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Original Article

The investigation of drug repurposing for HDAC1 inhibitory effects by *in silico* and *in vitro* methods

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ABSTRACT

Background and Aims: Histone deacetylases (HDACs) modulate chromatin structure and regulate gene expression. The imbalance in chromatin acetylation and dysregulation of histone deacetylases are challenging in many pathologies, ranging from cancer to neurodegeneration. Computer-based *in silico* methods are becoming increasingly important in the determination of therapeutic targets and the development of personalized treatment approaches. This study aimed to investigate the HDAC1 inhibitory effects of chronic prescription drugs using *in silico* and *in vitro* methods.

Methods: Five chronically used prescription drugs were chosen: ipratropium bromide, metoprolol, leflunomide, nateglinide, and levothyroxine. Molecular docking was performed for each of the chosen drugs as well as the known inhibitor Trichostatin A on HDAC1. The binding pose with the best scores was saved for each compound and analyzed for its interaction with the protein. An HDAC1 inhibitor screening assay kit was used to determine the IC_{50} value for each drug.

Results: The IC_{50} values for HDAC1 inhibition by ipratropium bromide, metoprolol, leflunomide, nateglinide, and levothyroxine were found to be 352.10 µM, 255.70 µM, 219.80 µM, 289.50 µM, and 132.70 µM, respectively, whereas the value for the positive control Trichostatin A was 36.13 nM. GraphPad Prism 5 was used to conduct statistical analyses.

Conclusion: In this study, the in vitro HDAC1 inhibitory effect of ipratropium bromide, metoprolol, leflunomide, nateglinide, and levothyroxine is shown for the first time. *In silico* and *in vitro* methodologies used to show HDAC1 inhibitory activity in marketed drugs can provide insight into new drug discovery studies against cancer or neurodegenerative diseases.

Keywords: Docking, drug repurposing, HDAC1, *in silico*, molecular modeling

INTRODUCTION

Epigenetics is a research area that focuses on the study of modifications that occur in gene expression and function without a change in the genetic code. Histone modifications, DNA methylation, hydroxymethylation, and regulation of gene expression by non-coding RNAs are examples of epigenetic mechanisms. All these mechanisms mediate the effects of aging and environmental factors on the genome and play a crucial role in the development of disorders (Cacabelos & Torrellas, 2014; Lardenoije et al., 2015). Post-translational modification of specific amino acids in histone proteins causes changes in chromatin structure. Chromatin architecture, which is modulated by the antagonistic activity between histone deacetylases (HDACs) and histone acetyltransferases (HATs), plays a decisive role in transcriptional regulation. HDACs suppress transcription through chromatin condensation by deacetylating both histone and non-histone proteins, whereas HATs activate transcription through chromatin decondensation (Ganai, Abdullah, Rashid, & Altaf, 2017).

The HDAC enzymes take the acetyl group off of lysine residues in the N-terminal histone tails. According to their structural characteristics, eighteen human HDACs are divided into four classes and assigned numbers based on the chronology in which they were discovered: Class I (HDAC1, 2, 3, and 8), Class II (HDAC4, 5, 6, 7, 9, and 10), Class III (sirtuins), Class IV (HDAC11) (Park & Kim, 2020). The yeast Rpd3 (reduced potassium dependence 3) protein and the Class I proteins share sequence similarities. In 1996, HDAC1 the first histone deacetylase was discovered and cloned (Taunton, Hassig, &

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Schreiber, 1996). HDAC1 has >80% homology with HDAC2 (sister protein) and their embryonic knockout is lethal (Dovey, Foster, & Cowley, 2010). HDAC1 and HDAC2 play a major role in the deacetylation of histone as well as many other nuclear proteins involved in transcriptional regulation (Serebryannyy, Cruz, & de Lanerolle, 2016). HDAC1 has been proven to be involved in the pathogenesis of several diseases. It has been reported to play an important role in carcinogenesis (Müller et al., 2013). Moreover, previous studies have shown an association between HDAC1 overexpression and schizophrenia (Bahari-Javan et al., 2017). The development of novel HDAC1 inhibitors is, therefore, a promising approach to the treatment of such diseases (Johnstone, 2002).

