Applications of Computational Methods and African Natural Product Databases to Search for Novel Inhibitors of Histone Deacetylases

DISSERTATION

zur Erlangung des akademischen Grades
Doctor rerum naturalium (Dr. rer. nat.)
der Naturwissenschaftlichen Fakultät I Biowissenschaften
der Martin-Luther-Universität Halle-Wittenberg

von

Herrn M.Sc. Conrad Simoben, Veranso

geboren am 19. Apr. 1990 in Nkambe

Gutachter:

Prof. Dr. Wolfgang Sippl, Halle

Prof. Dr. Stefan Günther, Freiburg

Prof. Dr. Dusanka Janezic, Ljubljana

Datum der Disputation: 23 March 2021

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Abstract

Computational methods have been proven to be shortcuts in developing novel lead compounds and drug candidates. They can cut down the cost as well as reduce the time involved. For example, computer-aided drug discovery (CADD) methods have been used to guide the discovery of histone deacetylase (HDAC) inhibitors. HDACs represent an interesting class of Zn-dependent enzymes and they have been demonstrated to play important physiological roles associated with genome functions that have been linked to several diseases. In this thesis, several computational methods were applied to suggest novel molecules that could target HDAC isoforms. First, a new class of HDAC small inhibitor ("reverse hydroxamate" as zinc-binding motif) was discovered by virtual screening and confirmed to inhibit *Schistosoma mansoni* HDAC8 (*sm*HDAC8) with IC_{50} values in the low micromole range. Additional testing of the molecules indicated that they were equally potent inhibitors of human HDAC1, 6 and 8. The binding mode of the best hit could be confirmed by X-ray crystallography. Second, using virtual screening, a set of molecules with reverse HIV latency was discovered. They may act as a starting point in a structure-based design and/or in chemical optimization efforts to improve the HIV shock-and-kill-based strategy. Third, a procedure of using computed binding free energy was developed to generate quantitative structure-activity relationship (QSAR) models to optimize benzhydroxamates as *sm*HDAC8 inhibitors based on *in silico* predictions. Simultaneously, screening of novel modulators of HDACs from natural sources was carried out. For this purpose, the current work contributed in constructing an online database for natural products (NPs) isolated from African source species (http://african-compounds.org/anpdb/). Analysis of the developed databases showed that African NPs occupy chemical spaces that were not previously reported in published NP databases and contain compounds similar to HDAC inhibitors. This renders the developed database as a possible source of novel HDAC modulators and compounds with other biological activities. Thus, the presented results demonstrate and support the idea that computational approaches can readily identify novel HDAC modulators (for example to treat parasitic diseases and HIV) as well as provide the digital source ready to search for such biologically active molecules.

Keywords: African NP Database, CADD, Cheminformatics, Histone Deacetylase Inhibitor, Human Immunodeficiency Virus, Latency Reversal Agent, Molecular Dynamics, Natural Product, Pharmacoinformatic, QSAR, Schistosomiasis.

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Kurzfassung

Es hat sich gezeigt, dass computer-basierte Methoden eine Abkürzung für die Markteinführung neuer Arzneistoffe sind. Sie sind in der Lage, sowohl die Kosten als auch den Zeitaufwand für die Entwicklung neuer Wirkstoffe zu reduzieren. In ähnlicher Weise wurde in dieser Arbeit computergestütztes Wirkstoffdesign (CADD - Computer-Aided Drug Discovery) genutzt, um die Entdeckung von Histondeacetylase (HDAC)-Modulatoren zu beschleunigen. HDACs sind eine interessante Klasse von Zn-abhängigen Enzymen. Sie spielen wichtige physiologische Rollen im Zusammenhang mit Genomfunktionen, die mit verschiedenen Krankheiten in Verbindung gebracht werden. In der aktuellen Arbeit wurden mehrere computer-basierte Methoden angewandt, um neue Moleküle vorzuschlagen, die auf HDACs abzielen könnten. Erstens wurde eine neue Klasse von HDAC Inhibitoren ("Reverse-Hydroxamat" als zinkbindendes Motiv) durch virtuelles Screening entdeckt, die eine Hemmung von *Schistosoma mansoni* HDAC8 (*sm*HDAC8) mit IC_{50}-Werten zwischen 4,4 und 20,3 µM zeigte. Ein zusätzliches Screening der Moleküle zeigte, dass sie gleich starke Inhibitoren der menschlichen Isoformen HDAC1, 6 und 8 waren. Zweitens wurde eine Reihe von Molekülen mit umgekehrter HIV-Latenzzeit mittels virtuellem Screening entdeckt. Diese Verbindungen können als Ausgangspunkt für ein strukturbasiertes Design und/oder chemische Optimierungsbemühungen zur Verbesserung der auf dem ,,HIV-Schock-und-töten‘‘-basierten Strategie dienen. Drittens wurden berechnete freie Bindungsenergie zur Erzeugung quantitativer Struktur-Wirkungs-Beziehungsmodelle (QSAR) benutzt, um Benzhydroxamaten als *sm*HDAC8-Inhibitoren auf der Grundlage von *in silico*-Vorhersagen weiter optimierten zu können. Gleichzeitig waren wir an der Suche nach neuartigen Modulatoren von HDACs aus natürlichen Quellen interessiert. Zu diesem Zweck trugen wir zum Aufbau einer Online-Datenbank für Naturstoffe (NPs) bei, die aus Afrikanischen Ausgangsspezies isoliert wurden (http://african-compounds.org/anpdb/). Die Analyse der entwickelten Datenbank zeigte, dass die Afrikanische NPs chemische Räume besetzen, die bisher in anderen veröffentlichten NP-Datenbanken nicht erfasst wurde und dass sie Verbindungen enthalten, die beschriebenen HDAC-Inhibitoren ähnlich sind. Unsere Datenbank ist somit eine vielversprechende Quelle für die Suche nach neuen HDAC-Modulatoren und auch Verbindungen mit anderen biologischen Aktivitäten.. Die vorgestellten Ergebnisse demonstrieren und unterstützen die Idee, dass computer-gestützte Ansätze sowohl neue HDAC-Modulatoren (z.B. zur Behandlung von parasitären Krankheiten und HIV) identifizieren können als auch die digitalen Quellen für die Suche nach solchen biologisch aktiven Molekülen bereitstellen können.

Schlagwörter: Afrikanische NP-Datenbank, CAAD, Chemoinformatik, Histon-Deacetylase-Inhibitor, Humanes Immundefizienz-Virus, Latenz-Umkehrmittel, Molekulardynamik, Naturprodukt, Pharmakoinformatik, QSAR, Schistosomiasis.

Acknowledgements

I am deeply grateful to my supervisor Prof. Dr Wolfgang Sippl whose enthusiasm and energy transformed my vision of this project into reality. His commitment and sense of mission have continuously elevated me. I also would love to extend sincere gratitude to the network of collaborations and collaborators especially Prof. Dr Manfred Jung, Prof. Dr Stephan Günther, Prof. Dr Raymond J. Pierce, Dr Christophe Romier to achieve the results presented herein.

I express special thanks:

- To Dr Dina Robaa and Dr Fidele Ntie Kang for their unstoppable support. I heartily appreciate the time you dedicated to discussing scientific problems, teaching and helping me grow, I say thank you.
- To my colleagues, for the professional and friendly atmosphere they created.
- To all my friends. I am thankful for all the supports and encouragements you rendered in keeping me on the path to achieving the successes we longed for.
- To my family. My parents who are my fervent pillars lifting me higher every day. To my siblings, for their encouragement and trust in me. To my in-laws, for the perfect timing in making things right.
- And most of all, to the Martin-Luther-Universität Halle-Wittenberg for providing the conducive and reliable environment for the pursuit of the projects reported herein; and finally my deepest gratitude goes the German Academic Exchange Service (DAAD) for financial support throughout my research stay for the realization of the projects whose results are presented herein.

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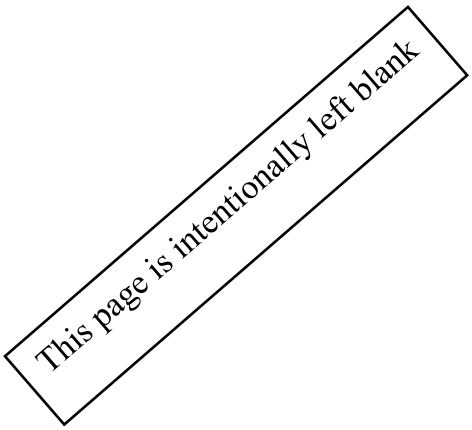
Dedication

To God and my parents; Richard Mengnjo Veranso and Emelda Kingah Venyena; who have raised me to be the person I am today. You were my first teachers and have been with me through every step of the way. Thank you for all the unconditional love.

"I think the Chinese proverb 'One picture is worth ten thousand words' is the best way to describe why the computer is so important to pharmacology. Except that the Chinese probably underestimated the number of words."

~Robert Langridge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List of Abbreviation

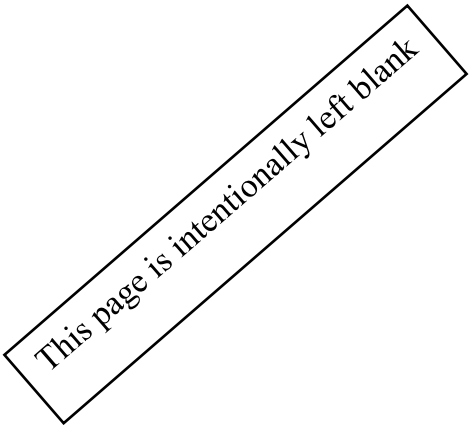
Abbreviation	Full
2D	Two-dimensional
3D	Three-dimensional
AIDS	Acquired immunodeficiency syndrome
AMBER16	Assisted model building with energy refinement version 16
ANPDB	African Natural Product Database
ART	Antiretroviral therapy
CADD	Computer-aided drug discovery
cART	Combination antiretroviral therapy
CD4+ T cells	Cluster of differentiation 4 on the T-helper cells
Chinese NMPA	Chinese National Medical Products Administration
EANPDB	Eastern African Natural Product Database
FDA	Food and Drug Administration
GAFF	Generalized amber force field
GB	Generalized Born
Hda1 yeast protein	Histone deacetylase gene 1 yeast protein
HDAC	Histone deacetylase
HDACi	Histone deacetylase inhibitor
HIV	Human immunodeficiency virus
HTS	High-throughput screening
IC_{50}	Half-maximal inhibitory concentration
LBDD	Ligand-based drug design
LRA	Latency reversing agents
MD	Molecular dynamics
MM	Molecular mechanics
MM/QM-GB/SA	Quantum mechanics/molecular mechanics Poisson-Boltzmann (Generalized Born) surface area.

MM-GB/SA	Molecular mechanics generalized Born surface area
MM-PB(GB)/SA	Molecular mechanics Poisson-Boltzmann (generalized Born) surface area.
MOE	Molecular operating environment
MW	Molecular weight
NAD^+	nicotinamide adenine dinucleotide
NANPDB	Northern African Natural Product Database
NP	natural product
PB	Poisson-Boltzmann
PDB	Protein data bank
PM3	Parameteric method 3
QM	Quantum mechanics
QM/MM	Hybrid quantum mechanics/molecular mechanics
QSAR	Quantitative structure-activity relationship
RCSB	Research collaboratory for structural bioinformatics
RGC	Receptor guanylate cyclase
rmsd	Root mean squared deviation
Rpd3 yeast protein	Reduced potassium dependency 3 yeast protein
SA	Surface area
SASA	Solvent-accessible surface area
SBDD	Structure-based drug design
SMILES	Simplified molecular-input line-entry system
vdW	van de Waal
VS	Virtual Screening
ZBG	Zinc-binding group

1. Introduction

"All who have meditated on the art of governing mankind have been convinced that the fate of empires depends on the education of youth."

~ Aristotle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1.1 HDACs in Biology

Epigenetic modifications such as the post-translational histone modifications by acetylation and/or deacetylation processes regulate gene expression *via* chromatin remodelling.[1,2] The acetylation status of histones is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). An upset in this balance leads to hyperacetylation (gene overexpression) or hypoacetylation (gene suppression) by HATs or HDACs, respectively. This upset has been associated with the pathology of several diseases, including cancer, neurodegenerative disorders, as well as parasitic and viral infections.[3-6] Hypoacetylation of lysine residues *via* the removal of acetyl groups by HDACs leads to positively charged histone lysine residues which then binds tightly to the negatively charged DNA phosphate group resulting in chromatin compaction (Figure 1).[7-9]

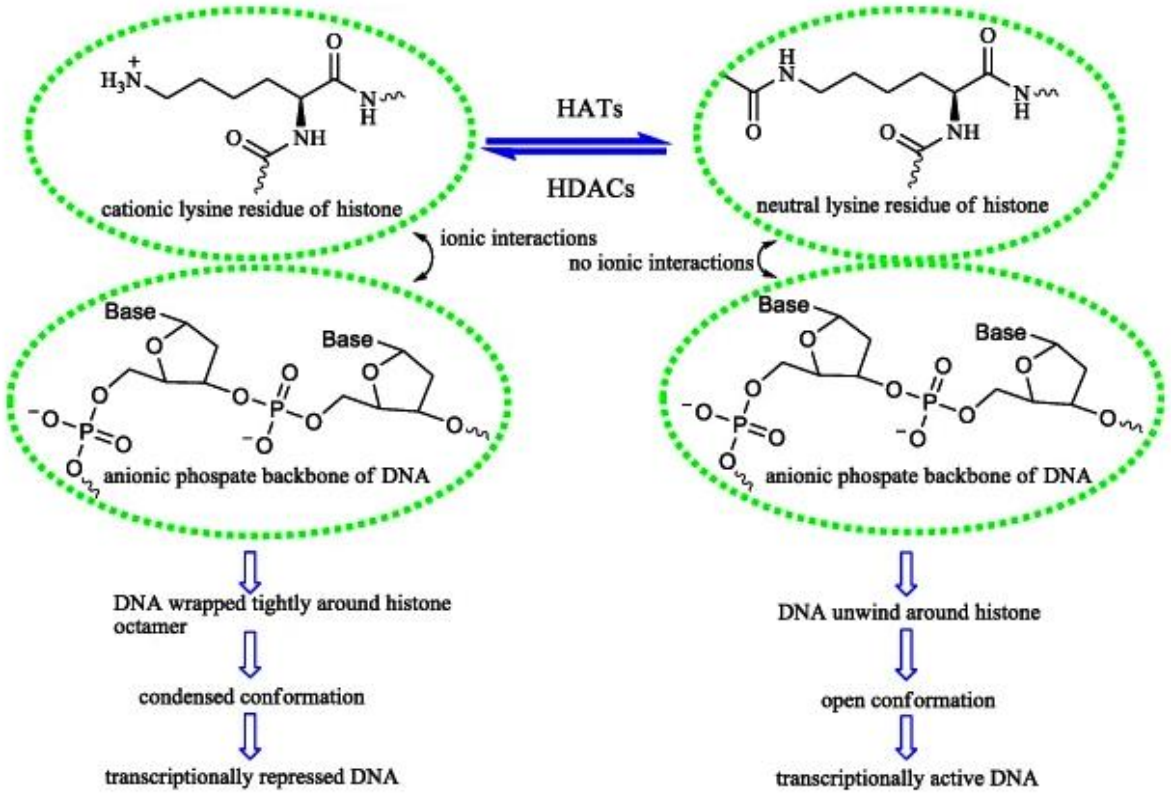


Figure 1: The effect of acetylation and deacetylation of histone (figure reproduced with permission).[10]

1.1.1 Classification of HDACs

Detailed biochemical studies show that 18 mammalian HDACs have been identified so far, and are divided into 4 classes, based on their sequence homology to yeast.[11] The different classes have distinct and/or unique characteristics such as cofactor(s), subcellular localization, and tissue distribution.[11-14] For instance, classes I, II, and IV HDACs require zinc ion as a cofactor, whereas class III HDACs (known as the sirtuins) are NAD^+-dependent enzymes. In this study, our focus will be on the zinc-dependent HDACs (popularly referred to as the classical HDACs) which are grouped as follows: i) class I HDACs (HDAC1, 2, 3 and 8) are located mainly in the nucleus and are homologous to the Rpd3 yeast protein, ii) class II HDACs are homologous to the Hda1 yeast protein and can shuttle between cytoplasm and nucleus, they are further grouped into class IIa (HDAC 4, 5, 7, and 9) and class IIb (HDAC 6 and 10) and iii) class IV HDACs predominantly located in the nucleus is solely occupied by HDAC11.

