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In Silico Identification of Novel HDAC2 Inhibitors for Reinstating Synaptic wiring in Autism Spectrum Disorder

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Abstract

Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder with an increasing incidence rate. For the treatment of ASD, several pharmaceutical approaches, dietary supplements, and behavioral therapy have been proposed, but a definitive cure remains elusive. Histone deacetylases (HDACs) are critical epigenetic regulators whose molecular and pharmacological roles are being studied extensively in medically significant ailments such as neuropsychiatric disorders, neurodegenerative diseases, and cancer on a global scale. HDAC2's specific expression pattern in CNS makes it an appealing therapeutic target for neurological illnesses such as ASD.

Methods: The study used PyRx software (version 0.8) to screen a library of 401 alkaloid compounds against HDAC2. In addition, the DS software was used to predict the physicochemical and DMET properties of the compound library.

Results: Valaciclovir hydrochloride, Dihydrocapsaicin, Guanosine, Santacruzamate A, and 2'-Deoxyguanosine monohydrate compounds exhibited strong interactions with HDAC2. These compounds had a higher binding energy than the control compound, and they have promising drug-like properties and ADMET characteristics.

Conclusion: These identified compounds could be used as HDAC2 inhibitors for the management of ASD, however, experimental research is needed to optimize them as HDAC2 inhibitors.

Introduction

Autism Spectrum Disorder (ASD) is a medical condition distinguished by an array of symptoms associated with the development of the nervous system. ASD has three primary characteristics: persistent social communication and interaction deficits, repetitive behaviors, and narrow interests [1]. According to the most recent epidemiological study, the global prevalence of ASD is 0.62% [2]. Autism is a chronic, life-long condition that cannot be reversed [3]. The etiological basis of ASD is still unknown. In contrast, pharmacological intervention, comprehensive educational strategies, rehabilitative training, sensory integration techniques, and nutritional therapy are commonly used therapeutic modalities. Unfortunately, these therapies do not have a track record of clinical efficacy in addressing the core features of ASD. Herbal medicine and acupuncture, which are categorized as complementary and alternative medicine, have become increasingly popular as treatment options for children with ASD. This is because they are believed to have a lower likelihood of causing adverse reactions compared to conventional drugs [4]. Notably, the number of Randomized Controlled Trials on herbal medicine are similar to those on contemporary therapeutic approaches, demonstrating the widespread global use and acceptance of herbal medicine in the management of ASD [5].

The etiology of ASD is unknown; however, it is widely assumed that gene-environment interactions mediated by epigenetic mechanisms play a significant role [6]. Empirical evidence has established links between prenatal environmental factors and an increased risk of ASD, associated with detectable changes in histone deacetylases (HDACs) or acetylation levels. The interplay between epigenetic modifications and gene expression patterns in the context of ASD suggests that changes in histone acetylation, which leads to changes in gene transcription, may play a key role in the pathogenesis of ASD. Notably, prenatal HDAC inhibition has been shown to alter messenger RNA levels in ASD-associated risk genes such as Nlgn1, Shank2, Shank3, and Cntnap2 [7]. Among the eleven HDAC isoforms, HDAC2 is responsible for modulating the mRNA and protein levels of EAAT2 as well as regulating synaptic plasticity, which results in the amelioration of behavioral and cognitive impairments [8]. The study by Tamanini et al. observed an increase in the levels of HDACs, specifically HDAC2, in the brains of individuals with Alzheimer's disease and a mouse model of Alzheimer's disease (CK-p25 and 5xFAD mice). This phenomenon leads to a reduction in the acetylation of histones and a decrease in the expression of critical genes associated with the processes of learning and memory. The effects observed in CK-p25 mice are mitigated through HDAC2 knockdown, leading to an upregulation of crucial genes, an augmentation of synaptic plasticity, and eliminating memory deficits linked to neurodegeneration [9].

