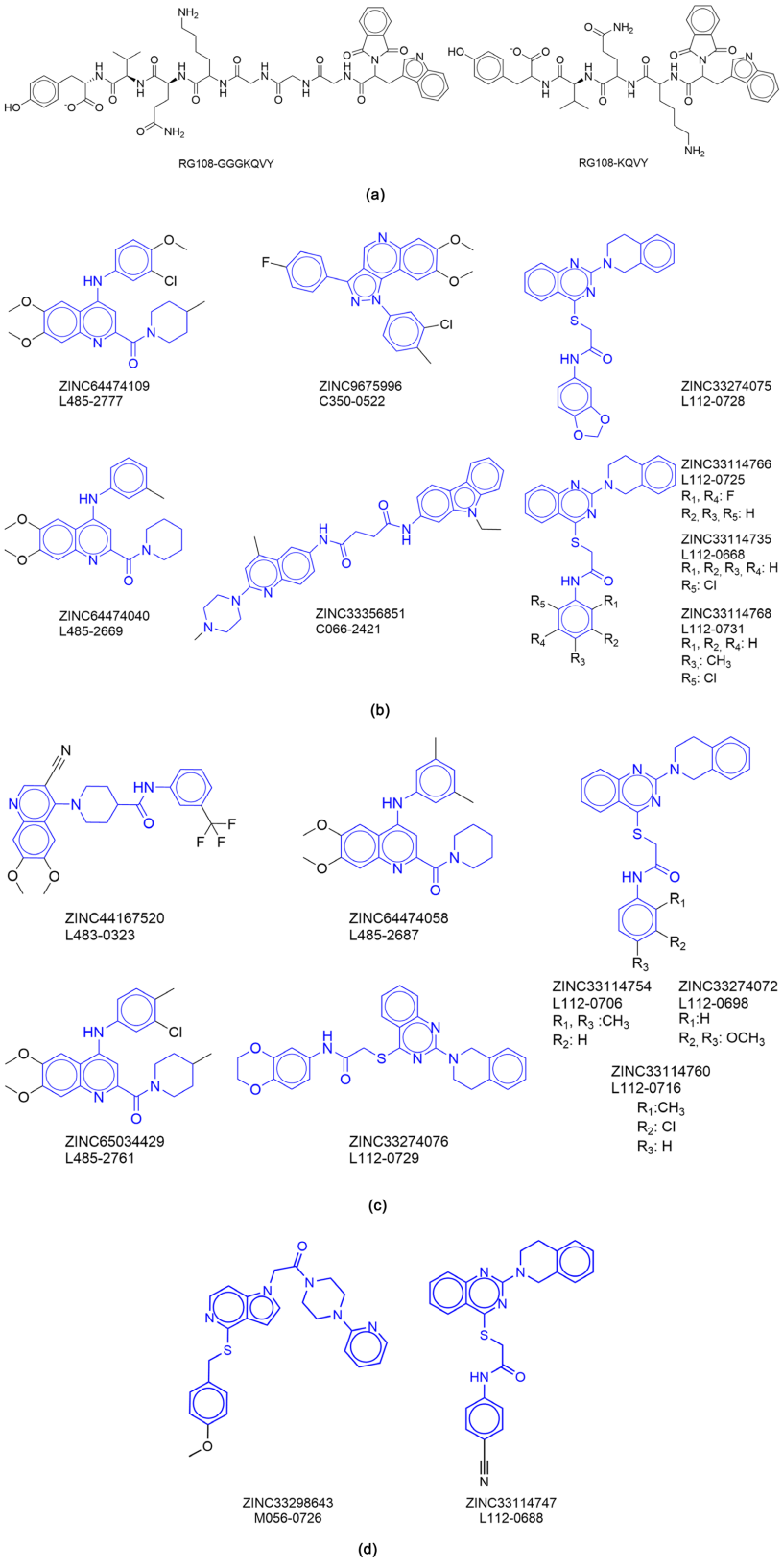

Figure 1. Chemical structures of activators of the enzymatic activity of DNMT1. (a) RG108 inhibitory molecule with peptides caused enzymatic activation of DNMT1. Compounds with an enzymatic activation (b) Greater than 400%; (c) Greater than 150%; and (d) Greater than 100%. Each compound's core scaffold (Bemis and Murcko) is highlighted.

We implemented a ligand-based approach to identify additional DNMT1 activators. We did not conduct a structure-based approach because the mechanism of enzymatic activation of the small molecules at the structural level is not known. The ligand-based approach was a fingerprint-based similarity search of ChemDiv, a commercial screening library taking as a reference eight known DNMT1 activators, namely, **RG108-GGGKQVY, RG108-KQVY**, **L112-0668**, **L112-0728**, **L485-2669**, **L483-0323**, **L485-2761**, and **M056-0726** (**Figure 1**) most of them previously uncovered in a virtual screening intended to find inhibitors (vide supra) [7]. At the time of the study, ChemDiv contained 220,000 compounds (https://www.chemdiv.com/; accessed March, 2023). The similarity search was done with two fingerprints of different designs: dictionary (MACCS Keys, 166-bits) and topological (Extended Connectivity Fingerprint, Radius 6, ECFP6) [8]. Similarity searching hits were selected from ChemDiv if they had a Tanimoto similarity [9] value equal to or above 0.80 (with MACCS keys) or equal to or above 0.50 (with ECFP6). For ECFP6, we considered a similarity value lower than MACCS keys because of the higher resolution of the former [10]). **Figure S2** in the Supplementary Material illustrates the general approach to identifying the DNMT1 enzyme activators reported in this study.