1.1.2 Structure of HDACs

HDACs share a common structural similarity, although they are grouped into different subgroups, based on their sequence homology to yeast and their diverse functionality.[11,15] Members of the class I subgroup (HDAC1, 2, 3 and 8), for example, have a high degree of sequence homology to yeast Rpd3 with approximately 80 % similarity and 66 % identity.[16,17] The proteins in this group consist of ~ 400 amino acid residues with short N- and C-terminii making up almost the entire catalytic domain.[16,17] On the other hand, yeast Hda1 is the founding member of class II HDACs (class IIa: HDAC4, 5, 7 and 9; class IIb: HDAC6 and 10).[18-22] Class II HDACs are characterized with about 600 to 1000 amino acid residues forming an extended N-terminus with regulatory functions in addition to the C-terminus (class IIa)[18,21,22] or they possess two deacetylase domains (Class IIb).[19-21] It is noteworthy to mention that studies on the two deacetylase domains of HDAC6 remain indecisive if both domains are catalytically active although both domains might be required for the proper functioning of the enzyme.[19] HDAC10 similarly possesses two catalytic domains; one catalytic domain on its N-terminus functions as polyamine deacetylase while the putative second catalytic domain on the C-terminus lacks the conserved HDAC features and enzymatic activity.[20] Finally, the youngest classified member of the HDAC family is HDAC11, which is the only representative of class IV, due to its overall low sequence similarity to class I and II HDACs or the SIR2 family.[23] HDAC11 is estimated to be of similar length (347 amino acid residues) to class I HDACs and has a proven catalytic domain situated at the N-terminus. Conclusively, for every HDAC, there is at least one deacetylase domain present which represents a key structural feature of this protein family.

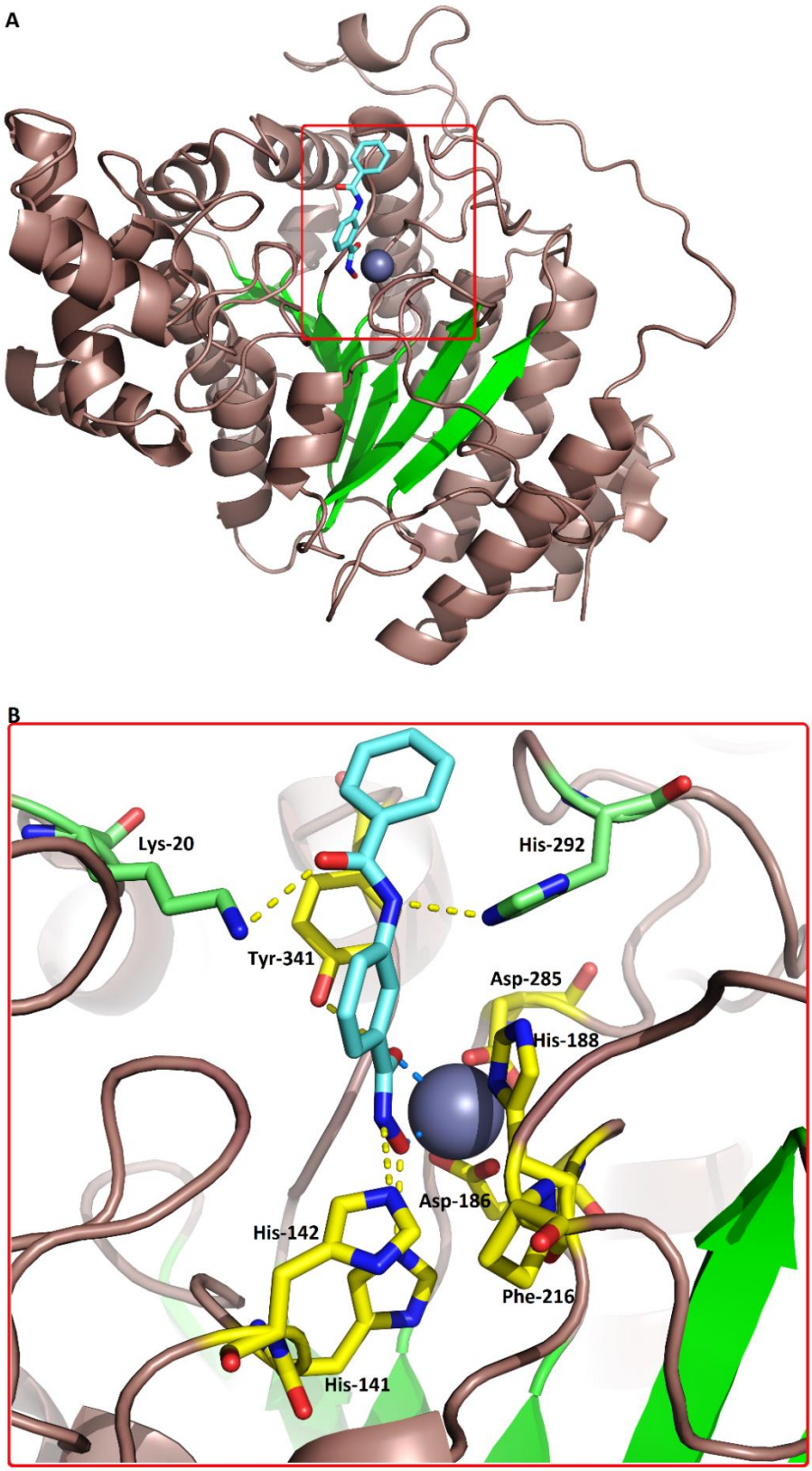


Figure 2: Representation of the general structure of HDACs using the crystal structure of *sm*HDAC8 (PDB ID: 5FUE). A) The central β-sheets (green) sandwiched by α-helices (brown) and surrounded by loops (brown ribbons). B) Zoom-in of the catalytic pocket showing some of the conserved residues (yellow sticks). Coordination to zinc ion by the co-crystallized ligand is represented with light blue dashed lines while the hydrogen bond interactions between the ligand and the protein are shown as yellow dashed lines. In both figures, the ligand and the catalytic zinc ion are shown as cyan sticks and bluish-grey sphere, respectively.

Resolved crystal structures of the deacetylase domain confirm that there is a conserved architecture (Figure 2A). The general structure of the deacetylase domain contains a central eight-stranded parallel β-sheet sandwiched between several α-helices connected by loops of varying lengths. The binding pocket (called the lysine-binding channel) found in all HDACs is located at the centre of the domain and adopts a funnel-shape. This pocket is characterized by conserved amino acid residues (His-141, His-142, Asp-186, His-188, Phe-216, Asp-285 and Tyr-341) (Figure 2B) organized around a catalytic zinc ion and participates in the catalytic process of enzymatically active HDACs. Two of the conserved residues (Asp-186 and Asp-285) plus an additional non conserved residue (His-188) coordinate the active site zinc ion. The binding pocket is around 11 Å deep and is surrounded by flexible loops at the rim.[24-26] An interesting aspect of the binding pocket is the possibility of extending it by opening sub-pockets that are closed depending on the HDAC isoform. Examples of such pockets include the foot pocket present in class I HDACs and the lower pocket present in class IIa HDACs.[24-26]

1.1.3 Catalytic Mechanism of the Classical HDACs

The search for specific substrate for the classical HDACs remains challenging due to the difficulties associated with the low measurable HDAC activity after purification of HDACs to homogeneity as well as the functional redundancy (the compensation of activity of one classical HDAC by another) of many HDACs.[27,28] For example, according to Hu *et al*.,[29] HDAC8 preferentially deacetylate H3 and H4 while HDAC11 might specifically deacetylate Histone H3 Lysine 9 (H3K9) and Histone H3 Lysine 14 (H3K14). Similarly, the deacetylation of none histone proteins by HDACs have also been reported and this process controls several cellular process.[27] However, since the class I, II and IV HDACs are Zn-dependent enzymes, they are thought to proceed through a similar catalytic mechanism.[19,30] Conserved amino acid residues (two His-Asp dyads, Tyr) within the binding site control the catalytic process of the enzymes. Figure 3 illustrates the proposed mechanism within the active site of HDAC8.[30] The process is activated when the catalytic Zn^{2+} ion and the Tyr-341 residue polarize the carbonyl group of the acetylated lysine tail by coordinating to the acetyl oxygen. A nucleophilic attack on this polarized acetyl carbonyl carbon by a water molecule activated by a His-141 residue results in a tetrahedral intermediate that is stabilized by the Zn^{2+} ion and the Tyr-341. Worthy to be noted is the reported reduced catalytic activity of class IIa HDACs, which is explained by the replacement of this Tyr-341 residue by a His residue in the binding site.[28] The tetrahedral intermediate subsequently collapses when the protonated His-141 residue acts as the general acid to protonate the ε-amine leaving group, yielding acetate and lysine products.

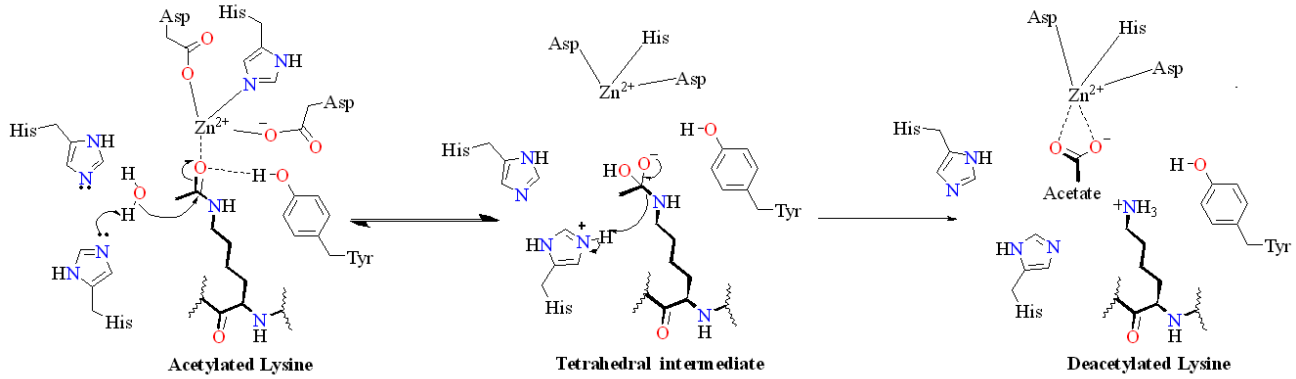


Figure 3: Proposed catalytic mechanism of classical HDACs.

1.2 HDACs in the Treatment of Diseases

Illnesses and conditions have been with man for millennia, and is still, and will be an obvious challenge to human health as predicted by experienced observers.[31-37] This is a serious burden to the scientific community, pulling the investment of more funds in the search of new drugs to treat them, especially the rare and globally threatening ones. In recent years, HDACs have been validated as promising targets for the treatment of several ailments.[3-6] Interest in these HDACs has led to the approval of several HDAC inhibitors (HDACis) for the treatment of cancer while many other drug candidates are in clinical trials.[38] Indeed, the successful targeting of HDACs for treating cancer has been extended to a multitude of diseases including neurodegenerative disorders, parasitic and viral infections which involve major modifications such as morphology, gene expression amongst others.[3-6] Interestingly, several HDACis have been shown to manifest a broad spectrum antiprotozoal activity. This thesis focuses mainly on schistosomiasis and human immunodeficiency viruses (HIV); their implications, how they affect the community and new strategies to treat them by targeting HDACs.

1.2.1 Schistosomiasis

Schistosomiasis (bilharzia) is an intravascular parasitic infection in humans caused by *Schistosoma spp*. It is one of the neglected parasitic diseases ravaging hundreds of millions of humans in underprivileged communities (e.g. in parts of the Middle East, South America, Southeast Asia and, particularly, in sub-Saharan Africa) for many decades now.[39,40] Parasites require a vector and one or more intermediate hosts to be carried around/transported as part of their life cycle. In the case of schistosomes (Figure 4),[39,40] the miracidia (ciliated larvae within the schistosome egg) are hatched into water (usually slowly flowing water). The hatched miracidia incubate freshwater snails to develop and release fork-tailed cercariae that will swim up to the surface of the water and fall back towards the bottom until they find a human host. These fork-tailed cercariae access their host by penetrating the skin of humans paddling, bathing

and/or washing in the water. Although schistosomiasis is a preventable disease, it still affects millions of people with an estimated 290 million people requiring preventive treatment and about 98 million reported by WHO to have been treated in 2018.[41]

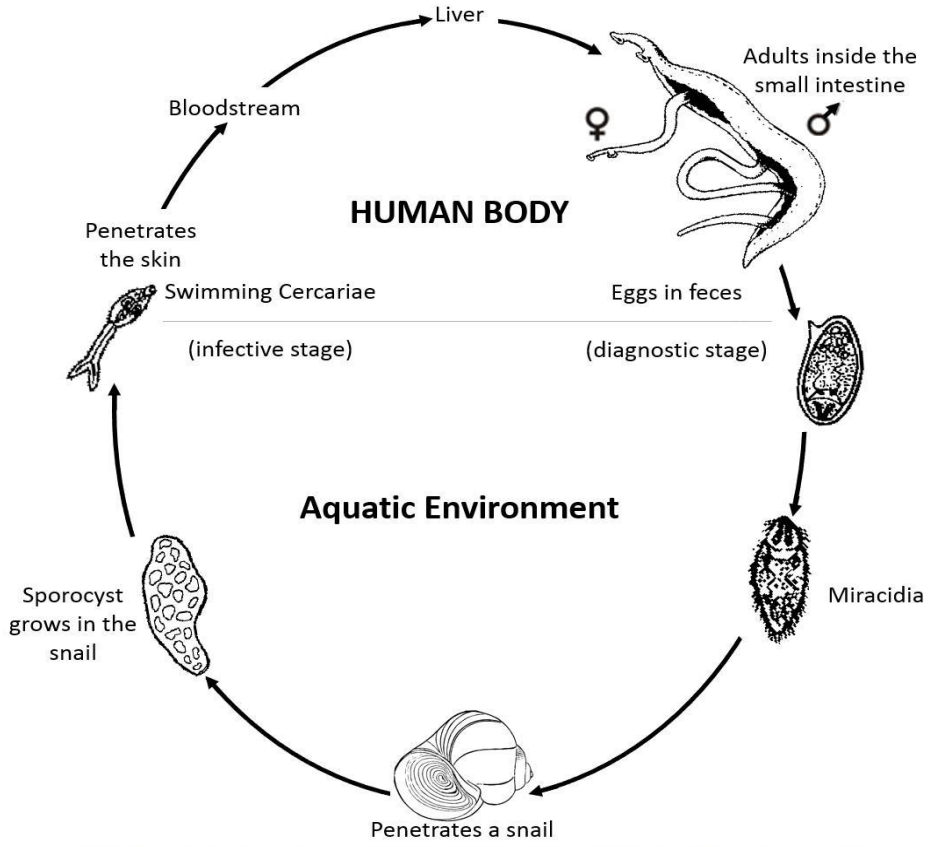


Figure 4: The life cycle of *Schistosoma mansoni* (reproduced with permission from https://www.flickr.com/photos/gtzecosan/15893494112 (accessed 10 June 2020))

Chronic infection of schistosomiasis is associated with long-term under-nutrition, anaemia, organ scarring and fibrosis, resulting in disabling patient symptoms.[42-44] Many programs (national and international) to control and/or prevent *Schistosoma* infections have been implemented. However, with no available vaccine and the only drug of choice "praziquantel" (a low-cost and highly effective orally administered drug against all *Schistosoma spp* as a single dose with no notable side effects[45,46]) is already having negative reports in terms of selectivity and resistance.[47-49] Thus, there is a need to search for new small molecules to treat the disease.[42,46,47,50] Interestingly, several studies in recent years have confirmed the emergence of HDACs as attractive therapeutic targets to target the parasite's epigenome as a means to treat schistosomiasis amongst other parasitic ailments.[3,6,51,52]

Interestingly, parasitic Zn- and NAD^+-dependent HDACs have been identified and shown to play crucial roles in modulating the expression of genes in several major human-infecting parasites.[6,52] Hence, parasitic HDACs have emerged as novel potential antiparasitic targets, because some of the gene expressions are pro-survival for several parasites under various

conditions.[6,50-52] Downregulation of HDACs in *Schistosoma mansoni*, for example, is confirmed to reduce the survival chances of the pathogen in infected mice.[53] Moreover, testing of several HDACis demonstrated that they do penetrate the parasite and induce mortality.[54] Several research groups have, thus, embarked on this route to search for novel molecules to treat schistosomiasis.[46,51,55,56] While looking for HDACis to treat parasitic infections, the pathogen's HDACs become the focus (main target) and the human HDACs are treated as off-targets potentially causing unwanted side effects. The most studied and targeted isoform for *S. mansoni* is its HDAC8 (*sm*HDAC8). This has been an interesting route so far in the search of new *sm*HDAC inhibitors, because the human counterpart (*hs*HDAC8) is only expressed in a high amount within unhealthy/infected human cells.[57-61] Thus, the development of small-molecule *sm*HDAC8 inhibitors represents a promising approach for the treatment of schistosomiasis.