Natural products (NPs) and their structural analogues have been important in developing therapeutic agents for various diseases [10]. NPs are widely recognized as privileged structures in the context of protein-drug target interactions. Despite the pharmaceutical industry's reduced emphasis in this area, their distinct properties and structural heterogeneity continue to captivate researchers, fostering the pursuit of NP-derived medicinal compounds [11]. Using in silico tools, this study aimed to find natural compound HDAC2 inhibitors to combat ASD.

Methods

Identification and preparation of target protein

Based on the literature review findings, HDAC2 has emerged as a potential target for addressing ASD. In this study, the 3D structure of the HDAC2 protein was acquired from the Protein Data Bank (PDB ID: 5IX0). The PDB model under consideration exhibits the HDAC2 protein, which possesses a molecular weight of 131.25 kDa and a primary sequence consisting of 369 amino acid residues. Furthermore, the crystal structure comprises the co-crystallized inhibitor molecule 6EZ (BRD-7232) within its arrangement. The HDAC2 protein was refined and prepared using Discovery Studio 2020 (DS 2020) software and then saved as a .pdb-formatted protein structure file.

Alkaloid compounds library retrieval and preparation

A comprehensive collection of 401 alkaloid compounds were obtained from Selleck Chemicals, a reputable source (https://www.selleckchem.com/). It is noteworthy to mention that a specific subset of these compounds has obtained approval from the FDA. These compounds demonstrate a wide range of structural diversity, possess medicinal properties and can permeate cell membranes. The compounds were downloaded in .sdf format.

Filtration of compounds

The DS 2020 software was employed to calculate and analyze the physicochemical and DMET properties of the alkaloid compounds library. The selection of compounds was initially based on their physicochemical and drug-like properties, which met the criteria outlined by Lipinski's rule of five. The compounds underwent ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) evaluation. The virtual screening study was restricted to compounds exhibiting favorable ADMET properties.

Structure-based virtual screening (SBVS)

SBVS has solidified its position as a key component in drug discovery and development over the past decade [12]. Utilizing computational methods, VS involves the identification of potential drug candidates from vast chemical structure databases [13]. Over the past decade, SBVS has emerged as a crucial element in drug discovery, encompassing the use of computational methods to identify potential drug candidates from extensive libraries of chemical structures [14]. The AutoDock Vina program integrated within PyRx software (version 0.8) [15] was used to screen the prepared compound library against the active site of the HDAC2 protein. After that, comprehensive interactions and visual inspections were performed using PYMOL and DS 2020 to identify complexes with lower binding energy (BE) values, thereby determining the complexes with the highest stability.

Results

In this study, computational methodologies, such as drug-likeness prediction and SBVS, were utilized to identify prospective HDAC2 inhibitors. The SBVS study utilized the 3D conformation of HDAC2 in conjunction with an inhibitor (BRD-7232). The binding coordinates of this inhibitor were employed to screen a library of 401 alkaloid compounds. The initial filtration of the library consisting of 401 compounds was conducted based on physicochemical properties and Lipinski's rule of five criteria, which include molecular weight less than or equal to 500 Da, logarithm of partition coefficient (log P) less than or equal to 5, hydrogen bond donor count less than or equal to 5, and hydrogen bond acceptor count less than or equal to 10. As a result, 336 compounds successfully met these filtration criteria and were retained for further analysis.

These 336 compounds were minimized and prepared for SBVS against the active site residues of HDAC2. The coordinates of the binding pocket were 67.539250, 30.033357, and 1.110750 for the x, y, and z axes, respectively. The docking protocol was validated to ascertain the precision and dependability of the screening procedure. The alignment between the re-docked complex and the initially downloaded complex from the PDB, as depicted in Figure 1A, serves as evidence for the reliability and effectiveness of the molecular docking protocol. After confirming the docking protocol's accuracy, SBVS was performed on a compound library consisting of 336 compounds. This screening was conducted against the binding pocket of HDAC2.