2.2. Enzymatic DNMT1 Activity Assay

Selected computational hits were purchased from ChemDiv and experimentally tested at the Reaction Biology Company in an enzymatic activity methyltransferase assay using the HotSpotSM platform [11]. Our group has reported the methodology and results of this biochemical assay, including the identification of 7-amino alkoxy-quinazolines [3]. Briefly, HotSpotSM is a low-volume radioisotope-based assay that employs tritium-labeled AdoMet -3H-SAM- as a methyl donor. The test compounds diluted in dimethyl sulfoxide were added using acoustic technology (Echo550, Labcyte, San Jose, CA, USA) into an enzyme/substrate mixture in the nano-liter range. The reactions were started by adding tritium-labeled AdoMet and incubated at 30˚C. Total final methylations on the substrate (Poly dI-dC) were identified by a filter binding method implemented in Reaction Biology. Data analysis was conducted using Graphed Prism software (La Jolla, CA, USA) for curve fit. The enzymatic inhibition assays were carried out with the DNMT1 enzyme at a concentration of 25 nM, 1 μM of S-adenosyl methionine, SAM. The standard positive control was S-adenosylhomocysteine, SAH. The compounds were tested in 10-concentration IC50 (effective concentration to inhibit enzymatic activity by 50%) with a threefold serial dilution starting at 100 μM.

2.3. Pharmacophore Hypothesis

Five activators of the enzymatic activity of DNMT1 were used to build a pharmacophore hypothesis: **L112-0668**, **L112-0728**, **L485-2669**, **L112-0731**, and **RG108-KQVY** (**Figure 1**). These compounds were classified as high activators for their pronounced activation potential (higher than 150%) for the initial four

compounds. Furthermore, RG108-KQVY was ascribed the classification of "highly activating." Three nucleotide molecules were used to perform the pharmacophore model of the inhibitory compounds: decitabine, zebularine, and azacitidine. Another pharmacophore model was constructed using the experimentally confirmed hit molecules and queries that had greater than 400%: **C066-2421**, **C350-0522**, **L112-0668**, **L112-0725**, **L112-0728**, **L112-0731**, **L485-2669**, and **L485-2777**. The pharmacophore models were constructed using the Molecular Operating Environment (MOE) 2022.02 program with the pharmacophore elucidator and consensus function. The default settings were employed.

3. Results and Discussion

3.1. Small Molecule Activators of the Enzymatic Activity of DNMT1

Table 1 summarizes the results of the similarity-based virtual screening of ChemDiv, including the percentage of enzymatic activity of DNMT1. The table also summarizes the results of the enzymatic activity of the compounds previously identified as activators during a docking-based virtual screening to identify inhibitors (vide supra) [7]. Table S1 in the Supplementary Material shows the results of the similarity values for each of the six hit compounds from the similarity searching.

Results of the similarity searching indicated that six out of the nine molecules selected from ChemDiv activated the enzymatic activity of DNMT1 in a biochemical assay. The molecules that turned out to be inactive, despite having a high Tanimoto coefficient value based on MACCS keys fingerprints (0.87 - 0.92), had low Tanimoto coefficient values based on ECFP6 fingerprint (0.16 - 0.24). This suggests that the ECFP6 fingerprint has a greater impact on assessing molecular similarity for virtual screening applications. Indeed, in previous benchmark studies, it has been noticed the efficiency of circular fingerprints such as ECFP6 [12]. This could be because ECFP6 fingerprints consider not only the presence or absence of chemical groups, as MACCS keys [8], but also the connections between them, providing a more certain measure of similarity between molecules [13]. It is also why the similarity values computed with ECFP are generally lower than those computed with MACCS keys (166 bits), as they evaluate the entire molecule, including the connectivity of its chemical groups [13].

The difference in activation percentage between the activators ranged from 191% to 672%. This depended on the molecule's activation percentage, to which they had the highest similarity. It is important to mention that the molecules that had more activation percentage were those that had similarity with the molecules published by Martínez-Fernández [7], the molecules that were considered as references for this virtual screening were the ones that are mentioned in Section 2.3. The molecules were classified in three categories based on their activation percentage: "activators" were those with over 100% activation, "high activators" greater than 150%, and "strong activators" with an activation

Table 1. Summary of the DNMT1 enzymatic activity of small molecules.