1.2.2 Human Immunodeficiency Virus (HIV)

The human immunodeficiency virus (HIV) is the causative agent of the acquired immunodeficiency syndrome (AIDS) which breaks down the immune system, thus paving the way to opportunistic infections affecting millions of persons.[62,63] Transmission of HIV is mainly *via* the exchange of a variety of body fluids (e.g. blood, breast milk, semen and vaginal secretions) from infected people to healthy people.[64,65] Although the survival rate from HIV/AIDS infections has increased due to the continuous efforts put in place to sensitize and educate people, the disease has claimed ~ 32 million lives so far.[66,67] By the end of 2018, it was estimated that about 37.9 million people were living with HIV (with over two-thirds of these people residing in the WHO African Region).[66,67] One of the key approaches in fighting HIV/AIDS infection is currently with the use of a highly active combination antiretroviral therapy (cART) to durably control/suppress HIV, thus, reducing the chances of infected individuals to infect others.[68-70] A drawback to the use of only cART is that the patients are prone to take it for their whole life. This is because the cART does not act on resting CD4+ T cells containing latent proviral reservoirs which can reactivate at any time to produce infectious virus.[71,72] Therefore, new approaches to eradicate HIV are needed.

A recent strategy, "shock-and-kill",[71,73-75] has been proposed as a means of identifying and eliminating HIV reservoir-containing cells.[76] This is a strategy to eradicate the pool of latently infected cells.[71,73-76] The "shock-and-kill" strategy uses latency (period of non-productive replication) reversal agents (LRAs) to initially stimulate the virus production (i.e., "shock") by activating cells harboring HIV.[71,75] These identified cells are then eliminated through apoptosis or immune-enhancing mechanisms ("kill"), while concurrently using cART to prevent reservoir

re-seeding.[68,72] An important functional class of the LRAs are inhibitors of class I HDACs.[4,74,75,77-79] It has been presented with evidence that HDACs regulate the transcription of numerous cellular and viral genes, resulting in the removal of important docking signals that are required for binding of activating transcription factors.[80] Remarkably, overexpression of class I HDACs in resting CD4+ T cells have been described.[81,82] Hence, targeting proviral sites of the CD4+ T cells in HIV infected individuals with class I-selective and improved efficacy HDACis as potent inducers of the viral expression is a promising strategy. Indeed, several works in this regard have yielded the identification and crystallization of novel molecules such as the ethyl ketones (PDB ID: 6WBW, 6WBZ) acting on HDAC1 as LRAs.[83] Thus, giving hopes to HIV/AIDS patient that the administration of cART might no longer be a life sentence anymore.

1.3 HDAC Inhibitors (HDACis)

Inhibitors have been successfully used to regulate the activity of HDACs. Interestingly, five HDACis (vorinostat (SAHA) (**1**),[84] romidepsin (FK228) (**2**),[85] belinostat (PXD-101) (**3**),[86] panobinostat (LBH-589) (**4**)[87] and chidamide (**5**)[88] (Figure 5)) have been approved for the treatment of several cancer types while several other drug candidates are in clinical trials.[89,90] Many of these drugs, which are pan HDACis also show unfavourable properties for drug development such as low potency and poor selectivity.[91] Reported HDACis respect the widely accepted simple classical pharmacophore model proposed by Jung *et al*.[92,93] This pharmacophore model consists of three main pharmacophoric features: the zinc-binding group (ZBG; to coordinate the catalytic zinc ion), the linker (placed in the hydrophobic substrate-binding tunnel) and the capping group (cap; to interact with amino acid residues at the rim of the pocket).

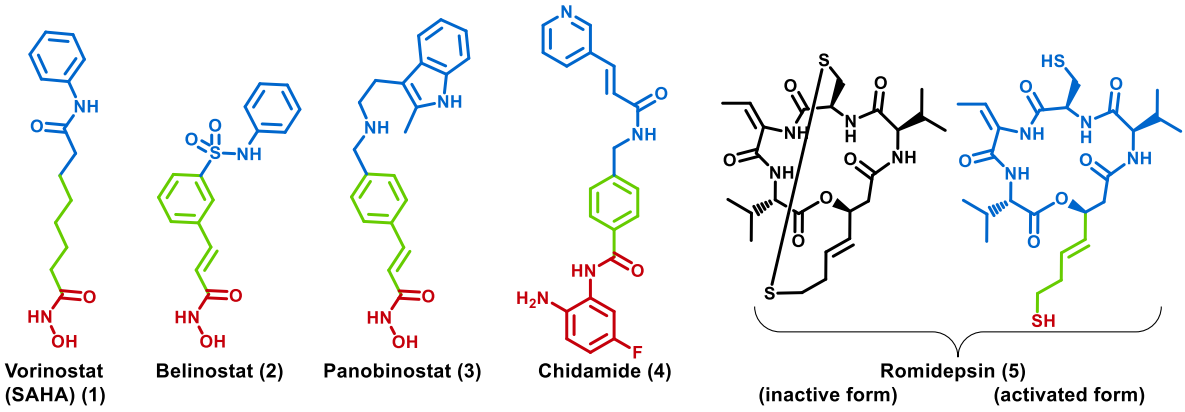


Figure 5: Structural features of approved HDACis. For each molecular structure, blue, green and red colours indicate the cap, linker and ZBG group respectively.

More insights into the HDAC structure has prompted new strategies that can be used to develop novel and/or isoform-selective HDACs. The exploration of target isoform-specific regions (such as Side Pocket,[94] Lower Pocket,[94] Foot Pocket[95]), which could either be closed or open within

and around the binding pocket are now being explored to generate more selective HDACis.[96] Curiosity to getting more insights into the effect of these molecules on HDACs has led to the crystallization of several complexes.

1.3.1 Synthetic HDACis

Chemically, most of the investigated HDACis as well as approved HDAC inhibiting drugs are synthetic molecules and an overview of these molecules and their synthetic routes have been provided by Peng *et al*.[97] Some of these synthetic HDAC inhibiting molecules include the approved HDACi drugs compounds **1** - **4** (Figure 5). Vorinostat (SAHA) (**1**) was the first FDA approved HDACi in 2006 and is used for the treatment of cutaneous T-cell lymphoma.[98] However, it is reported to act as a pan-HDACi with relatively low K_i values on all eleven human isoforms in the range of 20 - 173 nM. Similarly, panobinostat (**3**), approved by the FDA in 2015 for the treatment of multiple myeloma also inhibits a broad spectrum of HDACs with IC_{50} values ranging from 0.6 - 22 nM and is currently the most potent HDACi drug available in the market.[99,100] Interestingly, in 2014, two synthetic HDAC molecules; belinostat (**2**) and chidamide (**4**) were both approved for the treatment of relapsed or refractory peripheral T-cell lymphoma by the FDA and the Chinese NMPA respectively. While belinostat (**2**) demonstrated pan-HDAC inhibitory activity (IC_{50} values in the range of 10-59 nM against all eleven HDAC isoforms),[101] chidamide (**4**) on the other hand selectively inhibits HDACs 1, 2 and 3 with IC_{50} of 95, 160 and 67 respectively.[102,103]

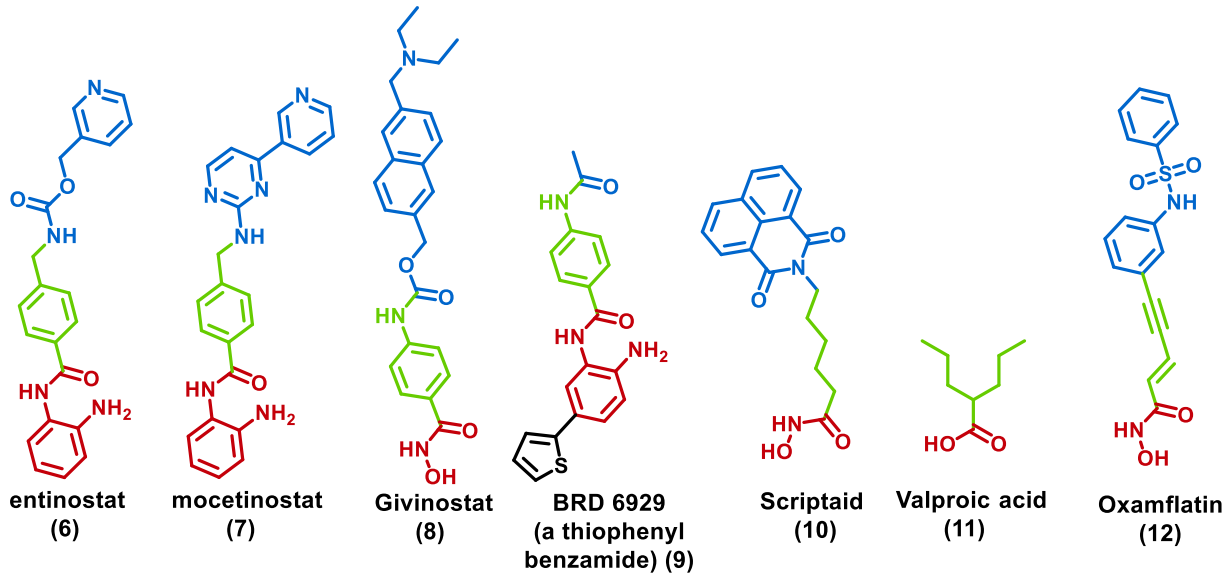


Figure 6: Some synthetic HDACis.

In a similar way, newly designed analogues (entinostat (**6**) and mocetinostat (**7**); Figure 6) of chidamide (**4**) with the same ZBG showed similar selectivity pattern. These analogues showed preferential inhibition towards HDAC1, 2, 3 and 11 with IC_{50} values of 250 - 2700 and 60 - 200

nM for **6** and **7**, respectively. For both molecules, no significant inhibition up to 30 µM concentration was observed for HDACs 4 and 7.[104,105] Interestingly, mocetinostat (**7**) is currently in phase III clinical trials for the treatment of lymphoma, urothelial carcinoma, relapsed and refractory, myelodysplastic syndrome and metastatic leiomyosarcoma. Another synthetic HDACi of interest is givinostat (ITF2357; **8**), a potent inhibitor of both class I and II HDACs and already in phase III clinical trials for the treatment of polycythemia vera, juvenile idiopathic arthritis, Duchenne muscular dystrophy (DMD), chronic myeloproliferative neoplasms, and polyarticular-course juvenile idiopathic arthritis.[106]

The thienyl benzamide (BRD 6929; **9**) has also been reported to show high selective potency (greater than 100-fold selectivity) against HDAC1 and HDAC2 ((IC_{50} = 10-60 nM)) when compared to other HDACs (including HDAC3).[107] The crystal structures of HDAC2 with **9** (PDB ID: 4LY1) shows that the thiophene group attached in *para*-position of the anilide moiety occupies a pocket at the foot of the binding cavity. This pocket is not present in other HDAC isoforms and could be a possible explanation for its selectivity. It also coordinates the catalytic Zn-ion with similar groups of atoms like in compounds **4**, **6** and **7**. Specific potency towards class I HDACs has also been reported for scriptaid (**10**) and valproic acid (**11**). Scriptaid (**10**) was identified by Su *et al*.,[108] *via* a high-throughput transcriptional screening and has the potential to be used for the treatment of one of the most challenging solid cancers; namely, glioblastoma multiforme (GBM), due to its ability to induce apoptosis in glioblastoma cells. Valproic acid (**11**) on the other hand has therapeutic mechanisms of actions that are still not well understood. Nevertheless, this branched short fatty acid inhibits HDAC1 (IC_{50} of 0.4 nM) and is being investigated for the treatment of HIV and various cancers.[109,110] Oxamflatin (**12**), a pan-activity HDACi, was shown to have strong cytostatic effects and potential toxicity against a variety of tumour cell lines such as the ovarian cancer cells at nM concentrations.[111]

1.3.2 Natural Products (NPs) as HDAC Modulators

The diversity of NPs (structurally, chemically, biologically) developed over millions of years of evolution explains their use in traditional medicines such as the Indian Ayurveda,[112] traditional Chinese medicine[113] or African herbal medicines[114] for centuries in primary health care system.[115-118] Thus, revisiting of nature (NPs), which had worked in the past as seen by the multiple drugs obtained from natural sources (Figure 7), can revolutionize and lead to the discovery of novel and potent drug molecules.

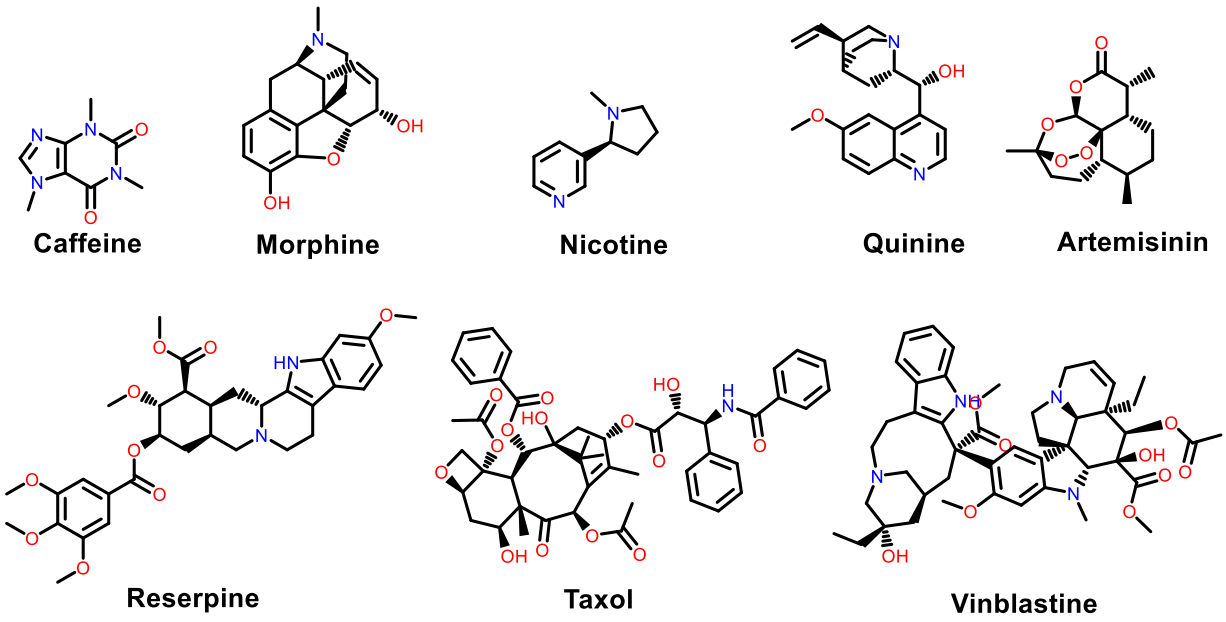


Figure 7: Some of the approved NP molecules for the treatment of diseases.