A total of 143 compounds were identified in SBVS using PyRx software, exhibiting favorable interaction and binding energy (BE) values surpassing -6.5. However, compared to the control compound, only 14 compounds exhibited higher BE as indicated in Table 1.

Compound name	Binding energy (Kcal/mol)
Valaciclovir hydrochloride	-9.9
Dihydrocapsaicin	-9.9
Guanosine	-9.7
Santacruzamate A	-9.6
2'-Deoxyguanosine monohydrate	-9.6
VIRA-A (vidarabine)	-9.6
Tropisetron	-9.6
Piperlongumine	-9.5
Halofuginone	-9.4
Kynurenic acid	-9.4
Gefitinib	-9.4
Piperine	-9.3
Nifuratel	-9.3
Anamorelin	-9.2
BRD-7232 (control)	-9.2

Table 1: Binding energy of top 14 screened compounds and control compounds.

Based on BE, the top 5 compounds were selected for in depth interaction analysis (Figure 1B). Valaciclovir hydrochloride was found to interact with Leu276, His145, Tyr308, Gly306, Phe155, Gly154, Cys156, Tyr29, Met35, Phe114, Leu144, Arg39, Ile40, Ala141, Trp140, Gly142, Trp317, Gly304, Asp181, His183, Asp269, Gly143, His146, Gly305, and Phe210 residues of HDAC2 protein. Among these, Tyr308, Phe155, His146, Gly142, and Ala141 residues were found to be involved in H-bonding with Valaciclovir hydrochloride (Figure 2a). Dihydrocapsaicin interacted with Asp104, Phe155, Phe210, His145, Gln265, Phe114, Tyr308, Gly154, Gln265, Phe114, Cys156, Gly143, Met35, Gly305, Leu144, Tyr29, Ala141, Arg39, Gly306, His146, Asp181, His183, Asp269, and Leu276 residues of HDAC2 protein. The Asp104, Gly154, His183, and His146 residues were H-bonded with Dihydrocapsaicin (Figure 2b). Guanosine was found to bind with Gly306, Asp181, Gln265, Gly143, Phe155, Tyr29, Leu144, Phe114, Arg39, Ala141, Met35, Ile40, Trp140, Trp317, Gly304, Gly142, Gly305, Gly154, Cys156, His146, Tyr308, His183, His145, and Asp269 residues of HDAC2 protein. Among these, Asp181, His145, Gly305, Ala141, and Trp140 residues were H-bonded with Isoguanosine (Figure 2c). Santacruzamate A was found to interact with Phe210, Asp104, Phe114, Arg39, Ala141, Leu144, Gly142, Gly305, Met35, Gly143, Cys156, Gly306, His183, Asp269, Asp181, His145, His146, Tyr308, Gly154, and Phe155 residues of HDAC2 protein. The Arg39, His183, His145, His146, and Gly154 residues were H-bonded with Santacruzamate A (Figure 2d). Further, 2'-Deoxyguanosine monohydrate was found to bind with Met35, Arg39, Ala141, Leu144, Ile40, Trp140, Gly142, Gly304, Trp317, Gly305, Gly306, Gly143, Gln265, His146, His183, Asp181, Asp269, Tyr308, His145, Gly154, Cys156, Tyr29, Phe155, Phe114, and Met35 residues of HDAC2 protein. The Phe155, Ala141, Trp140, Gly142, Gly305, Gln265, Asp181, and His145

residues were found to be involved in H-bonding with 2'-Deoxyguanosine monohydrate (Figure 2e).

Furthermore, the co-crystal control compound (BRD-7232) was found to interact with His146, His145, Gly306, Asp181, Gln265, Gly143, Gly305, Cys156, Met35, Leu144, Phe114, Tyr29, Phe210, His183, Leu276, Phe155, Gly154, Tyr308, and Asp269 residues of HDAC2 protein. The Gly154, and Asp181 residues were H-bonded with control compound (Figure 3f).