Strategy	Compound ID	% Activity (std dev)[a]
Docking-based virtual screening initially target to find inhibitors	ZINC9675996/C350-0522[e]	558.13 (16.26)
	ZINC33356851/C066-2421[e]	424.54 (55)
	ZINC33114735/L112-0668[b,c,e]	519.25 (163.12)
	ZINC33114747/L112-0688	121.28 (11.47)
	ZINC33114766/L112-0725[e]	525.03 (87.55)
	ZINC33274075/L112-0728[b,c,e]	467.42 (27.19)
	ZINC33114768/L112-0731[b,c,e]	509.10 (11.24)
	ZINC44167520/L483-0323[c]	230.74 (0.14)
	ZINC64474040/L485-2669[b,c,e]	442.42 (37.77)
	ZINC65034429/L485-2761[c]	151.49 (9.85)
	ZINC33298643/M056-0726[c]	131.66 (0.94)
Similarity	ZINC33274076/L112-0729[d]	191.42 (31.99)
	ZINC64474058/L485-2687[d]	243.27 (66.66)
	ZINC64474109/L485-2777[d]	672.64 (88.83)
	ZINC33114760/L112-0716[d]	221.10 (12.35)
	ZINC33114754/L112-0706[d]	250.28 (12.49)
	ZINC33274072/L112-0698[d]	334.73 (12.88)
	ZINC21146903/3909-9992[d]	95.85 (0.29)
	ZINC20103984/C066-0242[d]	94.04 (6.09)
	ZINC34921986/V011-9764[d]	71.16 (2.06)

[a]The averages of the DNMT1 enzyme activation values are shown in units of percentage and the standard deviation of their measurements. SAH was used as a positive control. [b]Compounds used to construct the pharmacophoric hypothesis. [c]Compounds considered as queries in the similarity searching. [d]Molecules from ChemDiv identified from similarity searching of reference compounds using the Tanimoto coefficient and ECFP6 and MACCS keys (166-bits) fingerprints. The results of the similarity searching are summarized in **Table S1**. [e]Molecules used for the pharmacophore hypothesis with the high activators molecules.

percentage over 400%. **Table S2** in the Supplementary Material summarizes the percentage of DNMT1 of selected hit compounds at different concentrations. Results indicated that, for some compounds such as **L112-0729**, **L112-0716**, **L112-0706**, and **L112-0698**, the activity was higher at lower compound concentrations. The overall increase in enzymatic activity at lower concentrations followed by a decrease in activity at even lower concentrations seem to be associated with the so-called biphasic response, wherein an automatic reduction or decrease in response occurs at high concentrations. The biphasic response results in an optimal range of stimulation for downstream effects [14]. Biphasic

responses in signaling are frequently sought after, as they can act as a safeguard against excessive activation, overexpression, and the dominance of mutants [14]. In any case, it is remarkable that **L485-2777** increases the activity of DNMT1 up to 627% at 11 μM. The activity of **L112-0729** is also notable: 275% of activation at 11 μM. **L112-0716**, **L112-0706**, and **L112-0698** increased the activity of DNMT1 between 261% - 393% at 5.6 μM (**Table S2**).

Figure 1(b) and **Figure 1(c)** shows the chemical structures of the activators, highlighting in blue the core (Bemis-Murcko) scaffolds [15]. The Figure indicates that a scaffold is common to all three categories of molecules according to the classification based on activity (*vide supra*). For example, the molecules with higher activity **L485-2777** (672%) and the molecule **L485-2761** (151.49%) share the same scaffold; however, the only difference between them lies in their substituent groups. **L485-2777** has a methoxy group and a chloride, whereas **L485-2761** has a methyl group and a chloride. Similarly, the molecules **L112-0725**, **L112-0668**, **L112-0728, L112-0731, L112-0706, L112-0698, L112-0716, L112-0688** with activity percentages ranging from 121% to 525% share the same scaffold but have different substituents, suggesting that it is the substituents that make the difference in activation capacity due to their electronegativity, and their donor or acceptor capacity, among other factors [16] [17]. In contrast, the inhibitory compounds (**Figure S1**) and activators do not share scaffolds.

3.2. Pharmacophore Hypothesis

Figure 2 shows the preliminary pharmacophoric points of DNMT1 modulators, including activators (**Figure 2(a)**), strong activators (**Figure 2(b)**), and nucleoside inhibitors (**Figure 2(c)**). The figure shows reference activator compounds **L112-0668** (**Figure 2(a)**) and **L485-2777** (**Figure 2(b)**), and the DNMT1 inhibitor azacitidine. The figure highlights the principal difference between the pharmacophore models of the activators and nucleoside inhibitors: the activators have more aromatic/hydrophobic groups, one donor group but in different positions. Something that the inhibitors and activators have in common is that both of them have two acceptor groups in the ring at the same position. Furthermore, the highly activating compounds share only one hydrophobic group with a hydrogen acceptor group with the inhibitors, while they share four hydrophobic/aromatic groups and only one hydrogen acceptor group with the activators. One hypothesis of the differences and similarities between these two models is based on the binding site. According to **Figure 1** and **Figure 2**, and **Figure S1** in the Supplementary Material, most of the activating compounds are larger than the nucleoside inhibitors, and if they have different binding sites, they have different groups because they need other interactions with amino acids found in that cavity. This hypothesis will be explored with a structure-based pharmacophore model [18] [19]. Of note, a structure-based pharmacophore model of activators of DNMT1 is yet to be explored since the mechanism of activation is not yet understood at the molecular level.