Even with the diminished focus on NPs by the major pharmaceutical companies, scientific studies show that NPs still account for about half of the drugs approved by the FDA, especially antibiotic and anticancer drugs (Figure 8).[119-128] This undisputable fair share contribution of NPs in modern drug discovery is attracting heavy investments that are leading to the isolation, characterization and possible biological evaluations and establishment of the mechanisms of actions of many NP molecules.[129-132] The bulkiness and complex structural representation of some NPs still challenges and inspires talented synthetic chemists of nowadays.[130,133-138] Several studies have confirmed the advantages of NPs over combinatorially synthesized compounds as a promising source for drug discovery with biologically relevant and privileged scaffolds.[139-146] For example, principal component analysis (PCA) of compounds using simple descriptive features like lipophilicity, the number of chiral centres, molecular weight (MW), the prevalence of aromatic rings, the introduction of complex ring systems, number of rotatable bonds, the degree of the saturation of the molecule, as well as the number and ratios of different heteroatoms have been used to analyze the chemical space of these molecules.[134,147-149]

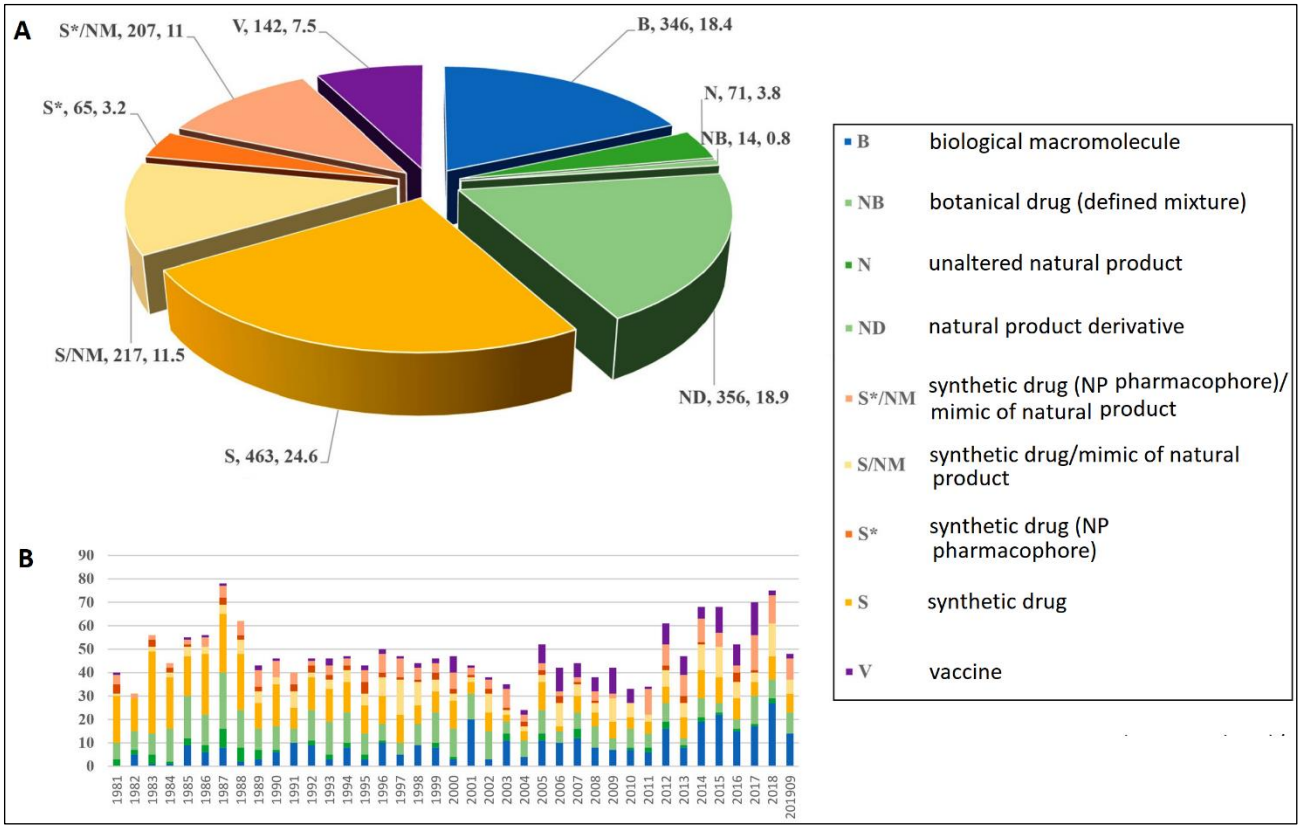


Figure 8: Distribution of natural, nature-inspired and non-natural drugs among all newly approved drugs from 1st January 1981 to 30th September 2019; n = 1881. A) Pie chart depiction for all source category and B) Bar graph by source/year (Figures reproduced with permission from ACS, https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01285).[125]

Interestingly, NP inspired molecules constitute part of HDACis (Figure 9).[38,85,90,150-156] These NP-based molecules have displayed modulating activities against several HDAC isoforms; although the inhibitory mechanisms are not clearly understood for all of them.[157] Macrocyclic compounds represent one very important class of HDACis and have received a lot of attention. For example, the macrocyclic compound trapoxin A (**13**, Figure 9) isolated from the fungus *Helicoma ambiens* RF-1023 has been found to possess interesting HDAC inhibitory activity.[152,158,159] Compound **13** is said to cause irreversible inhibition of the deacetylating enzymes leading to the accumulation of acetylated histones in various mammalian cell lines.[160] *In vitro* inhibitory assays confirmed this molecule to be very potent with a selectivity index of over 600-fold for HDAC1 when compared to HDAC6.[161] Apicidin (**14**, Figure 9), also isolated from a fungus (*Fusarium pallidoroseum*) is another macrocyclic molecule which is very similar to trappoxin A (**13**).[162] *In vitro* investigation of the inhibitory activity of apicidin also revealed that it is a potent and selective class I HDACi (IC_{50} of 1-2 nM) when compared to HDAC8 (IC_{50} = 750 nM) and HDAC6 (IC_{50} > 10 μM).[163]

Other interesting macrocyclic compound is one of the first approved HDAC inhibiting drug, romidepsin (**5**, Figure 5).[85] This depsipeptide, romidepsin (**5**), produced by *Chromobacterium*

violaceum was reported to selectively inhibit HDAC1 and HDAC2 with IC_{50} values of 1.6 and 3.9 nM, respectively, when compared with HDAC4 and HDAC6 with IC_{50} values of 25 and 790 nM, respectively. Another depsipeptide compound of natural origin is largazole (**15**, Figure 9), which showed similar HDAC activities as romidepsin. Largazole (**15**) was isolated from the marine cyanobacterium *Symploca sp.* and was demonstrated to selectively inhibit HDACs.[164,165] For instance, picomolar range inhibitions of HDACs 1, 2 and 3 isoforms have been reported (IC_{50} = 0.4-0.9 nM) for largazole, while nanomolar range IC_{50} values were reported for HDAC6 and HDAC8, but with no significant inhibition of HDAC5 at concentrations up to 1 µM. Both **5** and **15** undergo *in vivo* activation to produce the active thiol forms of the molecules. The thiol group which is also well-represented in NPs is a well-explored ZBG towards the development of HDACis. Santacruzamate A (**16**, Figure 9) is a potent and selective inhibitor of HDACs isolated from the cyanobacterium *Symploca sp.*[166] This molecule selectively inhibits HDAC2 with IC_{50} value of 0.119 nM, while no observable effect against HDAC4 and HDAC6 for IC_{50} values of ~ 1 µM and 434 nM was also reported.

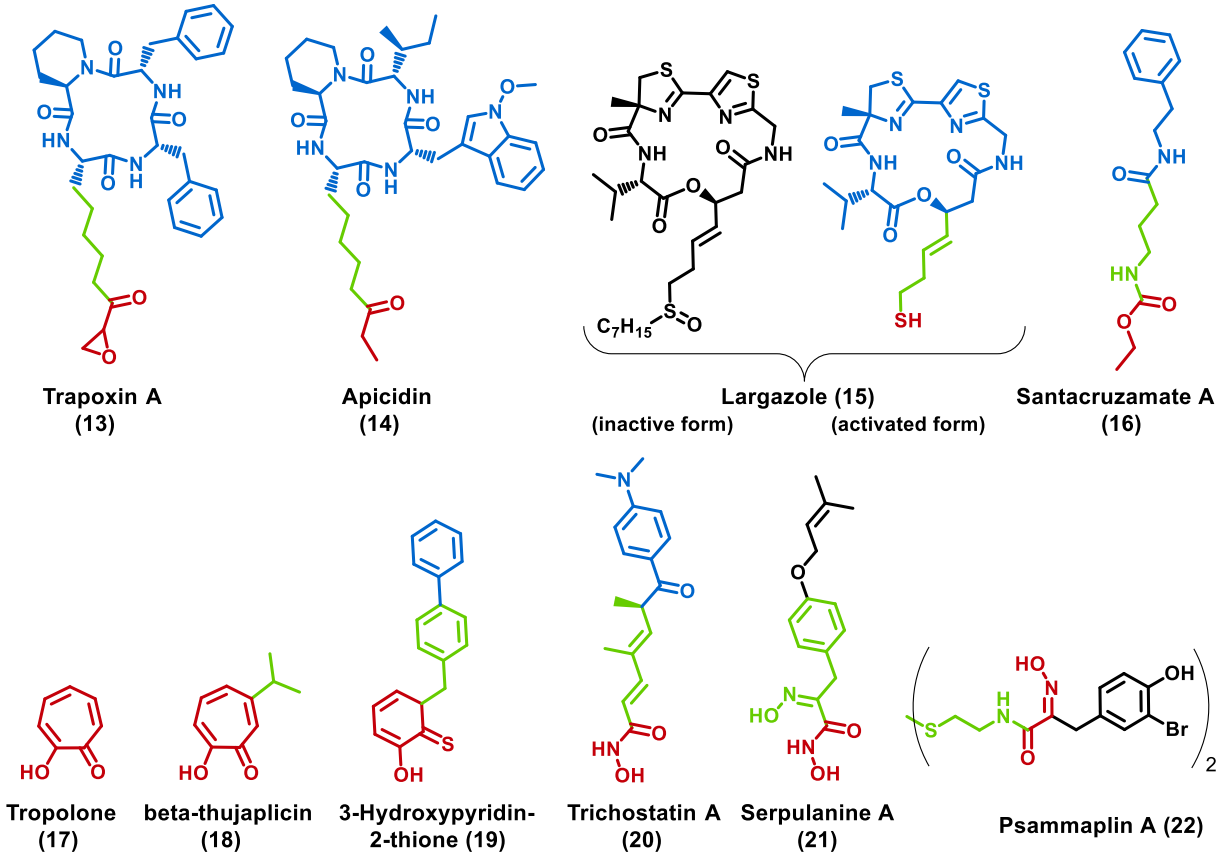


Figure 9: Examples of NP-based HDACis.

Tropolones have also received some attention in the search of novel HDACis. For instance, the tropolone derivative (**17**, Figure 9) (inspired by beta-thujaplicin, **18**, Figure 9),[167] was developed as a novel and selective HDAC2 inhibitor with the potentials of inhibiting the growth of T-cell lymphocyte cell lines. Modification of the ZBG of tropolone led to the development of the

3-hydroxypyridin-2-thione derivative (**19**, Figure 9) as a novel and selective HDACi (IC_{50} of 0.681 and 3.675 μM for HDAC6 and HDAC8 respectively, but with no HDAC1 inhibitory activity.[168] Trichostatin A (**20** is one of the first discovered HDACi from nature (isolated from *Streptomyces hygroscopicus*). *In vitro* studies have proven the strong HDAC inhibitory activity of trichostatin A (**20**, Figure 9) against all the eleven HDAC isoforms with IC_{50} values in the range of 0.4-90 nM.[169,170] Compound **20** is one of the first discovered HDAC from nature (isolated from *Streptomyces hygroscopicus*). Serpulanines are naturally occurring HDACis with a rare (*E*)-2-hydroxyimino hydroxamic acid functional group array. They have so far been isolated only from extracts of a rare Sri Lankan macrofungus *Serpula sp*. collected from a wooded area in the Monaragala District.[171] Evaluation of the HDAC inhibitory activity of serpulanine A (**21**, Figure 9) confirmed that this compound could inhibit HDACs like other hydroxamic acid bearing molecules. Compound **21** was found to inhibit class I and II HDACs. Psammaplin A (**22**, Figure 9) is another very potent HDAC1 selective inhibitor (IC_{50} of 0.9 nM) that is found in several marine sponges.[172-176] Compound **22** was reported to be 360-fold selective for HDAC1 over HDAC6 and more than 1000-fold less potent against HDAC7 and HDAC8.[176]

1.4 Natural Products Databases as Digital Sources for Novel HDAC Modulators.

Although significant progress has been made in the search of HDAC modulators from both synthetic and natural origin, challenges associated with potency, selectivity, pharmacokinetics and safety for the new molecules is still a problem to be solved. Thus, the exploration of new sources (such as NP libraries) and molecules bearing new scaffolds and pharmacophoric features with the help of rational design and *de novo* design is highly interesting. Nowadays, rational drug design-based methods to developed NP analogues with acceptable pharmacokinetics, pharmacodynamics properties, as well as low toxicity is gradually overtaking the use of pure NPs.[131,136,137,139-141] However, the place of NPs remains unique because it is impossible to design such analogues from scratch without consulting NPs. NPs can therefore help, in answering questions about which compounds should be prepared and how should such a diversity-oriented synthesis be planned?[131,139-141] The above listed successful stories and challenges prompted us to explore more NP sources to search for HDAC modulators.

1.4.1 Natural Product Databases

Modern drug discovery has seen a great interest in NPs.[120,128,131,146,177] This, to a certain degree, is because of their diversity and advantage in providing new lead compounds and scaffolds.[131,132,178-183] The growing interest in NPs has also invited huge investment in the search

(isolation, characterization, biological evaluation) of NPs in both academia and industrial sectors.[177,184] A result of this investment is seen with the increased number of isolated NPs.[177] One way of documenting and making available this information/findings to the community is through the collection of available data into several public and commercial NP databases and repositories.[185] A detailed review of existing NPs databases and where to find data in 2020 was recently provided by Sorokina and Steinbeck.[185] There are many examples of these NP databases; from comprehensive (general; including compounds from terrestrial, marine and microbial organisms) to focussed ones (based on a particular disease, or compounds from specific geographical regions or organism types). However, studies also reveal that these collections of virtual NP databases which contain about ~ 250,000 NPs only have about 10 % of readily available purchasable samples.[178] These databases were proven to be more diverse when compared with synthetic/combinatorial databases within the biologically relevant chemical space (Figure 10).[134,178,186,187] Disappointment in large combinatorial databases left a clear message to drug discovery researchers to either increase the diversity of the combinatorial databases *via* the improvement of the diversity of synthetic reactions and/or shift to NPs which have worked in the past.