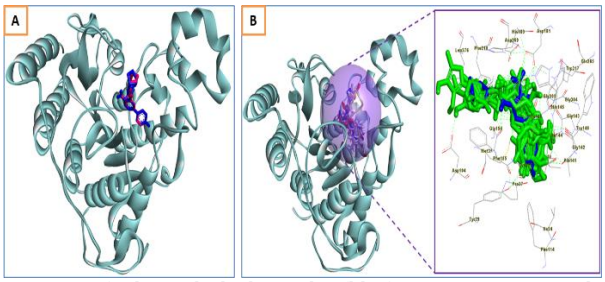

Figure 1: A) The re-docked complex (blue) superimposition with the original PDB structure (red) of HDAC2. **B)** A superimposed view presenting the structural alignment of the top 5 compounds alongside the control compound (BRD-7232) within the catalytic pocket of the HDAC2 protein.

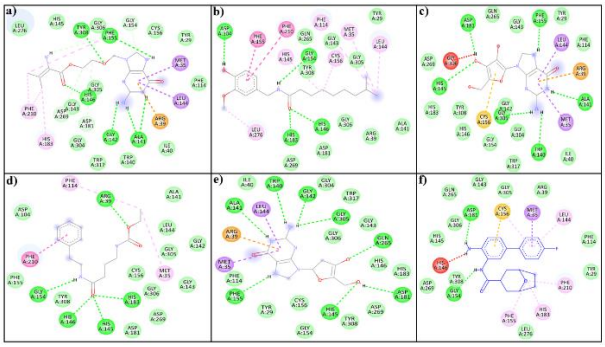

Figure 2: Interacting residues of HDAC2 protein with **A)** Valaciclovir hydrochloride, **B)** Dihydrocapsaicin, **C)** Guanosine, **D)** Santacruzamate A, **E)** 2'-Deoxyguanosine monohydrate, and **F)** control compound (BRD-7232).

Table 2 and Table 3 present the drug-likeness properties predicted by Lipinski's rule of five criteria and ADMET characteristics of the five compounds. The ADMET descriptors module in DS 2020 analyzes a range of characteristics, including AlogP98, PSA (polar surface area), PPB (plasma protein binding), intestinal absorption, aqueous solubility, blood-brain barrier (BBB) permeability, hepatotoxicity, and inhibition of the CYP2D6 enzyme. The solubility levels 0, 1, 2, 3, 4, and 5 correspond to the following descriptions: extremely low, no but possible, low, good, optimal, and too soluble. Similarly, BBB's levels 0, 1, 2, 3, and 4 represent very high, high, medium, low, and undefined, respectively. Lastly, the levels 0, 1, 2, and 3 for Absorption indicate good, moderate, poor, and very poor, respectively. The prediction of plasma protein binding (PPB) can be categorized as either true or false, indicating a high or poor level of binding, respectively. Similarly, the prediction of hepatotoxicity can be classified as true or false, indicating toxicity or non-toxicity respectively. Likewise, the prediction of CYP2D6 binding can be categorized as true or false, indicating inhibition or non-inhibition (Table 3).

Discussion

ASD is characterized by enduring deficiencies in social interaction and communication, along with limited and repetitive behaviors, preferences, or interests [1]. For the treatment of ASD, a wide range of therapeutic modalities are available, including pharmacological interventions, educational strategies, rehabilitative training, sensory integration approaches, and dietary interventions. Although there are currently no interventions that directly target the fundamental characteristics of ASD, specific medications and behavioral therapies have been identified as effective treatments for managing symptoms such as hyperactivity, depression, inattention, or seizures [16,17].