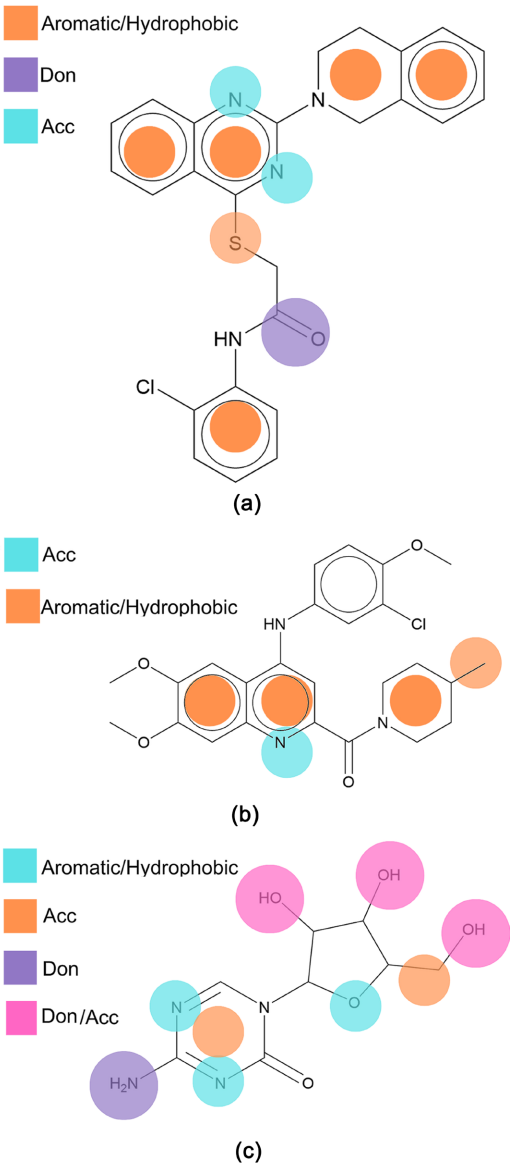

Figure 2. Potential pharmacophoric points of DNMT1 modulators. (a) Ligand-based hypothesis of activators based on the structures of L112-0668, L112-0728, L485-2669, L112-0731, and RG108-KQVY (compound shown: L112-0668). (b) Ligand-based hypothesis of high activators based on the structures of C066-2421, C350-0522, L112-0668, L112-0725, L112-728, L112-0731, L485-2669, L485-277 (compound shown: L485-2777). (c) Ligand-based model of nucleoside inhibitors derived from the structures of azacitidine, decitabine, and zebularine (compound shown: azacitidine).

3.3. Visualization of the Chemical Multiverse

A chemical multiverse is a group of multiple chemical spaces, each one defined by a given set of descriptors [20]. Therefore, multiple chemical representations provide a more comprehensive view of the coverage of the chemical space of compound sets. The chemical multiverse has been used to analyze the relationship and diversity of compound datasets [21]. The chemical multiverse of DNMT1 modulators is shown in **Figure 3**, described by ECFP6, MACCS Keys