1.4.2 Natural Products from African Sources

The African continent is magnificently gifted with richness and diversity in flora and fauna. This gift, in turn, justifies how a significant proportion of the African population relies on diverse traditional means in treating ailments as their primary source of healthcare and needs. The dependence on traditional methods of treatment can be attributed to socioeconomic reasons, personal beliefs or the difficulty in accessing pharmaceutical products. Scientific exploration of the known traditional methods as well as the source species represents one of the possible means in documenting and safeguarding the information and/or standardizing and improving the quality of the products being consumed. In this line, several ethnopharmacological, pharmacoinformatic and chemoinformatic studies to validate claims from African source species have been reported.[186-204] It was generally observed that most of the isolated molecules were of the alkaloid, flavonoid, phenolic, quinone and terpenoid compound classes.[186,187,191-203] One of the most investigated topics, are claims of African traditional medicine approaches for the treatment of malaria.

Likewise, claims that many of the African medicinal plants have the potential to heal fevers and malaria in traditional medicine have been the most investigated.[186,190,192-201] This confirms to an extent, how the indigenes are fighting against parasitic ailments which is a serious burden to the people claiming thousands of lives annually.[205] Chemoinformatic analysis of available NP

databases using PCA revealed that only a small portion of the already annotated NPs originating from Africa occupy a similar chemical compared to the rest of the world. For example, comparative study to analyze the chemical space (Figure 10) between two focused NP databases; AfroCancer (compounds with demonstrated *in vitro* and/or *in vivo* anticancer, cytotoxic and antiproliferative activities from the African medicinal plants) and NPACT (Naturally Occurring Plant-based Anticancer Compound-Activity-Target) databases showed that their chemical spaces diverge in different directions.[205]

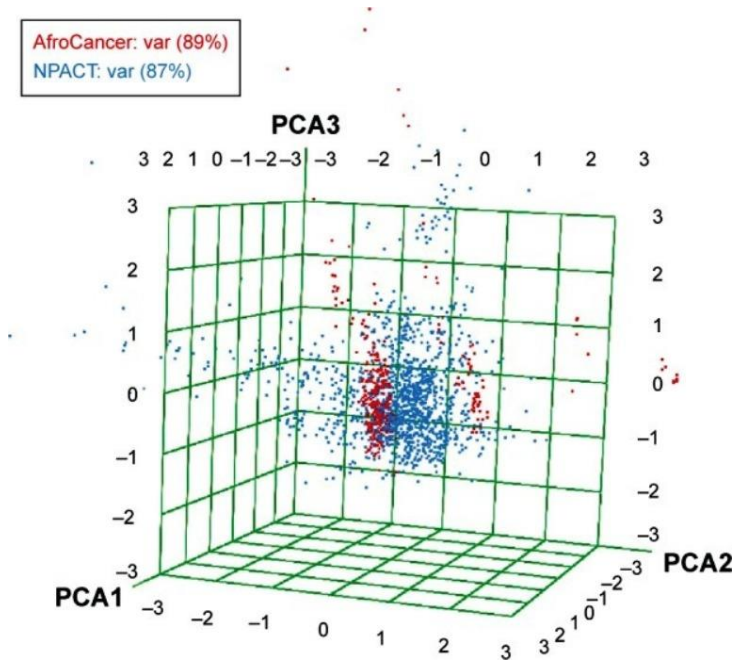


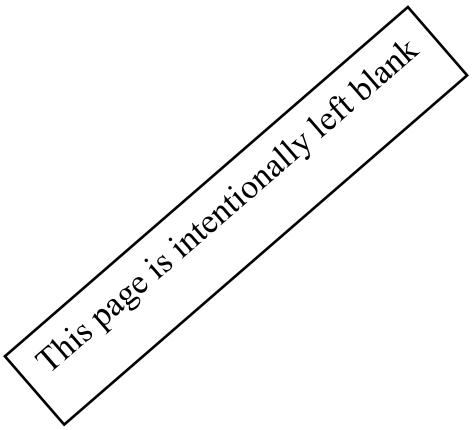
Figure 10: 3D plot using the best 3 PCA to compare the chemical space occupied by the NPs in the AfroCancer (red) and those in the NPACT (cyan) databases (Figure reproduced with permission).[205]

Taking into consideration the poor overlap between existing databases of natural compounds with those from African sources, it appears that new collections would greatly contribute to cover a broader chemical space. Such an information source could also contain HDACis of a novel origin. The multiple cases of natural compounds being discovered as HDACis and optimized into drugs or drug-like molecules also inspired us to develop a new database of African NPs as a possible source for new HDACis and other biologically active molecules.

2. The Objectives of the Work

"If you're not prepared to be wrong, you'll never come up with anything original."

~ Ken Robinson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Overall Objective

As described above, targeting HDACs has emerged as a promising therapeutic strategy to treat several ailments including cancer, parasitic diseases and viral infections.[1,3,4,52,90] Fascinatingly, compounds of both synthetic and natural origin have provided considerable contribution in the search of novel HDAC inhibitors. The richness of the African fauna and flora stands as a good starting point for the search of novel HDAC inhibitors since the exploration of literature sources shows that the appropriate attention it deserved was not given. In this study, the main aim was to design a reliable and efficient computational approach to search for novel HDAC modulators for the treatment of schistosomiasis and HIV/AIDS as well as to develop a database of African NPs which could serve as a novel source for such compounds. To achieve the main aim of this project, the following specific objectives were applied.

1. Studying of the classical HDACs and their inhibitors. This helped to identify structural similarities and differences, how HDACs function and their proposed catalytic mechanism, reported interactions that are conserved across particular classes of inhibitors.
2. Designing and performing structure-based *VS*. This process was important to find novel hits for the parasitic HDAC8 (*sm*HDAC8) and the human HDAC1 (*hs*HDAC1) targets. Selection of hits that can possess increased potency and selectivity was based on the knowledge gathered specifically from the previous objective. This is complemented with the evaluation of docking poses and stability of the small molecules within the active site of the targets of interest (*sm*HDAC8 and *hs*HDAC1).
3. Developing a quantitative structure-activity relationship to account for the inhibitory activity of smHDAC8 inhibitors of the benzhydroxamate type in order to suggest new molecules for synthesis that might have higher activity, based on the prediction.
4. Developing a database consisting of NPs from African source species, by manual curation of information (such as source organisms, reported biological activities amongst others) from published peer-reviewed literature sources.
5. *VS* of the newly developed African natural product database and the proposing of NPs that can act as modulators of smHDAC8.
6. Biological screening of suggested molecules and resolving of a crystal structure. Interdisciplinary collaboration with other groups to validate theoretical results remains very important and indispensable in the drug discovery pipeline.

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3. Results and Discussions

The results of this thesis include the following scientific manuscripts.

"The fewer the hypotheses needed to explain existing observations and predict new phenomena, the more 'elegant' the theory."

~ Occam's razor

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3.1 Design of Selective Histone Deacetylase Inhibitors: Rethinking Classical Pharmacophore

Jelena Melesina, Lucas Praetorius, **Conrad Veranso Simoben**, Dina Robaa, Wolfgang Sippl

Future Medicinal Chemistry, **2018**; 10(13), 1537-1540.
https://doi.org/10.4155/fmc-2018-0125

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Editorial

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Future Medicinal Chemistry

Design of selective histone deacetylase inhibitors: rethinking classical pharmacophore

Jelena Melesina[1], Lucas Praetorius[1], Conrad V Simoben[1], Dina Robaa[1] & Wolfgang Sippl*,[1]

[1]Institute of Pharmacy, Martin Luther University of Halle-Wittenberg, 06120 Halle/Saale, Germany

*Author for correspondence: wolfgang.sippl@pharmazie.uni-halle.de

"the research community is more and more enthused to address a certain class of HDACs or a specific HDAC isoform by designing selective inhibitors"

For two decades, a classical pharmacophore model comprising a zinc binding group, a linker and a cap group, has been used for the development of histone deacetylase (HDAC) inhibitors. However, some of the recently reported selective HDAC inhibitors targeting additional, usually subtype specific, cavities in the binding pocket show supplementary features which do not fit this classical pharmacophore. We, therefore, propose an extended pharmacophore model, which can describe almost all currently known HDAC inhibitors. This pharmacophore consists of six pharmacophoric features and should be helpful for the classification and design of selective HDAC inhibitors.

First draft submitted: 16 April 2018; Accepted for publication: 20 April 2018; Published online: 3 July 2018

HDAC inhibitors as drugs

Histone modifying enzymes epigenetically regulating gene expression have emerged as targets with good prospects for cancer treatment. Especially promising are histone deacetylases (HDACs), since their activity is upregulated in many types of cancer. The eleven human HDAC isoforms have been classified into four classes: I, IIa, IIb and IV based on their homology with orthologs identified in yeast (class III, which is more commonly called sirtuins, is structurally distinct and is not discussed in this paper) [1]. Several HDAC inhibitors have already been approved for cancer treatment. The hydroxamic acid derivative vorinostat targeting multiple HDAC isoforms was the first HDAC inhibitor approved in 2006 for the treatment of cutaneous T-cell lymphoma. Since then, three other HDAC inhibitors, namely romidepsin, belinostat and panobinostat got approval for clinical use in the USA and chidamide was approved for the treatment of peripheral T-cell lymphoma in China [2,3]. Besides the primary application as anticancer drugs, HDAC inhibitors show potential for the treatment of other disorders such as neurodegeneration, inflammation and parasitic diseases [1,2,4]. However, studies have shown that the above-mentioned pan-HDAC inhibitor vorinostat may cause numerous unwanted side effects. Thus, the research community is more and more enthused to address a certain class of HDACs or a specific HDAC isoform by designing selective inhibitors [1,5]. This remains a challenge due to the high sequence and structural similarity of the various HDAC isoforms and because of missing information about structural data of some isoforms.

Classical pharmacophore of HDAC inhibitors

Back in the 90s when the first potent HDAC inhibitors were discovered and before the first crystal structure of the bacterial HDAC-like protein was solved, a useful pharmacophore model of HDAC inhibitors was proposed by Jung *et al.* [6–8]. This model, which was based on available information and highlighted common features of known HDAC inhibitors, was widely accepted and was further developed by many authors. It introduced a new terminology, which facilitated the design of inhibitors for many years. The currently used classical pharmacophoric definition, which is supported by several released crystal structures of HDAC–inhibitor complexes, consists of the zinc binding group (ZBG) coordinating the catalytic zinc ion, the linker placed in the substrate binding tunnel, and the surface recognition domain also known as cap. Diverse research groups have embarked on modifying

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3.2 Strategies to Design Selective HDACis

Jelena Melesina, **Conrad Veranso Simoben**, Lucas Praetorius, Emre F. Bülbül, Dina Robaa, Wolfgang Sippl

ChemMedChem, **2021**; https://doi.org/10.1002/cmdc.202000934

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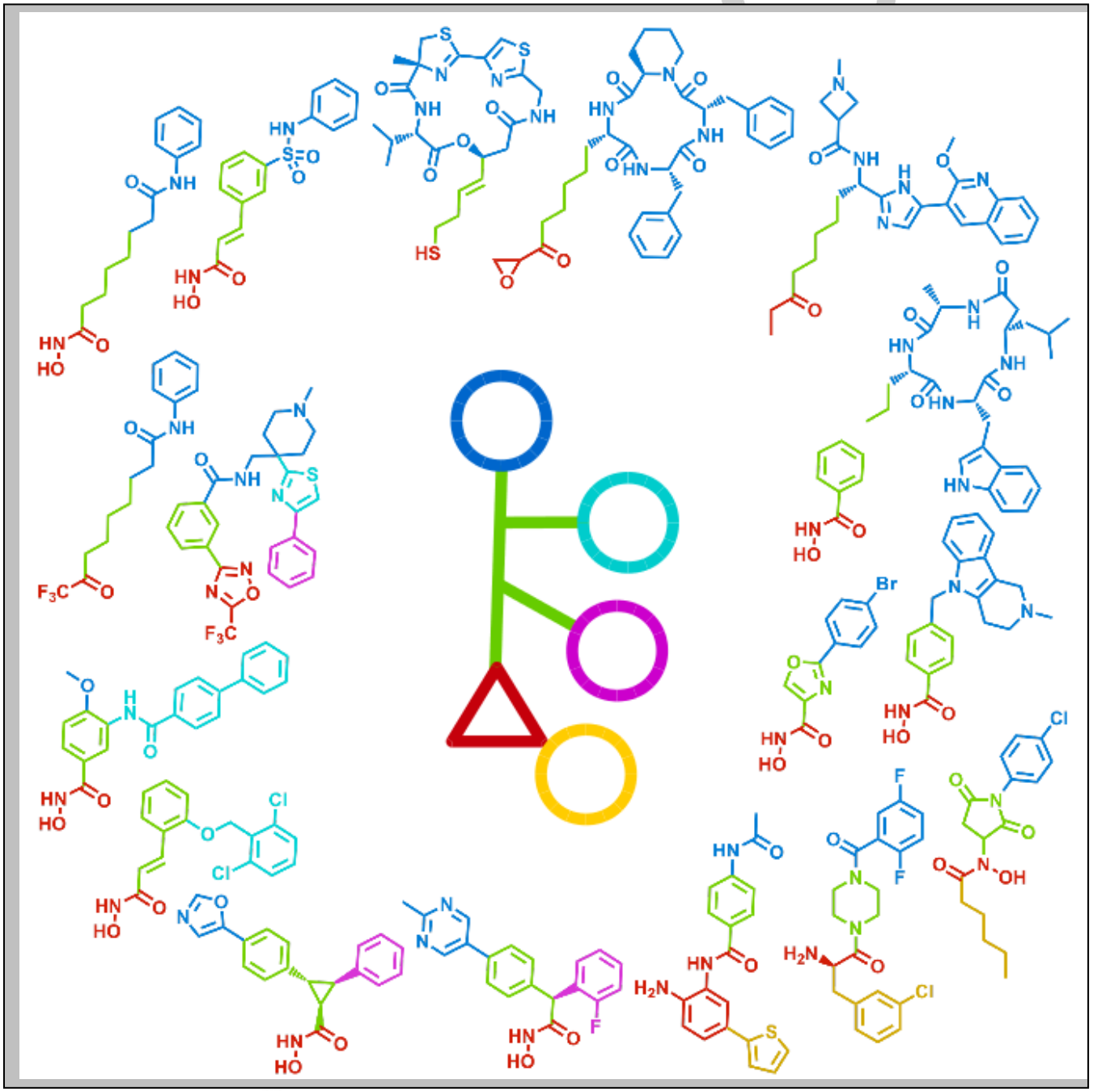
Strategies to Design Selective Histone Deacetylase Inhibitors

Jelena Melesina[a], Conrad V. Simoben[a], Lucas Praetorius[a], Emre F. Bülbül[a], Dina Robaa[a] and Wolfgang Sippl*[a]



[a] Dr. J. Melesina, C. V. Simoben, L. Praetorius, E. F. Bülbül, Dr. D. Robaa, Prof. Dr. W. Sippl
Institute of Pharmacy
Martin Luther University of Halle-Wittenberg
Kurt Mothes Str. 3, 06120 Halle/Saale, (Germany)
E-mail: wolfgang.sippl@pharmazie.uni-halle.de

Abstract: This review classifies drug design strategies successfully implemented in the development of histone deacetylase (HDAC) inhibitors, which have many applications including cancer treatment. Our focus is on especially demanded selective HDAC inhibitors and their structure-activity relationships in relation to corresponding protein structures. The main part of the paper is divided into six subsections narrating how optimization of the six corresponding structural features can influence inhibitor selectivity. It starts with the impact of the zinc binding group on selectivity, continues with the optimization of the linker placed in the substrate binding tunnel as well as the adjustment of the cap group interacting with the surface of the protein, and ends with addition of groups targeting class-specific sub-pockets – the side pocket targeting group, the lower pocket targeting group and the foot pocket targeting group. The review is enhanced with a conclusion and an outlook to the future of HDAC inhibitor design.