Drug repurposing (drug repositioning or re-tasking) is a new approach in drug design that reduces the high costs and attrition rates in clinical studies and speeds up the drug development process (Pushpakom et al., 2019). For example, Nelfinavir, an HIV-1 protease inhibitor, has been used to block the AKT pathway in cancer cells and is, therefore, a successful example of drug repurposing (Li & Jones, 2012; Guan, Fousek, & Chow, 2012). Molecular docking simulation predicts a ligand's pose within a macromolecular target's binding site and its binding affinity using a scoring system. The structure-based virtual screening's ranking of predicted ligand conformations is an essential component (Kitchen, Decornez, Furr, & Bajorath, 2004). This approach is used in many drug discovery studies, including those for novel HDAC inhibitors (Park, Kim, Kim, & Lim, 2010).

In this study, we aimed to identify drug candidates for repurposing as HDAC1 inhibitors. Furthermore, we intended to validate the ability of molecular docking to predict the activities of HDAC1 inhibitors. Through a preliminary docking study with 21 compounds, we selected five chronically used drugs with the highest Chemscore values from different pharmacological groups and studied them with the HDAC1 enzyme (Table 1). The drugs were: ipratropium bromide, used to treat chronic obstructive pulmonary disease and asthma; metoprolol, a selective β1 receptor blocker used in the treatment of high blood pressure; leflunomide, an immunosuppressive drug used in rheumatoid arthritis and psoriatic arthritis; nateglinide, a drug for the treatment of type 2 diabetes; and levothyroxine, used to treat thyroid hormone deficiency (hypothyroidism) and thyroid tumors. We then examined the in vitro HDAC1 inhibitory activities of the selected drugs. With this study, the HDAC1 enzyme inhibitory activities of different drugs that are used chronically are reported for the first time in the literature.

MATERIALS AND METHODS

In silico studies: Molecular docking

The structure of the HDAC1 protein was downloaded in PDB format from the Protein Data Bank (ID 1C3R) (https://www.rcsb.org) and processed with UCSF Chimera software (https://www.rbvi.ucsf.edu/chimera). The bound inhibitor, Trichostatin A, was extracted and saved in a separate PDB file. The Flare 6.0 software (Cresset, UK) was then used to load and process the PDB file. The best ionization states were assigned for each residue after the addition of hydrogens. The chemical structures of the tested compounds were downloaded in SDF format from Pubchem (https://pubchem.ncbi.nlm.nih.gov). To determine the best binding pose at the predicted binding pocket, molecular docking was performed with Gold and Flare software. Each compound in SDF file format was loaded into the program and processed using the default settings. The grid was selected to include the binding site of Trichostatin A. The binding pose with the best scores was saved for each compound and analyzed for its interaction with the protein. To validate the performance of the molecular docking experiments, we superimposed and compared the original extracted and docked poses of Trichostatin A.

In vitro HDAC1 Inhibitor Activity Studies

The inhibitory actions of the chosen drugs were evaluated using the HDAC1 inhibitor screening assay kit (Cayman Chemical, Item No. 10011564). The HDAC1 enzyme was first treated with an acetylated lysine substrate. Deacetylation makes the substrate sensitive enough that the second step's HDAC developer treatment results in the release of a fluorescent product. Using the CLARIOstar Plus microplate reader (BMG LABTECH, Ortenberg, Germany) and excitation and emission wavelengths of 340–360 nm and 440–465 nm, the fluorophore was examined.

For background fluorescence, 10 µL of solvent was added to 150 µL of buffer solution. For initial activity, 10 µL of diluted HDAC1 and 10 µL of solvent were added to 140 µL of buffer solution. A positive control was prepared by adding 10 µL of diluted HDAC1 and 10 µL of Trichostatin A to 140 µL of buffer solution. For inhibitory fluorescence, 10 µL of diluted HDAC1 and 10 µL of synthetic or naturally sourced active substances in different concentrations were added to 140 µL of buffer solution.