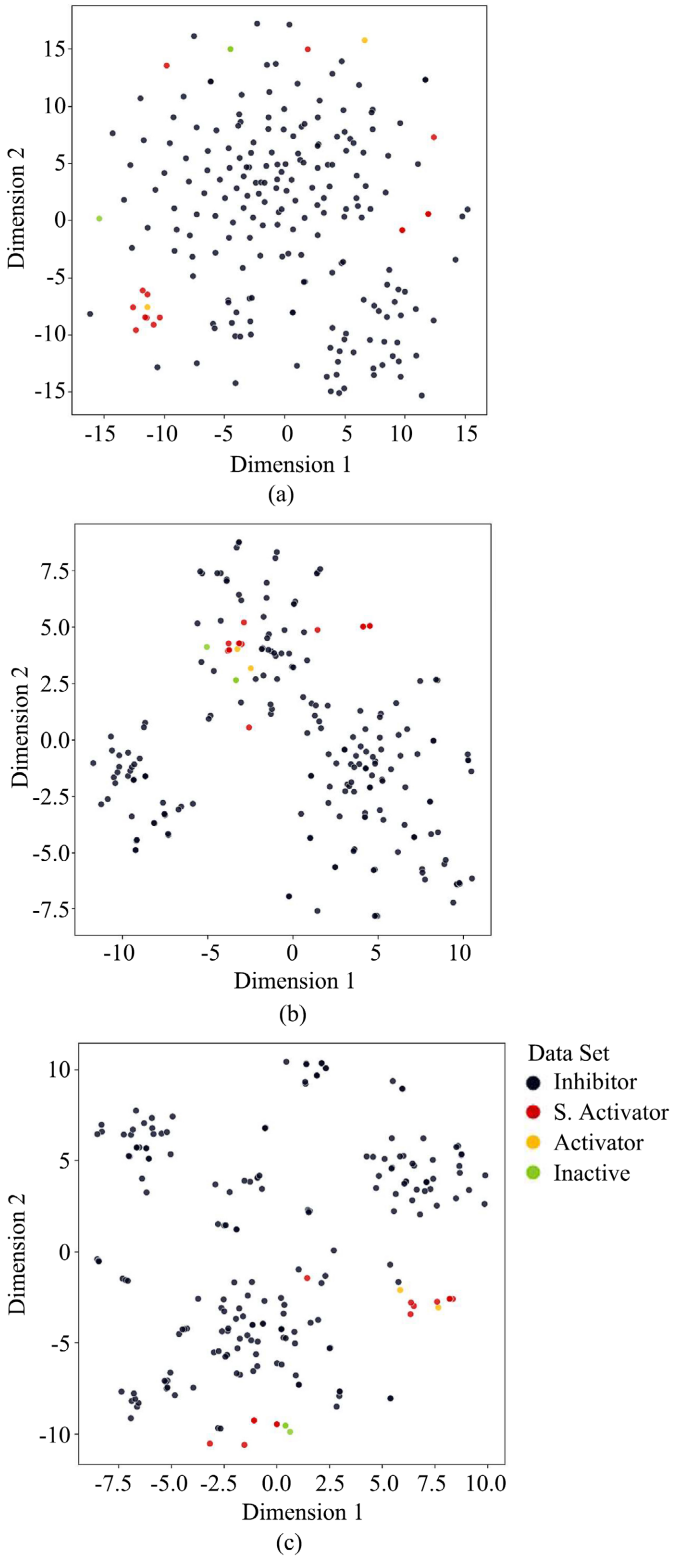

Figure 3. Visual representation of the chemical multiverse of modulators of the enzymatic activity of DNMT1 (15 strong activators in red; two activators in yellow; 197 inhibitors (from ChEMBL in purple; and three inactive compounds in green). The visual representation was done with tSNE based on the (a) ECFP6, (b) Ph2D, and (c) MACCS keys (166 bits) fingerprints.

(166 bits), and 2D pharmacophoric fingerprints (Fp2D). The visualization method was tSNE. The inhibitors used for the multiverse are nucleoside and non-nucleoside, shown in **Figure S1** in the Supplementary Material [22]. The visual representation of the chemical multiverse indicated that activators and inhibitors share common structural features among themselves. The characteristics in common are atom groups, the connectivity between the atom groups, and pharmacophoric features. This is why, within the chemical space described by various fingerprints, activating molecules arrange into distinct clusters within the groups formed by DNMT1 inhibitors.

3.4. Conclusions and Perspectives

Compounds with the ability to increase the enzymatic activity of DNMT1 have been disclosed on a limited basis. Since in most previous virtual screenings and optimization campaigns, the inhibitors are pursued, the activators might be considered as negative results or non-interesting results. Recently, another research group disclosed two compounds (hybrids, small molecules-peptides) that, in a serendipitous finding, could activate the activity of DNMT1. Herein, we discuss a total of seventeen small molecules with various levels of activation of enzymatic activity. The most potent activator of the enzymatic activity of DNMT1 was L485-2777 (623% enzyme activation at 11 μM of compound concentration). All compounds were tested in a biochemical assay for DNMT1 activity under the same conditions. Analysis of the chemical multiverse indicated that the inactive, activators and inhibitors occupy distinct positions in the chemical space sharing, within each group, common atomic groups, connectivity, and pharmacophoric characteristics, as captured by the different fingerprints. A preliminary pharmacophore hypothesis indicated by the compounds employed in the model's construction, it is notable that both inhibitory and activator compounds share three pharmacophoric features: one aromatic group and two hydrogen-bond acceptors. Conversely, the pharmacophore model of the highly activating molecules shares four hydrophobic points and one hydrogen-bond acceptor group with the model of the activating molecules. When compared to the model of the inhibiting molecules, it only shares one aromatic group with one hydrogen-bond acceptor. Activator compounds contain a higher quantity of hydrophobic groups in their structure, while nucleosidic inhibitors contain a higher number of hydrogen bond acceptor and donor groups. These differences are partly due to the fact that activators and inhibitors do not share common scaffolds. Given this observation, we posit the hypothesis that activating molecules typically possess a larger molecular size when contrasted with nucleoside inhibitors. However, among the different groups of activators evaluated (activators > 100%, super activators > 150%, and strong activators > 400%), they share common core structures. The primary distinctions among them lie in their functional groups. Additionally, they share pharmacophoric features, exhibiting fewer pharmacophoric points as the molecules become more activating. Consequently, the binding sites

for these two categories of compounds are probably dissimilar. The mechanism of enzymatic activation of the small molecules at the structural level is not known. For that purpose, it is necessary to conduct a structure-based approach.

Perspective of this study is performing more replicates of the enzymatic assays to obtain a more reliable value of the percentage activation and the evaluation of the newly identified small molecules as activators of the enzymatic activity of other DNMTs (e.g., DNMT3A, 3B). Other perspective is to conduct cell-based assays. Examples of cell-based assays are the live-cell DNA methylation assays, immunofluorescence for DNA methylation [23], protein-compound interaction assays [24], flow cytometry assays [25], and global methylation profiling assays [26] [27].

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Conflicts of Interest

Authors declare that they do not have any conflict of interest related to this work.

References

[1] Ganesan, A., Arimondo, P.B., Rots, M.G., Jeronimo, C. and Berdasco, M. (2019) The Timeline of Epigenetic Drug Discovery: From Reality to Dreams. *Clinical Epigenetics*, **11**, Article No. 174. https://doi.org/10.1186/s13148-019-0776-0

[2] Target Report Card. https://www.ebi.ac.uk/chembl/target_report_card/CHEMBL1993/

[3] Medina-Franco, J.L., López-López, E. and Martínez-Fernández, L.P. (2022) 7-Aminoalkoxy-Quinazolines from Epigenetic Focused Libraries Are Potent and Selective Inhibitors of DNA Methyltransferase 1. *Molecules*, **27**, Article No. 2892. https://doi.org/10.3390/molecules27092892