1. Introduction

Histone deacetylases (HDACs) and HDAC-like proteins are ancient enzymes found ubiquitously in various organisms from bacteria to mammals.[1] Among other functions, they participate in epigenetic regulation of gene transcription by removing the acetyl moieties from lysine residues of histones. Largely due to this important role, HDACs are engaged in multiple physiological processes and are promising drug targets for various pathological conditions, such as cancer, cardiac and neurodegenerative diseases, inflammation, metabolic and immune disorders, viral and parasitic infections.[1-2] Several HDAC inhibiting drugs have been approved for cancer treatment and novel HDAC inhibitors are intensively being developed. Since targeting multiple HDAC isoforms might simultaneously cause unwanted side effects as observed for the approved broad spectrum HDAC inhibiting drugs, isoform selective compounds are gaining more attention, especially in the field of non-cancer diseases.[1-2, 3]

Human HDACs are represented in eighteen isoforms, subdivided into four classes: class I (HDAC1-3, HDAC8), class II (IIa: HDAC4-5, HDAC7, HDAC9 and IIb: HDAC6, HDAC10), class III (SIRT1-7) and class IV (HDAC11). Class III HDACs are mostly called sirtuins; they are structurally and biochemically different from other HDACs and do not fall within the scope of this paper. The HDACs discussed in this review (classes I-II and IV) are also known as classical HDACs or zinc-dependent HDACs, or simply HDACs (as they are referred to further). They vary in size, subcellular localization, expression patterns and substrate recognition.[1-2] For instance, class I HDACs (HDAC1-3 and HDAC8) as well as class IIb member HDAC6 are efficient deacetylases. Class IIa HDACs (HDAC4-5, HDAC7 and HDAC9) are weak deacetylases but readily accept non-physiological trifluoracetylated substrates. Class IIa HDACs have lost their essential catalytic tyrosine, replaced by a histidine, and are, therefore, thought to play mainly a scaffolding role in macromolecular complexes.[4] HDAC10 has been shown to mainly work as polyamine deacetylase, while HDAC11 is known as fatty acid deacylase.[5] It has been observed by several authors that the activity on HDAC10 and HDAC11 isoforms could not be measured using the peptide-based acetylated and trifluoracetylated substrates commonly used for other HDACs and suggested that previously measured activities might be influenced by co-purified HDACs.[5a-c, 6]. Despite the distinct substrate specificity and other mentioned differences of HDACs, they are structurally very similar and share a common catalytic domain.[1-2] Thus, the intriguing question, which has been bothering many scientists working in the field of HDAC inhibitor development, is how to achieve selectivity among structurally similar HDAC isoforms.

The architecture of the catalytic HDAC domain is conserved as seen in around two hundred solved crystal structures of human, zebrafish, parasitic, plant and bacterial HDACs and HDAC-like proteins stored in the Protein Data Bank (PDB), URL rcsb.org.[7] The fold of the domain consists of a central β-sheet surrounded by an ensemble of α-helices and interconnecting loops. Several loops form the catalytic pocket which contains the catalytic zinc ion (although some authors suggest that there might be another metal ion in physiological conditions).[8] The catalytic pocket can be subdivided into several parts (Fig. 1) : 1) the main pocket (A) consisting of the acetate binding cavity, the substrate binding tunnel and the rim of the pocket and 2) the sub-pockets, such as the side pocket (B), the lower pocket (C) and the foot pocket (D).[9] The main pocket is present in all crystal structures of HDACs, while the sub-pockets could either be opened or closed depending on the bound ligand and HDAC isoform.[10] In addition to the first catalytic domain, HDACs might have a second catalytic or pseudocatalytic domain (class IIb HDACs), C- or N-terminal extensions and other domains (e.g., a unique zinc-finger domain in HDAC6).[1-2, 5b]

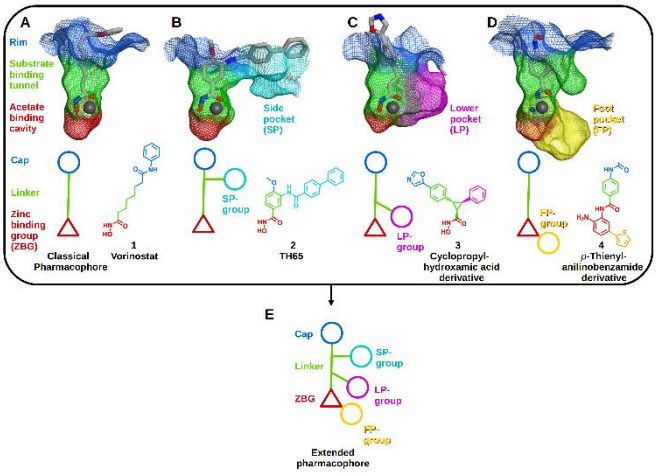


Figure 1. Extended pharmacophore model based on the plasticity of the HDAC binding pocket and the corresponding pharmacophoric features of bound

3.3 A Novel Class of *Schistosoma mansoni* Histone Deacetylase 8 (HDAC8) Inhibitors Identified by Structure-Based Virtual Screening and *In Vitro* Testing

Conrad Veranso Simoben, Dina Robaa, Alokta Chakrabarti, Karin Schmidtkunz, Martin Marek, Julien Lancelot, Srinivasaraghavan Kannan, Jelena Melesina, Tajith B. Shaik, Raymond J. Pierce, Christophe Romier, Manfred Jung, Wolfgang Sippl

Molecules, **2018**; 23(3), 566.
https://doi.org/10.3390/molecules23030566

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A Novel Class of *Schistosoma mansoni* Histone Deacetylase 8 (HDAC8) Inhibitors Identified by Structure-Based Virtual Screening and In Vitro Testing

Conrad V. Simoben [1], Dina Robaa [1], Alokta Chakrabarti [2,†], Karin Schmidtkunz [2], Martin Marek [3,‡], Julien Lancelot [4], Srinivasaraghavan Kannan [1,§], Jelena Melesina [1], Tajith B. Shaik [3], Raymond J. Pierce [4], Christophe Romier [3], Manfred Jung [2] and Wolfgang Sippl [1,*]

1 Department of Pharmaceutical Chemistry, University Halle-Wittenberg, 06120 Halle/Saale, Germany; veranso.conrad@gmail.com (C.V.S.); dina.robaa@pharmazie.uni-halle.de (D.R.); raghavk@bii.a-star.edu.sg (S.K.); jelena.melesina@pharmazie.uni-halle.de (J.M.)

2 Institute of Pharmaceutical Sciences, University of Freiburg, 79104 Freiburg, Germany; alokta.chakrabarti@gmail.com (A.C.); karin.schmidtkunz@pharmazie.uni-freiburg.de (K.S.); manfred.jung@pharmazie.uni-freiburg.de (M.J.)

3 Département de Biologie Structurale Intégrative, Institut de Génétique et Biologie Moléculaire et Cellulaire (IGBMC), Université de Strasbourg, CNRS, INSERM, B.P. 10142, 67404 Illkirch CEDEX, France; martin.marek@recetox.muni.cz (M.M.); shaik@igbmc.fr (T.B.S.); romier@igbmc.fr (C.R.)

4 Institut Pasteur de Lille, U1019—UMR 8204-CIIL-Centre d'Infection et d'Immunité de Lille, CNRS, Inserm, CHU Lille, Universite de Lille, F-59000 Lille, France; julien.lancelot@pasteur-lille.fr (J.L.); Raymond.Pierce@pasteur-lille.fr (R.J.P.)

* Correspondence: wolfgang.sippl@pharmazie.uni-halle.de; Tel.: +49-345-552-5040

† Current address: ProQinase GmbH, 79106 Freiburg, Germany

‡ Current address: Loschmidt Laboratories, Department of Experimental Biology & Recetox, Masaryk University, 625 00 Brno, Czech Republic

§ Current address: Bioinformatics Institute (A*STAR), #07-01 Matrix, 138671 Singapore, Singapore

Received: 30 January 2018; Accepted: 28 February 2018; Published: 2 March 2018

Abstract: A promising means in the search of new small molecules for the treatment of schistosomiasis (amongst other parasitic ailments) is by targeting the parasitic epigenome. In the present study, a docking based virtual screening procedure using the crystal structure of histone deacetylase 8 from *Schistosoma mansoni* (smHDAC8) was designed. From the developed screening protocol, we were able to identify eight novel *N*-(2,5-dioxopyrrolidin-3-yl)-*n*-alkylhydroxamate derivatives as smHDAC8 inhibitors with IC_{50} values ranging from 4.4–20.3 μM against smHDAC8. These newly identified inhibitors were further tested against human histone deacetylases (hsHDAC1, 6 and 8), and were found also to be exerting interesting activity against them. In silico prediction of the docking pose of the compounds was confirmed by the resolved crystal structure of one of the identified hits. This confirmed these compounds were able to chelate the catalytic zinc ion in a bidentate fashion, whilst showing an inverted binding mode of the hydroxamate group when compared to the reported smHDAC8/hydroxamates crystal structures. Therefore, they can be considered as new potential scaffold for the development of new smHDAC8 inhibitors by further investigation of their structure–activity relationship.

Keywords: epigenetics; crystal structure; docking; histone deacetylase (HDAC) inhibitors; schistosomiasis; virtual screening

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3.4 Binding Free Energy (BFE) Calculations and Quantitative Structure-Activity Relationship (QSAR) Analysis of *Schistosoma Mansoni* Histone Deacetylase 8 (*sm*HDAC8) Inhibitors

Conrad Veranso Simoben, Ehab Ghazy, Patrik Zeyen Daniel Herp, Christophe Romier, Dina Robaa, Manfred Jung, Wolfgang Sippl

(*Submitted to Preprintsever*)

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Binding free energy (BFE) calculations and quantitative structure-activity relationship (QSAR) analysis of *Schistosoma mansoni* histone deacetylase 8 (smHDAC8) inhibitors

Conrad V. Simoben[a]; Ehab Ghazy[a]; Patrik Zeyen[a]; Salma Darwish[a]; Chris Romier[c]; Dina Robaa[a]; Wolfgang Sippl[a]

[a]*Institute of Pharmacy, Martin-Luther University of Halle-Wittenberg, 06120 Halle/Saale, Germany*
[b]*Institute of Pharmaceutical Sciences, Albert-Ludwigs-University of Freiburg, 79104 Freiburg, Germany*
[c] *Département de Biologie Structurale Intégrative, Institut de Génétique et Biologie Moléculaire et Cellulaire (IGBMC), Université de Strasbourg (UDS), CNRS, INSERM, 1 rue Laurent Fries, B.P. 10142, 67404 Illkirch Cedex IGBMC*

Abstract:

Histone modifying proteins have been identified as promising targets to treat several diseases including cancer and parasitic ailments. *In silico* methods have been incorporated within a variety of drug discovery programs to facilitate the identification and development of novel lead compounds. In this study, we explore the binding modes of a series of benzhydroxamates derivatives developed as histone deacetylase inhibitors of *Schistosoma mansoni* (*sm*HDAC) using molecular docking and binding free energy (BFE) calculations. The developed docking protocol was able to correctly reproduce the experimentally established binding modes of resolved *sm*HDAC8-inhibitor complexes. However, as has been reported in former studies, the obtained docking scores weakly correlate with the experimentally determined activity of the studied inhibitors. Thus, the obtained docking poses were refined and rescored using the Amber software. From the computed protein-inhibitor BFE, different QSAR models could be developed and validated using several cross validation techniques. Some of the generated QSAR models with good correlation could explain up to ~ 73 % variance in activity within the studied training set molecules. The best performing models were subsequently tested on an external test set of newly designed and synthesized analogs. *In vitro* testing showed a good correlation between the predicted and experimentally observed IC_{50} values. Thus, the generated models can be considered as interesting tools for the identification of novel *sm*HDAC8 inhibitors.

1. Introduction:

Neglected parasitic diseases have been responsible for morbidity and mortality of hundreds of millions of humans in underprivileged communities especially in parts of the Middle East, South America, Southeast Asia and, particularly, in sub-Saharan Africa for over many decades.(1) Amongst these neglected parasitic diseases is schistosomiasis (bilharzia), which is a common intravascular parasitic infection in humans caused by *Schistosoma spp*.(2) Despite being a preventable illness, chronic infection is associated with long-term undernutrition, anaemia, organ scarring and fibrosis, resulting in disabling patient symptoms.(2-4) According to the World Health Organization (WHO), an estimated 206.5 million people required preventive treatment for schistosomiasis, out of which more than 89 million people were reported to have been treated.(3, 4) Several detailed studies/reviews explaining the epidemiology of

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3.5 Novel Histone Deacetylase Inhibitors and HIV-1 Latency-Reversing Agents Identified by Large-Scale Virtual Screening

Donya Naz Divsalar, **Conrad Veranso Simoben**, Cole Schonhofer, Khumoekae Richard, Wolfgang Sippl, Fidele Ntie-Kang and Ian Tietjen

Frontiers in Pharmacology, **2020**; 11, 905.
https://doi.org/10.3389/fphar.2020.00905

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frontiers in Pharmacology

ORIGINAL RESEARCH
published: 17 June 2020
doi: 10.3389/fphar.2020.00905

Novel Histone Deacetylase Inhibitors and HIV-1 Latency-Reversing Agents Identified by Large-Scale Virtual Screening

Donya Naz Divsalar[1†], *Conrad Veranso Simoben*[2,3†], *Cole Schonhofer*[1], *Khumoekae Richard*[1], *Wolfgang Sippl*[3], *Fidele Ntie-Kang*[2,3*] *and Ian Tietjen*[1,4*]

[1] Faculty of Health Sciences, Simon Fraser University, Burnaby, BC, Canada, [2] Department of Chemistry, University of Buea, Buea, Cameroon, [3] Instutite of Pharmacy, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany, [4] The Wistar Institute, Philadelphia, PA, United States

Current antiretroviral therapies used for HIV management do not target latent viral reservoirs in humans. The experimental "shock-and-kill" therapeutic approach involves use of latency-reversal agents (LRAs) that reactivate HIV expression in reservoir-containing cells, followed by infected cell elimination through viral or host immune cytopathic effects. Several LRAs that function as histone deacetylase (HDAC) inhibitors are reported to reverse HIV latency in cells and in clinical trials; however, none to date have consistently reduced viral reservoirs in humans, prompting a need to identify new LRAs. Toward this goal, we describe here a virtual screening (VS) approach which uses 14 reported HDAC inhibitors to probe PubChem and identifies 60 LRA candidates. We then show that four screening "hits" including (S)-*N*-Hydroxy-4-(3-methyl-2-phenylbutanamido)benzamide (compound **15**), *N*-(4-Aminophenyl)heptanamide (**16**), *N*-[4-(Heptanoylamino)phenyl]heptanamide (**17**), and 4-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-*N*-(2-hydroxyethyl)butanamide (**18**) inhibit HDAC activity and/or reverse HIV latency *in vitro*. This study demonstrates and supports that VS-based approaches can readily identify novel HDAC inhibitors and LRAs, which in turn may help toward inhibitor design and chemical optimization efforts for improved HIV shock-and-kill-based efforts.