All samples received 10 µL of HDAC substrate before the reaction could begin. After that, samples were incubated for 30 mins at 37°C. 40 µL of HDAC developer was added at the end of the incubation period, and the mixture was then incubated for an additional 15 mins at room temperature. Fluorescence was finally detected at the designated emission wavelengths. According to the following formula, the HDAC1 percent inhibition values of the drugs under investigation were determined:

$$\%Inhibition = \left[\frac{InitialActivity - Sample}{InitialActivity}\right] x100 \quad (1)$$

Table 1. Pharmacological properties of selected active substances **(Source: DrugBank; https://go.drugbank.com/drugs)**

Drug	Indication	Mechanism of Action	Toxicity	Targets
Ipratropium bromide	Anticholinergic drug used in the control of symptoms related to bronchospasm in chronic obstructive pulmonary disease (COPD).	Antagonist of the muscarinic acetylcholine receptor	LD_{50} 1500 mg/kg (in mice, oral administration)	Muscarinic acetylcholine receptor M1, Muscarinic acetylcholine receptor M2, Muscarinic acetylcholine receptor M3
Metoprolol	Beta-blocker used in the treatment of hypertension and angina, and used to reduce mortality due to myocardial infarction.	Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors.	LD_{50} in the range of 3090 to 4670 mg/kg (in rats, oral administration)	Beta-1 adrenergic receptor inhibitor
Leflunomide	Pyrimidine synthesis inhibitor indicated to treat rheumatoid arthritis.	Leflunomide is a prodrug that is rapidly and almost completely metabolized following oral administration to its pharmacologically active metabolite. The mechanism of action of leflunomide has not been fully determined, but appears to primarily involve regulation of autoimmune lymphocytes.	LD_{50} 100-250 mg/kg (in rats, oral administration)	Mitochondrial dihydroorotate dehydrogenase inhibitor.
Nateglinide	For the treatment of non-insulin dependent-diabetes mellitus in conjunction with diet and exercise.	Nateglinide activity is dependent on the presence of functioning β cells and glucose. The insulinotropic effects of nateglinide are highest at intermediate glucose levels and it does not increase insulin release already stimulated by high glucose concentrations.	LD_{50} > 2000 mg/kg (in mice, oral administration)	ATP-binding cassette sub-family C member 8 inhibitor
Levothyroxine	Levothyroxine is indicated as replacement therapy in primary (thyroidal), secondary (pituitary) and tertiary (hypothalamic) congenital or acquired hypothyroidism	Levothyroxine is a synthetically prepared levo-isomer of the thyroid hormone thyroxine (T4, a tetra-iodinated tyrosine derivative) that acts as a replacement in deficiency syndromes such as hypothyroidism	LD_{50} 20 mg/kg (in rats, oral administration).	Integrin alpha-V; Integrin beta-3; Thyroid hormone receptor alpha agonist; Thyroid hormone receptor beta agonist

RESULTS

In silico studies: Molecular docking

As shown in Table 2, the molecular docking results for both Flare and Gold software were comparable. However, the correlation between the docking scores and IC_{50} values, shown in Table 3, was poor. This implies that these docking scores are not sufficient to predict the activities of HDAC1 inhibitors and that other parameters involved in binding should be considered. Such parameters include solvation and desolvation parameters as well as the flexibility of residues in the binding site.

Ipratropium (Figure 1A) formed two hydrogen bonds (GLY 128 and MET 130) and fourteen hydrophobic interactions (PHE 141, CYS 142, LEU 23, and TYR 17). Metoprolol (Figure 1B) formed five hydrogen bonds (ARG 27, GLY 294, MET 130, ALA 127, and TYR 297), an ion-dipole interaction with zinc, one aromatic-aromatic interaction (HIS 131), and nine hydrophobic interactions (ALA 106, PHE 141, TYR 17, and CYS 142). Leflunomide (Figure 1C) formed four hydrogen bonds (HIS 131, HIS 132, GLY 140, and GLY 295), an ion-dipole interaction with zinc, three aromatic-aromatic interactions (PHE 141, HIS 170, and HIS 132), and seven hydrophobic interactions (PHE 141, PHE 198, CYS 142, and LEU 265). Nateglinide (Figure 1D) formed four hydrogen bonds (HIS 131, HIS 132, GLY 295, TYR 297, and GLY 140), an ion-dipole interaction with zinc, two aromatic-aromatic interactions (HIS 131 and HIS 132), and twenty hydrophobic interactions (PHE 141, LEU 23, CYS 142 and PHE 198). Levothyroxine (Figure 1E) formed five hydrogen bonds (HIS 131, HIS 132, TYR 297, and GLY 140), an ion-dipole interaction with zinc, five aromatic-aromatic interactions (LEU 265, HIS170, PHE 141, HIS 131 and HIS 132), and five hydrophobic interactions (PHE 141 and PHE 198). Trichostatin A (Figure 1F) formed two hydrogen

Table 2. Docking scores and interacting residues of selected compounds and positive control Trichostatin A.