[4] Almasri, J., Alkhateeb, H.B., Firwana, B., Sonbol, M.B., Damlaj, M., Wang, Z. and Al-Kali, A. (2018) A Systematic Review and Network Meta-Analysis Comparing Azacitidine and Decitabine for the Treatment of Myelodysplastic Syndrome. *Systematic Reviews*, **7**, Article No. 144. https://doi.org/10.1186/s13643-018-0805-7

[5] Rodríguez-Mejía, L.C., Romero-Estudillo, I., Rivillas-Acevedo, L.A., French-Pacheco, L., Silva-Martínez, G.A., Alvarado-Caudillo, Y. and Zaina, S. (2022) The DNA Methyltransferase Inhibitor RG108 Is Converted to Activator Following Conjugation with Short Peptides. *International Journal of Peptide Research and Therapeutics*, **28**, Article No. 79. https://doi.org/10.1007/s10989-022-10390-5

[6] Besselink, N., Keijer, J., Vermeulen, C., Boymans, S., de Ridder, J., van Hoeck, A. and Kuijk, E. (2023) The Genome-Wide Mutational Consequences of DNA Hypo-

methylation. *Scientific Reports*, **13**, Article No. 6874. https://doi.org/10.1038/s41598-023-33932-3

[7] Martínez-Fernández, L. (2023) Identificación de moduladores de DNA metiltransferasas de quimiotecas enfocadas [Identification of Modulators of DNA Methyltransferases with Focused Chemical Libraries]. Master's Dissertation, National Autonomous University of Mexico, Mexico.

[8] Kuwahara, H. and Gao, X. (2021) Analysis of the Effects of Related Fingerprints on Molecular Similarity Using an Eigenvalue Entropy Approach. *Journal of Cheminformatics*, **13**, Article No. 27. https://doi.org/10.1186/s13321-021-00506-2

[9] Bajusz, D., Rácz, A. and Héberger, K. (2015) Why Is Tanimoto Index an Appropriate Choice for Fingerprint-Based Similarity Calculations? *Journal of Cheminformatics*, **7**, Article No. 20. https://doi.org/10.1186/s13321-015-0069-3

[10] Maggiora, G., Vogt, M., Stumpfe, D. and Bajorath, J. (2014) Molecular Similarity in Medicinal Chemistry: Miniperspective. *Journal of Medicinal Chemistry*, **57**, 3186-3204. https://doi.org/10.1021/jm401411z

[11] Methyltransferase Assay Services|Reaction Biology. https://www.reactionbiology.com/services/target-specific-assays/epigenetic-assays/methyltransferase-assays

[12] Probst, D. and Reymond, J.-L. (2018) A Probabilistic Molecular Fingerprint for Big Data Settings. *Journal of Cheminformatics*, **10**, Article No. 66. https://doi.org/10.1186/s13321-018-0321-8

[13] Rogers, D. and Hahn, M. (2010) Extended-Connectivity Fingerprints. *Journal of Chemical Information and Modeling*, **50**, 742-754. https://doi.org/10.1021/ci100050t

[14] Suwanmajo, T. and Krishnan, J. (2013) Biphasic Responses in Multi-Site Phosphorylation Systems. *Journal of the Royal Society Interface*, **10**, Article ID: 20130742. https://doi.org/10.1098/rsif.2013.0742

[15] Bemis, G.W. and Murcko, M.A. (1996) The Properties of Known Drugs. 1. Molecular Frameworks. *Journal of Medicinal Chemistry*, **39**, 2887-2893. https://doi.org/10.1021/jm9602928

[16] Gupta, V.P. (2016) Characterization of Chemical Reactions. In: *Principles and Applications of Quantum Chemistry*, Elsevier, Amsterdam, 385-433. https://doi.org/10.1016/B978-0-12-803478-1.00012-1

[17] Ferreira de Freitas, R. and Schapira, M. (2017) A Systematic Analysis of Atomic Protein-Ligand Interactions in the PDB. *MedChemComm*, **8**, 1970-1981. https://doi.org/10.1039/C7MD00381A

[18] Muhammed, M.T. and Aki-Yalcin, E. (2021) Pharmacophore Modeling in Drug Discovery: Methodology and Current Status. *Journal of the Turkish Chemical Society Section A*: *Chemistry*, **8**, 749-762. https://doi.org/10.18596/jotcsa.927426

[19] Schaller, D., Šribar, D., Noonan, T., Deng, L., Nguyen, T.N., Pach, S. and Wolber, G. (2020) Next Generation 3D Pharmacophore Modeling. *Wiley Interdisciplinary Reviews*: *Computational Molecular Science*, **10**, e1468. https://doi.org/10.1002/wcms.1468

[20] Medina-Franco, J.L., Chávez-Hernández, A.L., López-López, E. and Saldívar-González, F.I. (2022) Chemical Multiverse: An Expanded View of Chemical Space. *Molecular Informatics*, **41**, Article ID: 2200116. https://doi.org/10.1002/minf.202200116

[21] Gómez-García, A., Jiménez, D.A.A., Zamora, W.J., Barazorda-Ccahuana, H.L.,

Chávez-Fumagalli, M.Á., Valli, M. and Medina-Franco, J.L. (2023) Navigating the Chemical Space and Chemical Multiverse of a Unified Latin American Natural Product Database: LANaPDB. *Pharmaceuticals*, **16**, Article No. 1388. https://doi.org/10.3390/ph16101388