Keywords: virtual screening, histone deacetylase, histone deacetylase inhibitor, HIV, latency reversal, drug discovery

OPEN ACCESS

Edited by:
Roberto Paganelli, University of Studies G. d'Annunzio Chieti and Pescara, Italy

Reviewed by:
Stevan Pecic, California State University, United States
Adam M. Spivak, The University of Utah, United States

***Correspondence:**
Fidele Ntie-Kang
fidele.ntie-kang@ubuea.cm
Ian Tietjen
itietjen@wistar.org

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Translational Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *23 January 2020*
Accepted: *03 June 2020*
Published: *17 June 2020*

Citation:
Divsalar DN, Simoben CV, Schonhofer C, Richard K, Sippl W, Ntie-Kang F and Tietjen I (2020) Novel Histone Deacetylase Inhibitors and HIV-1 Latency-Reversing Agents Identified by Large-Scale Virtual Screening. Front. Pharmacol. 11:905. doi: 10.3389/fphar.2020.00905

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3.6 Case Studies on Computer-Based Identification of Natural Products as Lead Molecules

Conrad Veranso Simoben, Fidele Ntie-Kang, Dina Robaa, Wolfgang Sippl

Physical Sciences Reviews, **2020**.
https://doi.org/10.1515/psr-2018-0119.

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Conrad V. Simoben[1] / Fidele Ntie-Kang[2,1] / Dina Robaa[1] / Wolfgang Sippl[1]

Case studies on computer-based identification of natural products as lead molecules

[1] Martin-Luther-Universität Halle-Wittenberg, Department of Medicinal Chemistry (AG Sippl), Institute of Pharmacy, Kurt-Mothes-Str. 3, 06120 Halle (Saale), Germany, E-mail: veranso.conrad@gmail.com, ntiekfidele@gmail.com, dina.robaa@pharmazie.uni-halle.de, wolfgang.sippl@pharmazie.uni-halle.de. https://orcid.org/0000-0002-5958-6961.
[2] Department of Chemistry, University of Buea, P. O. Box 63, Buea, Cameroon, E-mail: ntiekfidele@gmail.com

Abstract:
The development and application of computer-aided drug design/discovery (CADD) techniques (such as structured-base virtual screening, ligand-based virtual screening and neural networks approaches) are on the point of disintermediation in the pharmaceutical drug discovery processes. The application of these CADD methods are standing out positively as compared to other experimental approaches in the identification of hits. In order to venture into new chemical spaces, research groups are exploring natural products (NPs) for the search and identification of new hits and more efficient leads as well as the repurposing of approved NPs. The chemical space of NPs is continuously increasing as a result of millions of years of evolution of species and these data are mainly stored in the form of databases providing access to scientists around the world to conduct studies using them. Investigation of these NP databases with the help of CADD methodologies in combination with experimental validation techniques is essential to identify and propose new drug molecules. In this chapter, we highlight the importance of the chemical diversity of NPs as a source for potential drugs as well as some of the success stories of NP-derived candidates against important therapeutic targets. The focus is on studies that applied a healthy dose of the emerging CADD methodologies (structure-based, ligand-based and machine learning).

Keywords: CADD, drugs, ligand-based, machine learning, natural products, structure-based
DOI: 10.1515/psr-2018-0119

1 Introduction

A typical drug discovery and development process from concept to market takes about 13–15 years requiring approximately $2–3 billion on average [1, 2]. However, the increasing cost of the drug discovery and development process nowadays has not produced an exponential increase in the success rate of drugs approved annually as it has remained relatively flat or decreased over the past decade [1, 3]. Nevertheless, the development and application of new methodologies are on the point of disintermediation in the pharmaceutical drug discovery processes. Thereby, reducing billions of dollars of the industry's cost for the search of new drugs and a cut down on the time taken to get new medicines approved to just a few processing cycles (few years). The application of computational methodologies has been of tremendous importance at various stages of drug discovery especially in the identification of hits as compared to experimental approaches alone [4–15]. Thus, CADD methods have proven to be successful approaches for finding ligand hits, as well as in assisting in the lead optimization steps in discovery projects.

Exploration of natural products (NPs) for the discovery of new and more efficient leads, repurposing of known NPs, targeting of new targets on the basis of genome analysis, the revelation of mechanisms of action, and optimization of lead compounds are being achieved via the application of some of the developed CADD methodologies [16–24]. Humans had been using crude and/or pure medicinal plant extracts as well as isolated NPs for millennia in the treatment of several ailments before the advent of synthetic medicinal chemistry [17, 21, 24–28]. With the development of novel methodologies for the identification, isolation, and characterization of new and/or unique NPs [16], the chemical space has continuously increased as a result of millions of years of evolution of species. Thereby, opening new avenues for the discovery of new molecules that can target several ailments. The data for most of the identified NPs are made available as databases for usage in research and development (R&D) projects including drug discovery procedures [18, 29–33]. Numerous research groups have provided detailed reports on the growth of NP databases as well as insights into understanding some of the complex molecular scaffolds unparalleled in function, chemical diversity, and sample availability for some of

Conrad V. Simoben is the corresponding author.

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3.7 Computational Studies and Biosynthesis of Natural Products with Promising Anticancer Properties

Aurélien F. A. Moumbock, **Conrad Veranso Simoben**, Ludger Wessjohann, Wolfgang Sippl, Stefan Günther, Fidele Ntie-Kang

In Phytochemistry - Natural Products and Cancer, Edited by Farid A. Badria, **2017**; InTech Open. ISBN: 978-953-51-5277-4. DOI: 10.5772/67650

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Chapter 10

Computational Studies and Biosynthesis of Natural Products with Promising Anticancer Properties

Aurélien F.A. Moumbock, Conrad V. Simoben, Ludger Wessjohann, Wolfgang Sippl, Stefan Günther and Fidele Ntie-Kang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67650

Abstract

We present an overview of computational approaches for the prediction of metabolic pathways by which plants biosynthesise compounds, with a focus on selected very promising anticancer secondary metabolites from floral sources. We also provide an overview of databases for the retrieval of useful genomic data, discussing the strengths and limitations of selected prediction software and the main computational tools (and methods), which could be employed for the investigation of the uncharted routes towards the biosynthesis of some of the identified anticancer metabolites from plant sources, eventually using specific examples to address some knowledge gaps when using these approaches.

Keywords: anticancer, biosynthesis, computational prediction, natural products, plant metabolism

1. Introduction

An immense number of secondary metabolites (SMs) exist in nature, originating from plants, bacteria, fungi and marine life forms, serving as drugs for the treatment of many life-threatening diseases, including cancer [1–4]. Taxol, vinblastine, vincristine, podophyllotoxin and camptothecin, for example, are typically well-known drugs used in cancer treatment, which are of plant origin. The search for drugs against cancer has often resorted to plants and marine life for lead compounds. To illustrate this, Newmann and Cragg published a recent study in which it was shown that ~49% of drugs used in cancer treatment were either natural products

3.8 Compounds from African Medicinal Plants with Activities against Selected Parasitic Diseases: Schistosomiasis, Trypanosomiasis and Leishmaniasis

Conrad Veranso Simoben, Fidele Ntie-Kang, Sergi Herve Akone, Wolfgang Sippl

Natural Products and Bioprospecting, **2018**; 8(24), 151–169.
https://doi.org/10.1007/s13659-018-0165-y

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Natural Products and Bioprospecting (2018) 8:151–169
https://doi.org/10.1007/s13659-018-0165-y

REVIEW

Compounds from African Medicinal Plants with Activities Against Selected Parasitic Diseases: Schistosomiasis, Trypanosomiasis and Leishmaniasis

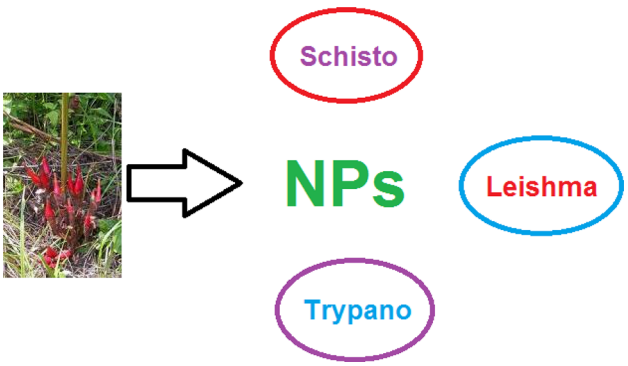
Conrad V. Simoben[1] · Fidele Ntie-Kang[1,2] · Sergi H. Akone[3,4] · Wolfgang Sippl[1]

Received: 22 February 2018 / Accepted: 26 April 2018 / Published online: 9 May 2018

Abstract

Parasitic diseases continue to represent a threat on a global scale, particularly among the poorest countries in the world. This is particularly because of the absence of vaccines, and in some cases, resistance against available drugs, currently being used for their treatment. In this review emphasis is laid on natural products and scaffolds from African medicinal plants (AMPs) for lead drug discovery and possible further development of drugs for the treatment of parasitic diseases. In the discussion, emphasis has been laid on alkaloids, terpenoids, quinones, flavonoids and narrower compound classes of compounds with micromolar range activities against *Schistosoma*, *Trypanosoma* and *Leishmania* species. In each sub-paragraph, emphasis is laid on the compound subclasses with most promising in vitro and/or in vivo activities of plant extracts and isolated compounds. Suggestions for future drug development from African medicinal plants have also been provided. This review covering 167 references, including 82 compounds, provides information published within two decades (1997–2017).

Graphical Abstract



Keywords African medicinal plants · Leishmaniasis · Natural products · Parasitic diseases · Schistosomiasis · Trypanosomiasis

✉ Fidele Ntie-Kang
ntiekfidele@gmail.com

[1] Institute of Pharmacy, Martin-Luther University of Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, 06120 Halle (Saale), Germany

[2] Department of Chemistry, Faculty of Science, University of Buea, P.O. Box 63, Buea 00237, Cameroon

[3] Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-University, Universitaetsstrasse1, Geb. 26.23, Duesseldorf 40225, Germany

[4] Department of Chemistry, Faculty of Science, University of Douala, PO Box 24157, Douala 00237, Cameroon

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3.9 Pharmacoinformatic Investigation of Medicinal Plants from East Africa

Conrad Veranso Simoben, Ammar Qaseem, Aurélien F. A. Moumbock, Kiran K. Telukunta, Stefan Günther, Wolfgang Sippl, Fidele Ntie-Kang

Molecular informatics, Accepted Author Manuscript.
https://doi.org/10.1002/minf.202000163

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molecular informatics

DOI: 10.1002/minf.202000163

Pharmacoinformatic Investigation of Medicinal Plants from East Africa

Conrad V. Simoben,*[a] Ammar Qaseem,[b] Aurélien F. A. Moumbock,[b] Kiran K. Telukunta,[c] Stefan Günther,[b] Wolfgang Sippl,[a] and Fidele Ntie-Kang*[a, d, e]

To Prof. Simon M. N. Efange for his contributions towards academic drug discovery in Sub-Saharan Africa

Abstract: Medicinal plants have widely been used in the traditional treatment of ailments and have been proven effective. Their contribution still holds an important place in modern drug discovery due to their chemical, and biological diversities. However, the poor documentation of traditional medicine, in developing African countries for instance, can lead to the loss of knowledge related to such practices. In this study, we present the Eastern Africa Natural Products Database (EANPDB) containing the structural and bioactivity information of 1870 unique molecules isolated from about 300 source species from the Eastern African region. This represents the largest collection of natural products (NPs) from this geographical region, covering literature data of the period from 1962 to 2019. The computed physicochemical properties and toxicity profiles of each compound have been included. A comparative analysis of some physico-chemical properties like molecular weight, H-bond donor/acceptor, $logP_{o/w}$, etc. as well scaffold diversity analysis has been carried out with other published NP databases. EANPDB was combined with the previously published Northern African Natural Products Database (NANPDB), to form a merger African Natural Products Database (ANPDB), containing ~6500 unique molecules isolated from about 1000 source species (freely available at http://african-compounds.org). As a case study, latrunculins A and B isolated from the sponge *Negombata magnifica* (Podospongiidae) with previously reported antitumour activities, were identified via substructure searching as molecules to be explored as putative binders of histone deacetylases (HDACs).

Keywords: database · drug discovery · Eastern Africa · medicinal plants · natural products (NPs)

1 Introduction

Historically, natural products (NPs), i.e. compounds derived from natural sources (bacterial, fungi, plants or animal species) possessing biological activities; have been the primary provenance of medicine globally.[1] Although the approval rate of new drugs from nature has not increased proportionally with the financial and technological investments on NP researches,[2] NPs still account for about half of the FDA-approved drugs..[2a,d,3] Thus, seeing the remarkable contribution of NPs as drugs, huge amounts of NPs are being isolated and characterized daily. Also, the biological evaluations of the isolated molecules are carried out in order to confirm the therapeutic claims. Further studies on the establishment of the mechanisms of actions of the isolated biologically interesting NPs are being carried out with the hope of getting the next generation lead compounds for drug discovery.[4]

One of the magnificent beauties of the African continent is its richness in flora and fauna. This richness offers the African population diverse traditional means in treating ailments based on what nature has presented to them. However, due to poor documentation, some of this traditional information is being lost nowadays. This is one of the main factors behind the scientific exploration of the known

[a] *C. V. Simoben, W. Sippl, F. Ntie-Kang*
Institute of Pharmacy, Martin-Luther University of Halle-Wittenberg, Kurt-Mothes-Str. 3, 06120 Halle/Saale, Germany
phone: +49 (0)3455525194
phone: +237 673872475
E-mail: veranso.conrad@gmail.com
fidele.ntie-kang@ubuea.cm

[b] *A. Qaseem, A. F. A. Moumbock, S. Günther*
Institute of Pharmaceutical Sciences, Research Group Pharmaceutical Bioinformatics, Albert-Ludwigs-University Freiburg, Hermann-Herder-Straße 9, 79104 Freiburg, Germany

[c] *K. K. Telukunta*
ELIXIR@PSB, VIB-UGent Center for Plant Systems Biology, Technologiepark 71, 9052 Ghent, Belgium

[d] *F. Ntie-Kang*
Department of Chemistry, Faculty of Science, University of Buea, P. O. Box 63, Buea CM-00237, Cameroon

[e] *F. Ntie-Kang*
Institut für Botanik, Technische Universität Dresden, ZellescherWeg 20b, 01217 Dresden, Germany

Supporting information for this article is available on the WWW under https://doi.org/10.1002/minf.202000163

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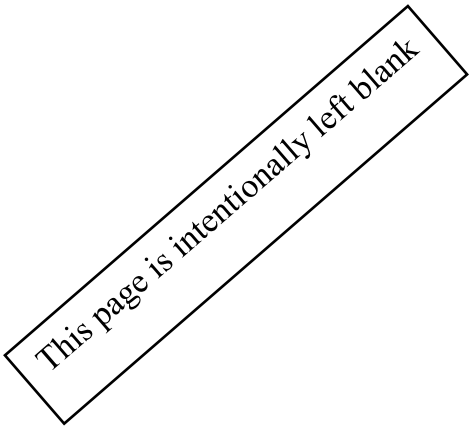
3.10 NANPDB: A Resource for Natural Products from Northern African Sources

Fidele Ntie-Kang, Kiran K. Telukunta, Kersten Döring, **Conrad Veranso Simoben**, Aurélien F. A. Moumbock, Yvette Imbole Malange, Leonel E. Njume, Joseph N. Yong, Wolfgang Sippl, Stefan Günther

Journal of Natural Products **2017**; 80(7), 2067-2076.
https://doi.org/10.1021/acs.jnatprod.7b00283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JOURNAL OF NATURAL PRODUCTS

Article

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NANPDB: A Resource for Natural Products from Northern African Sources