Drug	Gold Score	Virtual Score (Flare)	Interacting residues
Ipratropium bromide	48.8	-7.7	MET130, GLY128, GLY294, TYR297, PHE141, CYS142, LEU23, TYR17
Metoprolol	56.2	-10.2	HIS131, CYS142, ARG27, GLY294, ALA106, ALA127, TYR17, MET130, PHE141, TYR297
Leflunomide	59.1	-8.6	LEU265, HIS170, GLY140, TYR297, GLY129, GLY295, HIS132, CYS142, HIS131, PHE141, PHE141, PHE198,
Nateglinide	65.2	-13.6	PHE198, ASP168, GLY295, HIS131, HIS132, LEU23, CYS142, GLY140, TYR297, PHE141
Levothyroxine	64.6	-13.8	LEU265, HIS170, PHE198, HIS132, HIS131, TYR297, GLY140, PHE 141
Trichostatin A	62.1	-11.2	HIS131, HIS132, CYS142, LEU23, TYR17, MET130, PHE141

Table 3. IC_{50} values for HDAC1 inhibition by ipratropium bromide, metoprolol, leflunomide, nateglinide, levothyroxine, and Trichostatin A.

Drug	HDAC1 IC_{50} (μM)	SD	SE	%RSD
Ipratropium bromide	352.10	0.80	0.46	0.23
Metoprolol	255.70	0.83	0.48	0.32
Leflunomide	219.80	0.94	0.54	0.43
Nateglinide	289.50	0.72	0.42	0.25
Levothyroxine	132.70	0.58	0.34	0.44
Trichostatin A	36.13*	1.51	0.87	4.18

**nm concentration*

bonds (HIS 131 and HIS 132), an ion-dipole interaction with zinc, an aromatic-aromatic interaction (TYR 17), and nine hydrophobic interactions (MET 130, CYS 142, and LEU 23). As shown in Figure 2, the binding poses of the predicted binding pose after redocking are very similar to the original binding pose extracted from the complex from the Protein Data Bank.

In vitro HDAC1 Inhibitor Activity Studies

IC_{50} values for HDAC1 inhibition by Trichostatin A, ipratropium bromide, metoprolol, leflunomide, nateglinide, and levothyroxine were found to be 36.13 nM, 352.10 μM, 255.70 μM, 219.80 μM, 289.50 μM, and 132.70 μM, respectively (Table 3). The data were representative of three independent experiments. The values of mean, standard deviation (SD), and standard error (SE) were analyzed with GraphPad Prism 5. In our study, the IC_{50} value for HDAC1 inhibition by the reference Trichostatin A was lower than the IC_{50} values of the selected drugs. Daily-administered doses of ipratropium bromide, metoprolol, leflunomide, nateglinide, and levothyroxine can achieve values above the IC_{50}.

DISCUSSION

Epigenetic modifications are effective in almost all pathways in tumor development. HDAC inhibitors have been shown to prevent proliferation by inducing apoptosis and differentiation in many transformed or cancerous cell types by arresting the cell cycle in G1 or G2 (Lindemann, Gabrielli,& Johnstone, 2004; Marks, Miller, & Richon, 2003). It has been reported that HDAC1 is overexpressed in pancreatic, prostate, colorectal, gastric, and hepatocellular cancers, which correlates with a poor prognosis (Spiegel, Milstien, &Grant, 2012). The pathogenesis of neurodegenerative diseases is mediated by the epigenetic mechanisms involved in neuronal development. HDAC inhibitors have been demonstrated to prevent neuronal injury by lowering excitotoxicity, oxidative stress, and inflammation in various in vitro and in vivo models of cerebral ischemia (Wang, Yu, Tan, Jiang, & Tan, 2013; Hirata et al., 2018).