[22] Fujiwara, Y., Ida, Y., Kanai, S., Kumagai, A. and Ueda, N. (2021) Fast Similarity Computation for t-SNE. 2021 *IEEE 37th International Conference on Data Engineering* (*ICDE*), Chania, 19-22 April 2021, 1691-1702. https://doi.org/10.1109/ICDE51399.2021.00149

[23] Yamagata, K. (2010) DNA Methylation Profiling Using Live-Cell Imaging. *Methods*, **52**, 259-266. https://doi.org/10.1016/j.ymeth.2010.04.008

[24] McFedries, A., Schwaid, A. and Saghatelian, A. (2013) Methods for the Elucidation of Protein-Small Molecule Interactions. *Chemistry & Biology*, **20**, 667-673. https://doi.org/10.1016/j.chembiol.2013.04.008

[25] McKinnon, K.M. (2018) Flow Cytometry: An Overview. *Current Protocols in Immunology*, **120**, 5.1.1-5.1.11. https://doi.org/10.1002/cpim.40

[26] Kurdyukov, S. and Bullock, M. (2016) DNA Methylation Analysis: Choosing the Right Method. *Biology* (*Basel*), **5**, Article No. 3. https://doi.org/10.3390/biology5010003

[27] Yong, W.S., Hsu, F.M. and Chen, P.Y. (2016) Profiling Genome-Wide DNA Methylation. *Epigenetics & Chromatin*, **9**, Article No. 26. https://doi.org/10.1186/s13072-016-0075-3

Supplementary Material

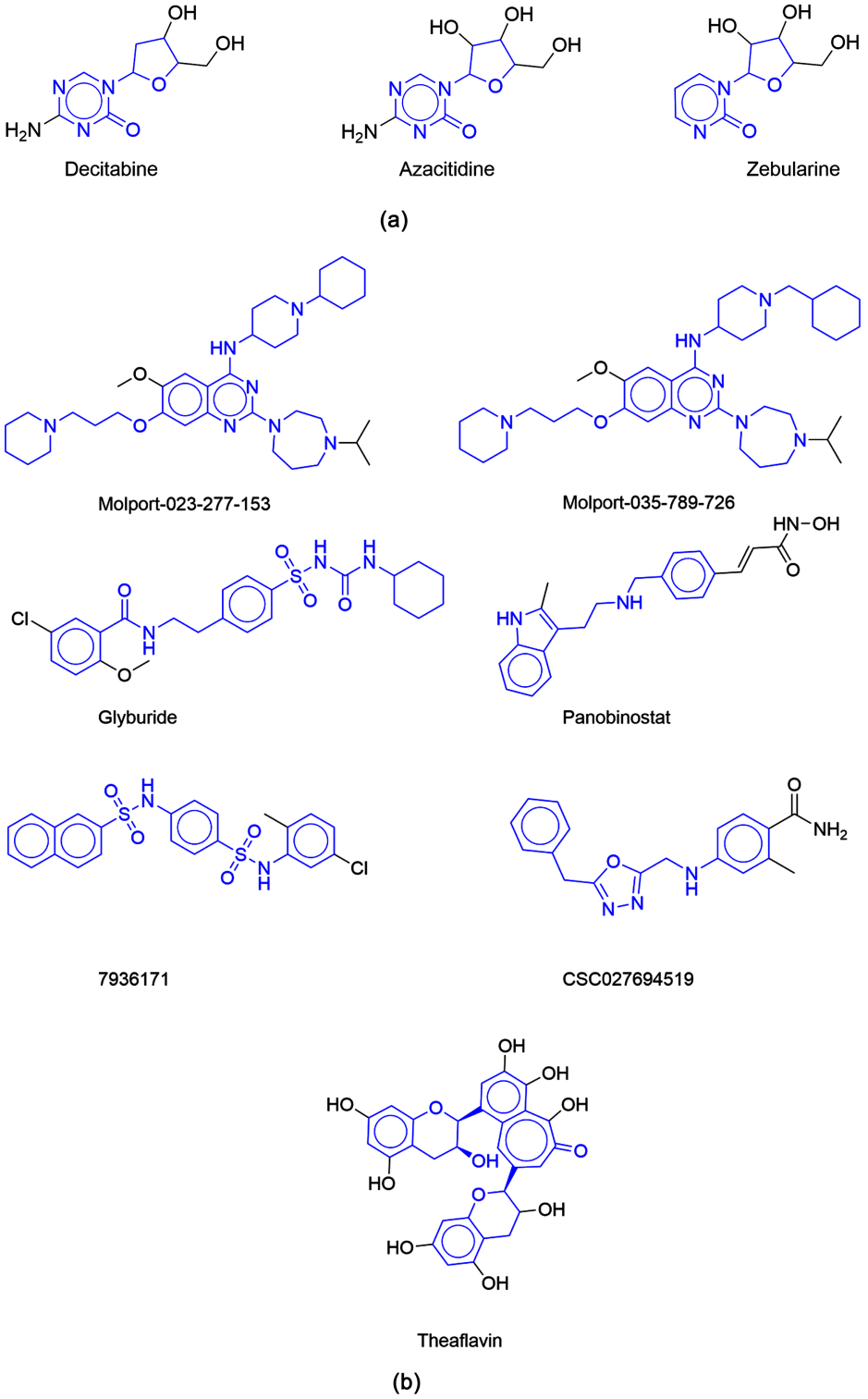

Figure S1. Chemical structures of representative DNMT1 inhibitors. The core scaffold (Bemis and Murcko) of each compound is marked in green. (a) Nucleotide compounds with inhibitory activity of DNMT1 that have been approved as drugs. (b) Representative non-nucleoside inhibitors of the enzymatic activity of DNMT1.