Compound Name	H_ Acceptors	H_ Donors	Molecular Weight	ALogP	Rotatable Bonds	Polar Surface Area
Valaciclovir hydrochloride	10	5	360.797	-0.296	8	146.85
Dihydrocapsaicin	4	2	307.428	4.354	10	58.55
Guanosine	10	6	283.241	-2.38	2	155.21
Santacruzamate A	5	2	278.347	1.899	9	67.43
2'-Deoxyguanosine monohydrate	9	5	267.241	-1.613	2	134.98

Table 2: Computationally predicted Drug-likeness properties of the top 5 compounds.

Compound Name	Solubility		AlogP98	BBB		CYP2D6		Hepatotoxic		Absorption Level	PPB	
	value	Level		value	Level	value	prediction	value	prediction		value	prediction
Valaciclovir hydrochloride	-2.255	3	0	-	4	-7.23597	F	-6.30472	F	2	-21.963	F
Dihydrocapsaicin	-3.704	3	0	0.245	1	-4.40848	F	-10.6646	F	0	-5.20975	F
Guanosine	-0.362	4	0	-	4	-4.13956	F	0.402279	T	3	-26.0682	F
Santacruzamate A	-1.968	4	0	-0.661	3	-6.5502	F	-11.4051	F	0	-3.15566	F
2'-Deoxyguanosine monohydrate	-0.694	4	0	-	4	-4.15139	F	1.47595	T	2	-24.79	F

Table 3: Predicted ADMET properties of the top 5 compounds.

Risperidone is the most commonly used treatment for addressing severe behavioral symptoms in children with autism [18]. Despite its observable benefits in reducing behavioral challenges, the outcomes of risperidone treatment remain inconclusive. They are accompanied by adverse events such as increased appetite, rhinorrhea, drowsiness, and excessive weight gain [19]. Herbal medicines, a component of Complementary and Alternative Medicine known for their relatively low adverse effects, have seen a surge in interest as a potential avenue for treating children with ASD [20].

HDAC2 regulates the mRNA and protein levels of EAAT2 as well as synaptic plasticity, resulting in the amelioration of behavioral and cognitive impairments; therefore, it is a promising target for ASD [8]. This study screened alkaloid compounds against HDAC2 to find potent natural HDAC2 inhibitors. 14 compounds had a higher BE than the control compound and a strong interaction with the HDAC2. Furthermore, PYMOL and DS 2020 were used to conduct detailed interaction studies (2D and 3D) and visual inspections of the top five compounds, namely Valaciclovir hydrochloride, Dihydrocapsaicin, Guanosine, Santacruzamate A, and 2'-Deoxyguanosine monohydrate. These compounds had a strong interaction with HDAC2 and interacted with key residues of HDAC2. The control compound (BRD-7232) interacted with His146, His145, Gly306, Asp181, Gln265, Gly143, Gly305, Cys156, Met35, Leu144, Phe114, Tyr29, Phe210, His183, Leu276, Phe155, Gly154, Tyr308, and Asp269 residues of HDAC2 protein. Notably, the top five compounds were also found to interact with these HDAC2 residues.

The evaluation of compounds for preliminary absorption, distribution, metabolism, and excretion (ADME) data, toxicity, and the application of drug-like property filters, such as Lipinski's rule, can enhance the efficiency of screening and testing protocols, thereby mitigating the occurrence of late-stage attrition. The top five compounds described in this study have been observed to exhibit favorable characteristics in terms of drug-like properties and ADMET properties.

This study looked for natural HDAC2 inhibitors by screening alkaloid compounds against HDAC2. Notably, Valaciclovir hydrochloride, Dihydrocapsaicin Guanosine, Santacruzamate A, and 2'-Deoxyguanosine monohydrate compounds demonstrated strong interactions with HDAC2 and possess promising drug-like properties and ADMET characteristics. These compounds could be used as HDAC2 inhibitors for the management of ASD, however more research is needed to optimize them as HDAC2 inhibitors.

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Author Contributions

SAA: Conceived the idea, collected and analyzed the data, prepared the graphs, tables and wrote the manuscript.

Conflict of Interest

Authors declared no conflict of interest.

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