The IC50 values for HDAC1 inhibition by ipratropium bromide, metoprolol, leflunomide, nateglinide, and levothyroxine were found to be 352.10 μM, 255.70 μM, 219.80 μM, 289.50 μM, and 132.70 μM, respectively. There are no previous stud-

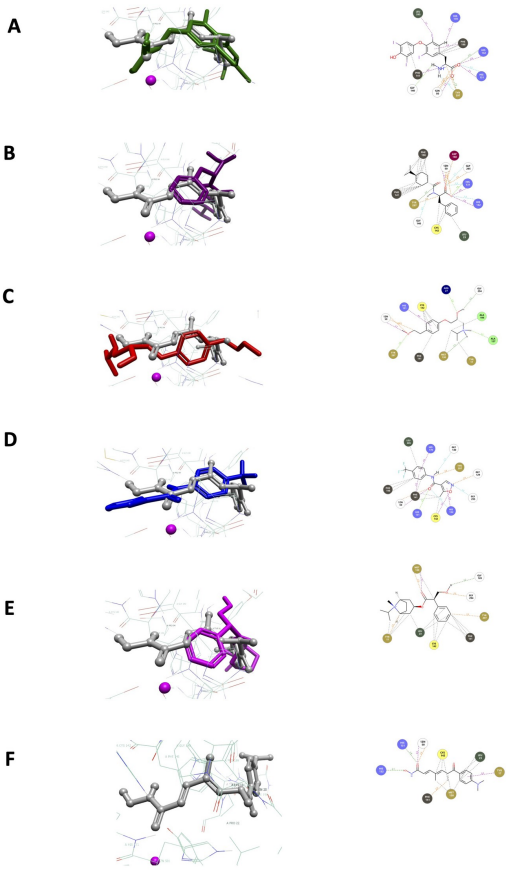

Figure 1. Docking poses of each selected compound and Trichostatin A in binding sites of HDAC1 proteins (PDB 1C3R): A. Ipratropium bromide, B. Metoprolol, C. Leflunomide, D. Nateglinide, E, Levothyroxine. and F. Trichostatin A. The panels on the left side show the docking poses of the compounds superimposed with the positive control Trichostatin A (grey). The panels on the right side show the 2D representations of the interactions, including strong (light green), average (dark green), and weak (cyan) H-bonds; hydrophobic contacts (grey); aromatic and ionic interactions (purple); and steric clashes (orange)

ies in the literature on the effects of these drugs on HDAC1. Our molecular docking study suggests that interactions with several residues are important for the inhibitory activity against HDAC1. These include the formation of hydrogen bonds with HIS 131 and HIS 132 and the ion-dipole interaction with zinc. These interactions were found in Trichostatin A and were common to all the investigated drugs except ipratropium bromide, which showed the lowest *in vitro* activity. Furthermore, the predicted binding pose of Trichostatin A was very similar to the original one, which implies that the molecular docking experiments were performed with good accuracy. Therefore, these interactions should be taken into consideration in the future design of novel HDAC1 inhibitors.

Ipratropium bromide, a derivative of the alkaloid atropine, is used in the treatment of asthma and chronic obstructive pulmonary disease. It acts as a muscarinic acetylcholine receptor