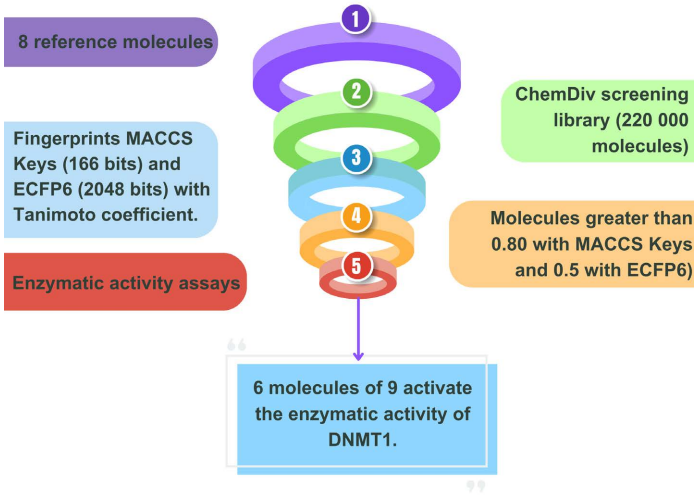

Figure S2. Similarity-based screening of the ChemDiv database using the Tanimoto coefficient and ECFP6 and MACCS Keys (166-bits) fingerprints. The reference (query) molecules were: L112-0668, L112-0728, L485-2669, L483-0323, L485-2761, M056-0726, RG108-KQVY, and RG108-GGGKQVY (chemical structures shown in **Figure 1**).

Table S1. Similarity values of the chemDiv compounds selected from the fingerprint-based similarity search.

ID hit/ compound	Fgpt	Reference (queries)							
		RG108-GGGKQVY	RG108-KQVY	L112-0668	L112-0728	L485-2669	L483-0323	L485-2761	M056-0726
L112-0729	MACCS[a]	0.51	0.49	0.78	0.94	0.66	0.65	0.61	0.71
	ECFP6[b]	0.09	0.08	0.62	0.84	0.13	0.13	0.13	0.14
L485-2687	MACCS	0.62	0.60	0.56	0.62	1.00	0.82	0.90	0.71
	ECFP6	0.08	0.08	0.10	0.11	0.75	0.21	0.52	0.12
L485-2777	MACCS	0.59	0.57	0.59	0.57	0.89	0.79	0.98	0.64
	ECFP6	0.09	0.09	0.14	0.12	0.54	0.23	0.82	0.13
L112-0716	MACCS	0.48	0.46	0.98	0.73	0.57	0.61	0.61	0.67
	ECFP6	0.10	0.08	0.78	0.58	0.14	0.13	0.15	0.15
L112-0706	MACCS	0.51	0.49	0.90	0.76	0.62	0.61	0.57	0.70
	ECFP6	0.10	0.09	0.71	0.61	0.16	0.13	0.14	0.15
L112-0698	MACCS	0.57	0.56	0.75	0.82	0.78	0.76	0.72	0.79
	ECFP6	0.11	0.09	0.63	0.68	0.18	0.18	0.18	0.17
3909-9992	MACCS	0.61	0.59	0.59	0.64	0.92	0.80	0.83	0.77
	ECFP6	0.10	0.10	0.17	0.14	0.18	0.20	0.15	0.16

Continued

C066-0242	MACCS	0.59	0.57	0.57	0.61	0.92	0.78	0.83	0.77
	ECFP6	0.11	0.10	0.11	0.11	0.16	0.17	0.13	0.20
V011-9764	MACCS	0.58	0.57	0.54	0.61	0.90	0.76	0.82	0.71
	ECFP6	0.10	0.10	0.11	0.13	0.24	0.19	0.20	0.17

[a]MACCS keys (166 bits). [b]ECFP6 (2048 bits).

Table S2. Percentage of activity of DNMT1 activators at different concentrations.

ID hit/compound	Concentration (μM)	% Activity (stddev)
L112-0729	100	190.42 (±33.40)
	33.3	214.275 (±51.09)
	11.1	275.445 (±61.60)
L485-2687	100	243.265 (±66.67)
	33.3	355.025 (±78.95)
	11.1	159.755 (±34.67)
L485-2777	100	672.635 (±88.85)
	33.3	501 (±126.19)
	11.1	623.17 (±135.82)
L112-0716	50	221.1 (±12.35)
	16.7	239.64 (±39.58)
	5.56	263.765 (±24.60)
L112-0706	50	250.29 (±12.49)
	16.7	214.435 (±24.20)
	5.56	261.705 (±20.30)
L112-0698	50	333.74 (±14.30)
	16.7	370.61 (±4.19)
	5.56	392.96 (±4.79)

The averages of the DNMT1 enzyme activation values are shown in units of percentage and the standard deviation of their measurements. The compounds L112-0698, L112-0716 and L112-0706, were tested at a starting concentration of 50 μM because they were insoluble at 100 μM.