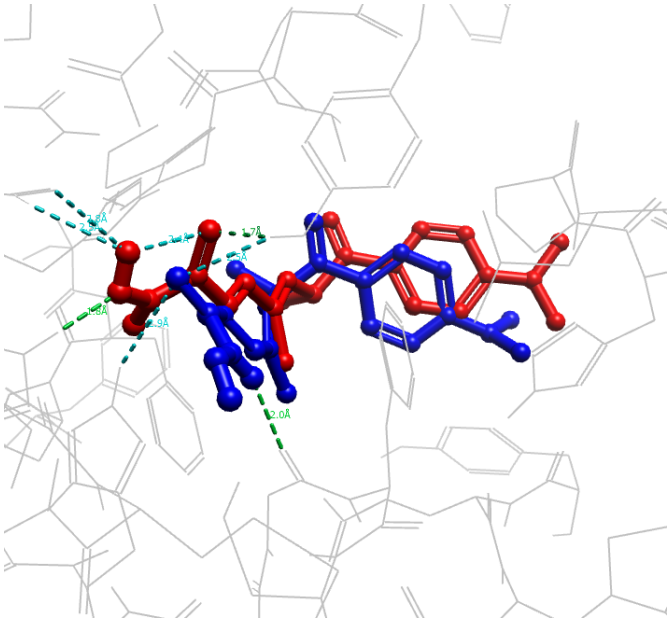

Figure 2. Representation of Trichostatin A in original binding pose (red) extracted from the original complex from the Protein Data bank (ID: 1C3R) superimposed with the predicted pose (blue) after redocking. Strong and weak hydrogen bonds are shown as green and cyan dotted lines, respectively.

antagonist to dilate bronchial smooth muscles and open the lungs' airways (Kazi, Reddy, &Singh, 2021). Metoprolol, a selective β1-blocker, is used to treat hypertension, chest pain, heart failure, palpitations, and arrhythmias, and to prevent migraine attacks (Grassi, 2018). However, metoprolol has been shown to increase the levels of Sirt1, a histone deacetylase that is nicotinamide adenine dinucleotide (NAD+) dependent, and to have a cardioprotective effect in the vasopressin-induced cellular aging model in cardiomyocytes (Li et al., 2022). Leflunomide is used in the treatment of rheumatoid arthritis and psoriatic arthritis when the disease cannot be controlled with other disease-modifying drugs. It acts by inhibiting the dihydroorotate dehydrogenase enzyme, decreasing intracellular pyrimidine levels, and the activity of tumor necrosis factor-α (Boyd, 2012). Leflunomide has been found to block UVB-induced Fyn kinase, which in turn blocks histone H3 phosphorylation. However, studies are reporting that HDAC inhibitors may be effective in the treatment of chronic inflammatory and autoimmune diseases (Vishwakarma et al., 2013). Nateglinide is an oral hypoglycemic agent that can be used alone or in combination with metformin in the treatment of type 2 diabetes. It is a derivative of D-phenylalanine, which stimulates insulin release from pancreatic beta cells (Halas, 2001). In an *in silico* simulation and drug repositioning study by Gao et al., nateglinide has been reported to have an inhibitory effect on the HDAC2 enzyme, which is an important therapeutic target in cancer and neurodegeneration (Gao, Yao, Wang, Yao, & Zhang, 2022). Levothyroxine is used in the treatment of hypothyroidism (Ianiro et al., 2014). In a study conducted by Cordeiro et al., it was demonstrated that

thyroid hormones controlled the expression of Sirt1 in mice subjected to calorie restriction (Cordeiro et al., 2013).

All tested drugs were found to show weaker *in vitro* activities than the reference standard agent, Trichostatin A. However, the tested concentrations of the studied drugs were extremely low compared to their daily recommended doses for regular treatment, where 1 mg/day, 200 mg/day, 20 mg/day, 360 mg/day, and 100 µg/day are the common doses for ipratropium bromide, metoprolol, leflunomide, nateglinide, and levothyroxine, respectively. Hence, with the regular use of these drugs for the treatment of chronic diseases, concomitant inhibitory effects against HDAC1 activity may also be clinically observed.

Due to regular or lifelong use of these drugs, possible concomitant activity against HDAC1 is of great importance. To the best of our knowledge, this study provides the first demonstration of the HDAC1 inhibitory action of ipratropium bromide, metoprolol, leflunomide, nateglinide, and levothyroxine using *in vitro* and *in silico* techniques.

CONCLUSION

Drug repurposing is a strategy for finding new indications for clinically-used drugs. In this study, we proposed that repurposing of lifelong used and FDA-approved drugs may also be effective on the HDAC1 enzyme. Our results showed that the chronically used drugs ipratropium bromide, metoprolol, leflunomide, nateglinide, and levothyroxine showed moderate inhibitory effects against the HDAC1 enzyme, which is an important therapeutic target in cancer and neurodegenerative diseases. We suggest that these drugs can be repurposed for the treatment of cancer and neurodegenerative diseases, concurrently with the indications for which they are used.

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REFERENCES

Bahari-Javan, S., Varbanov, H., Halder, R., Benito, E., Kaurani, L., Burkhardt, S. ... Fischer, A. (2017). HDAC1 links early life stress to schizophrenia-like phenotypes. *Proceedings of the National Academy of Sciences of the USA, 6;114(23):E4686-E4694*. https://doi.org/10.1073/pnas.1613842114

Boyd, A. S. (2012). Leflunomide in dermatology. *Journal of the American Academy of Dermatology, 66(4):673-679.* https://doi.org/10.1016/j.jaad.2011.08.025

Cacabelos, R., Torrellas, C. (2014). Epigenetic drug discovery for Alzheimer's disease. *Expert Opinion Drug Discovery, 9(9):1059-1086*. https://doi.org/10.1517/17460441.2014.930124

Cordeiro, A., de Souza, L. L., Oliveira, L. S., Faustino, L. C., Santiago, L. A., Bloise, F. F. . . . Pazos-Moura, C. C. (2013). Thyroid hormone regulation of Sirtuin 1 expression and implications to integrated responses in fasted mice. *Journal of Endocrinology, 216(2):181-193*. https://doi.org/10.1530/JOE-12-0420

Dovey, O. M., Foster, C. T., Cowley, S. M. (2010). Histone deacetylase 1 (HDAC1), but not HDAC2, controls embryonic stem cell differentiation. *Proceedings of the National Academy of Sciences of the USA, 107(18):8242-8247.* https://doi.org/10.1073/pnas.100047810

Ganai, S. A., Abdullah, E., Rashid, R., Altaf, M. (2017). Combinatorial *In Silico* Strategy towards Identifying Potential Hotspots during Inhibition of Structurally Identical HDAC1 and HDAC2 Enzymes for Effective Chemotherapy against Neurological Disorders. *Frontiers in Molecular Neurosciences, 10:357.* https://doi.org/10.3389/fnmol.2017.00357

Gao, Q., Yao, P., Wang, Y., Yao, Q., Zhang, J. (2022). Discovery of potent HDAC2 inhibitors based on virtual screening in combination with drug repurposing. *Journal of Molecular Structure, 1247(5):131399.* https://doi.org/10.1016/j.molstruc.2021.131399

Grassi, G. (2018). Metoprolol in the treatment of cardiovascular disease: a critical reappraisal. *Current Medical Research and Opinion, 34(9):1635-1643.* https://doi.org/10.1080/03007995.2018.1479245

Guan, M., Fousek, K., Chow, W.A. (2012). Nelfinavir inhibits regulated intramembrane proteolysis of sterol regulatory element binding protein-1 and activating transcription factor 6 in castration-resistant prostate cancer. *The FEBS Journal, 279(13):2399-2411*. https://doi.org/10.1111/j.1742-4658.2012.08619.x

Halas, C. J. (2001). Nateglinide. *American Journal of Health System Pharmacy, 58(13):1200-1205* https://doi.org/10.1093/ajhp/58.13.1200

Hirata, Y., Sasaki, T., Kanki, H., Choong, C. J., Nishiyama, K., Kubo, G. ... Uesato S. (2018). New 5-Aryl-Substituted 2-Aminobenzamide-Type HDAC Inhibitors with a Diketopiperazine Group and Their Ameliorating Effects on Ischemia-Induced Neuronal Cell Death. *Scientific Reports, 8(1):1400.* https://doi.org/10.1038/s41598-018-19664-9

Ianiro, G., Mangiola, F., Di Rienzo, T.A., Bibbò, S., Franceschi, F., Greco, A.V., Gasbarrini, A. (2014). Levothyroxine absorption in health and disease, and new therapeutic perspectives. *European Review for Medical and Pharmacological Sciences, 18(4):451-456.*

Johnstone, R.W. (2002). Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nature Reviews Drug Discovery, 1(4):287-299.* https://doi.org/10.1038/nrd772

Kazi, A. A., Reddy, B. V. S., Singh, L. R. (2021). Synthetic approaches to FDA approved drugs for asthma and COPD from 1969 to 2020. B*ioorganic & Medicinal Chemistry 41:116212.* https://doi.org/10.1016/j.bmc.2021.116212

Kitchen, D.B., Decornez, H., Furr, J. R., Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Reviews Drug Discovery, 3(11):935-949.* https://doi.org/10.1038/nrd1549

Lardenoije, R., Iatrou, A., Kenis, G., Kompotis, K., Steinbusch, H. W. M., Mastroeni, D. . . . Rutten, B. P. F. (2015). The epigenetics of aging and neurodegeneration. *Progress in Neurobiology, 131:21-64.* https://doi.org/10.1016/j.pneurobio.2015.05.002

Li, Q., Huang, K., Ma, T., Lu, S., Tang, S., Wu, M., Yang, H., Zhong, J. (2022). Metoprolol Protects Against Arginine Vasopressin-Induced Cellular Senescence in H9C2 Cardiomyocytes by Regulating the Sirt1/p53/p21 Axis. *Cardiovascular Toxicology, 22(2):99-107.* https://doi.org/10.1007/s12012-021-09704-8

Li, Y.Y., Jones, S. J. (2012). Drug repositioning for personalized medicine. *Genome Medicine, 4(3):27.* https://doi.org/10.1186/gm326

Lindemann, R. K., Gabrielli, B., Johnstone, R.W. (2004). Histone-deacetylase inhibitors for the treatment of cancer. *Cell Cycle, 3(6):777-786.* https://doi.org/10.4161/cc.3.6.927

Lindemann, R. K., Gabrielli, B., Johnstone, R.W. (2004). Histone-deacetylase inhibitors for the treatment of cancer. *Cell Cycle, 3(6):777-786.* https://doi.org/10.4161/cc.3.6.927

Müller, B. M., Jana, L., Kasajima, A., Lehmann, A., Prinzler, J., Budczies, J. . . . Denkert, C. (2013). Differential expression of histone deacetylases HDAC1, 2 and 3 in human breast cancer–overexpression of HDAC2 and HDAC3 is associated with clinicopathological indicators of disease progression. *BMC Cancer*, 13:215. https://doi.org/10.1186/1471-2407-13-215

Park, H., Kim, S., Kim, Y.E., Lim, S.J. (2010). A structure-based virtual screening approach toward the discovery of histone deacetylase inhibitors: identification of promising zinc-chelating groups. *ChemMedChem,* 5(4):591-597. https://doi.org/10.1002/cmdc.200900500

Park, S. Y., Kim, J. S. (2020). A short guide to histone deacetylases including recent progress on class II enzymes. *Experimental & Molecular Medicine, 52(2):204-212.* https://doi.org/10.1038/s12276-020-0382-4

Pushpakom, S., Iorio, F., Eyers, P.A., Escott, K.J., Hopper, S., Wells, A. . . . Pirmohamed, M. (2019). Drug repurposing: progress, challenges and recommendations. *Nature Reviews Drug Discovery, 18(1):41-58.* https://doi.org/10.1038/nrd.2018.168

Serebryannyy, L.A., Cruz, C.M., de Lanerolle, P. (2016). A Role for Nuclear Actin in HDAC 1 and 2 Regulation. *Scientific Reports, 6:28460.* https://doi.org/10.1038/srep28460

Spiegel, S., Milstien, S., Grant, S. (2012). Endogenous modulators and pharmacological inhibitors of histone deacetylases in cancer therapy. *Oncogene. 31(5):537-551.* https://doi.org/10.1038/onc.2011.267

Taunton, J., Hassig, C.A., Schreiber, S. L. (1996). A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science. 272(5260):408-411.* https://doi.org/10.1126/science.272.5260.408

Vishwakarma, S., Iyer, L.R., Muley, M., Singh, P.K., Shastry, A., Saxena, A., Narayanan, S. (2013). Tubastatin, a selective histone deacetylase 6 inhibitor shows anti-inflammatory and anti-rheumatic effects. *International Immunopharmacology, 16(1):72-78.* https://doi.org/10.1016/j.intimp.2013.03.016

Wang, J., Yu, J.T., Tan, M.S., Jiang, T., Tan, L. (2013). Epigenetic mechanisms in Alzheimer's disease: implications for pathogenesis and therapy. *Ageing Research Reviews, 12(4):1024-1041.* https://doi.org/10.1016/j.arr.2013.05.003

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