Fidele Ntie-Kang,[*,†,‡,#] Kiran K. Telukunta,[§,#] Kersten Döring,[§] Conrad V. Simoben,[†] Aurélien F. A. Moumbock,[‡] Yvette I. Malange,[‡] Leonel E. Njume,[∥] Joseph N. Yong,[‡] Wolfgang Sippl,[†] and Stefan Günther[*,§,⊥]

[†]Department of Pharmaceutical Chemistry, Martin-Luther University of Halle-Wittenberg, Wolfgang-Langenbeck Straße 4, 06120 Halle (Saale), Germany

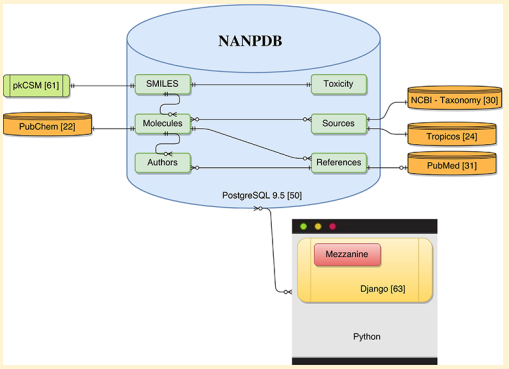
[‡]Department of Chemistry and [∥]Chemical and Bioactivity Information Centre, Department of Chemistry, Faculty of Science, University of Buea, P.O. Box 63, Buea, Cameroon

[§]Institute of Pharmaceutical Sciences, Research Group Pharmaceutical Bioinformatics, Albert-Ludwigs-University Freiburg, Hermann-Herder-Straße 9, 79104 Freiburg, Germany

[⊥]Freiburg Institute for Advanced Studies (FRIAS), University of Freiburg, Albertstraße 19, 79104 Freiburg i.B, Germany

Supporting Information

ABSTRACT: Natural products (NPs) are often regarded as sources of drugs or drug leads or simply as a "source of inspiration" for the discovery of novel drugs. We have built the Northern African Natural Products Database (NANPDB) by collecting information on ∼4500 NPs, covering literature data for the period from 1962 to 2016. The data cover compounds isolated mainly from plants, with contributions from some endophyte, animal (e.g., coral), fungal, and bacterial sources. The compounds were identified from 617 source species, belonging to 146 families. Computed physicochemical properties, often used to predict drug metabolism and pharmacokinetics, as well as predicted toxicity information, have been included for each compound in the data set. This is the largest collection of annotated natural compounds produced by native organisms from Northern Africa. While the database includes well-known drugs and drug leads, the medical potential of a majority of the molecules is yet to be investigated. The database could be useful for drug discovery efforts, analysis of the bioactivity of selected compounds, or the discovery of synthesis routes toward secondary metabolites. The current version of NANPDB is available at http://african-compounds.org/nanpdb/.



Natural products (NPs) are known to play an important role in drug discovery, as they often provide scaffolds as starting points for hit/lead discovery.[1,2] It has been verified from recent surveys that NPs from Northern African sources could constitute an important reservoir for the discovery of drugs,[3−5] due to the long history of the use of their source organisms in traditional medicine, which dates back to prehistoric and pharaonic times.[6,7] However, data for the use of the compound sources, collection points of compound sources, biological activities of tested isolates, access to compound samples for screening purposes, among others, are often unavailable and/or scattered in the literature. Some of these data are inaccessible to a majority of scientists. A smaller proportion of these literature sources includes M.Sc. and Ph.D. theses, which are often stored as hard copies in university libraries and inaccessible to the wider community of scientists working on natural products drug discovery. On the other hand, many NPs that have been identified from Northern African sources are known drugs or have been shown to have clinical potential. As a representative, the microtubule stabilizers should be mentioned; for example, taccalonomides A, B, E, and N, derived from *Tacca* species, are known to have a unique mode of action that does not involve direct binding to tubulin.[8]

It is noteworthy that the geographical region of Northern Africa differs significantly from the rest of the continent, covering a land surface area of about 9 million km^2,[9] most of which is occupied by the Sahara Desert (currently occupying a surface area of >50% of the total area of the region and is constantly expanding). The Northern African region includes Algeria, Egypt, Libya, Morocco, Sudan, South Sudan, Tunisia, Western Sahara, and parts of Northern Mali. It is therefore expected that the natural products from this part of the world will show some uniqueness with respect to structural diversity and biological activities when compared with the rest of the continent. The reason is that plants, animals, and fungi have

Received: March 31, 2017
Published: June 22, 2017

DOI: 10.1021/acs.jnatprod.7b00283
J. Nat. Prod. 2017, 80, 2067−2076

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4. Results Summary

"The computer is just a tool after all,
the most important thing is the person sitting in front of the screen"

~Robert Langridge

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4.1 Identification, Design and Bioactivity Prediction of *sm*HDAC8 Inhibitors using Computational Methods

This part of the work focused on the development of antiparasitic *sm*HDAC8 inhibitors using computer-based methods. First, the published *sm*HDAC8 inhibitors and the crystal structures of the target were studied which became available over the several last years mainly as part of international collaborations with partners of the Medicinal Chemistry group. In addition, information on available *hs*HDAC inhibitors and crystal structures were analyzed to highlight structural similarities and differences, understand structure-activity relationships and classify existing data. The conclusion, summarized in the first two articles (sections 3.1 and 3.2), was helpful to find a successful strategy to discover novel inhibitors. Investigation of the deacetylase catalytic domain of the ~ 200 deposited HDAC crystal structure from different species revealed that the main catalytic pocket (consisting of the acetate binding cavity, the substrate-binding tunnel and the rim of the pocket) is present in all the reported crystal structures, although the dimension of this pocket is different in every isoform.[24-26] Additional sites of interest that might be targeted for the development of selective HDAC inhibitors are the side pocket, the lower pocket and the foot pocket, which are not all present in every isoform and, in some cases, these pockets are closed due to the nature of the protein folding or depending on the bound ligand. In these studies, in combination with *in vitro* screening, several computational methods were applied to speed up the process of identifying and proposing novel *sm*HDAC8 inhibitors.

4.1.1 Identification of a Novel Class of *sm*HDAC8 Inhibitor

To identify novel smHDAC8 inhibitors (section 3.3), published crystal structures of *sm*HDAC8 (PDB ID: 4BZ8, 5FUE) were used to perform structure-based virtual screening of the Interbioscreen compound library comprising about 550,000 molecules. Filtration of the dataset e.g. removal of molecules with PAINS predicted endpoint, fragments with molecular weight < 250 Da, functional groups (ZBGs that we had already explored such as classical hydroxamic acids –C=O-NH-OH) resulted in a smaller set of 80 molecules that were docked into the *sm*HDAC8 binding pocket. Final selection of hits was based on docking scores and visual inspection of the predicted binding pose to confirm the presence of reported key interactions such as the ability of the molecules to coordinate the catalytic Zn^{2+}-ion and hydrogen bonding with conserved amino acid residues amongst others.

A series of *N*-(2,5-dioxopyrrolidin-3-yl)hydroxamate derivatives were selected because they possessed a ZBG that had not been previously investigated as HDACs inhibitor and were directed towards the foot pocket area. The predicted binding pose showed that this series of

molecules were able to chelate the catalytic Zn^{2+} in a bidentate fashion (Figure 11A), although, in an inverted manner when compared to the reported classical hydroxamate HDACis. The alkyl chain attached to the carbon atom of the hydroxamate could occupy the acetate-binding cavity while being stabilized by vdW interactions. The acetate binding cavity is situated near the area of the upper region of the foot pocket, which might be opened in *sm*HDAC8 by bulkier derivatives of such compounds. This information led to the identification of nine *N*-(2,5-dioxopyrrolidin-3-yl)-*n*-alkylhydroxamate derivatives (*n*-alkyl ranging from *n*-butyl to *n*-hexyl; Table 1) which were submitted to *in vitro* testing against *sm*HDAC8, the human orthologue *hs*HDAC8 and the major human HDAC isoforms (HDAC1 and -6) to assess selectivity. It was observed that the molecules had IC_{50} values ranging from 4.4–20.3 µM against *sm*HDAC8 and were equally active against *hs*HDAC1, 6 and 8. Also interesting, was the fact that, J1036 also induced dose dependent apoptosis on schistosomula. After 3 days at a dose of 100 µM, 67 % of larvae were affected. The interesting biological results prompted the necessity to crystallise one of our suggested compounds (J1036) with *sm*HDAC8 (Figure 11B). Analysis of the crystal structure of the *sm*HDAC8/J1036 complex shows that the inhibitor binds in the *sm*HDAC8 active-site pocket as predicted from the developed docking protocol. Similar binding modes were predicted by the docking experiment for human HDAC isoforms, explaining the lack of selectivity.

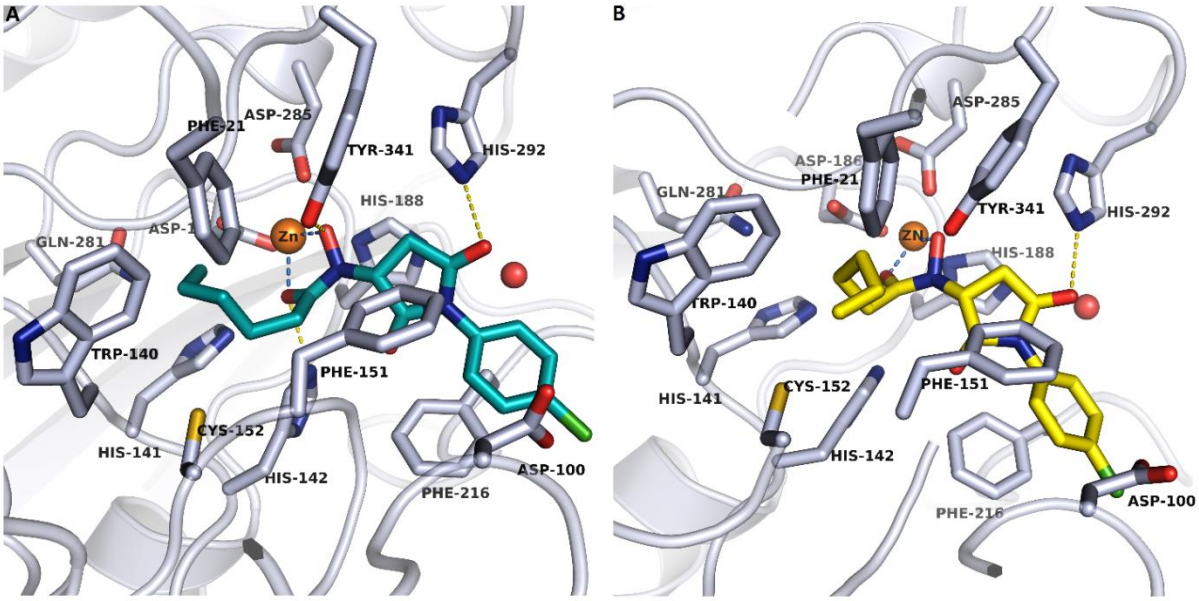
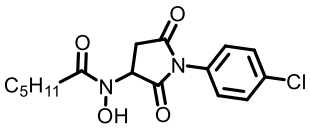
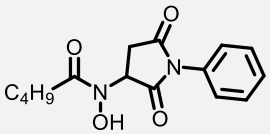
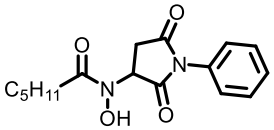
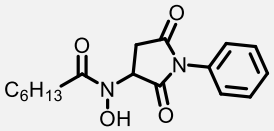
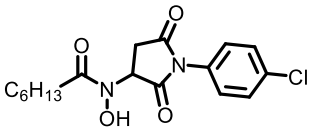
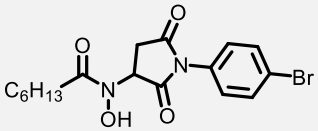
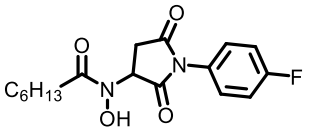
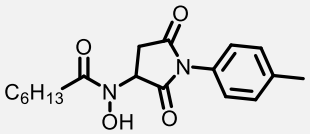
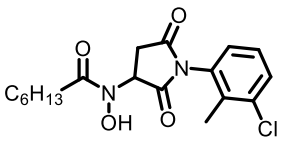
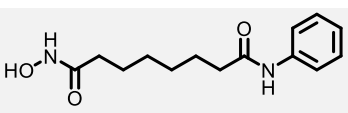


Figure 11: Predicted versus experimental binding mode of J1036 in the *sm*HDAC8 active-site cleft. A) Docking pose of J1036 in *sm*HDAC8 (PDB ID: 4BZ8). B) Crystal structure of *sm*HDAC8/J1036 complex (PDB ID: 6FU1). For both figures, protein backbones are shown as ribbon and amino acid residues within the active site are shown as white sticks. The catalytic zinc ion and conserved water molecule are respectively shown as an orange and red sphere. Coordinations to zinc ion by J1036 are shown with light blue dashed lines while the hydrogen bond interactions between the ligand and the protein are shown as yellow dashed lines.

Table 1: Summary of *in vitro* inhibitory activities of the *N*-(2,5-dioxopyrrolidin-3-yl)-*N*-alkylhydroxamates against *sm*HDAC8 and *hs*HDACs.

Code	***Structure***	***smHDAC8***	***hsHDAC 8***	***hsHDAC1***	***hsHDAC 6***
		IC_{50} (µM)	**IC_{50} (µM)**	**IC_{50} (µM)**	**IC_{50} (µM)**
J1036		4.40 ± 0.17	0.49 ± 0.18	6.76 ± 0.97	5.02 ± 0.31
J1057		13.18 ± 1.85	2.62 ± 0.19	42.1 ± 2.20	6.20 ± 0.34
J1058		20.30 ± 2.78	3.99 ± 0.74	25.96 ± 2.40	6.20 ± 0.41
J1060		11.47 ± 0.91	1.80 ± 0.24	5.00 ± 0.42	0.86 ± 0.12
J1061		5.5 ± 0.7	7.69 ± 3.30	3.98 ± 0.45	2.65 ± 0.29
J1063		5.9 ± 1.6	7.72 ± 4.42	1.42 ± 0.13	0.77 ± 0.09
J1064		7.79 ± 0.28	2.08 ± 0.34	4.30 ± 0.46	0.60 ± 0.12
J1065		20.2 ± 2.7	3.96 ± 0.60	8.40 ± 0.28	1.57 ± 0.37
J1066		13% inhib. at 25µM	n.d.	n.d.	n.d.
SAHA		1.56 ± 0.20	0.40 ± 0.10	0.12 ± 0.01	0.10 ± 0.01

4.1.2 Prediction of Activity of Novel Benzhydroxamates as *sm*HDAC8 Inhibitors using Computational Methods

Benzhydroxamate, which represents a class of molecules that have received a great deal of attention in the investigation of novel smHDAC8 inhibitors were optimized in this study (Section 3.4). For this purpose, *in silico* exploration of 34 previously reported *sm*HDAC8 inhibitors were used to generate predictive models. Initially, docking studies were performed to develop a docking protocol that would be able to reproduce the binding pose of the already crystallized molecules while suggesting the most probable binding pose for molecules with no crystal structures. Selection of binding pose for molecules with no reported crystal structure was based on confirmed interactions that have been reported and published for the chemical scaffold in question. The inability of the docking scores of the selected docking poses to explain the variation of the reported experimental activity of the molecules triggered us to perform more exhaustive calculations (binding free energy (BFE) calculations using different GB models, namely GB^{HCT} (igb=1), GB^{OBC} (igb=2), GB^{OBC2} (igb=5), and GBn (igb=8), as well as PB_mbondi (mbondi), PB_bondi (bondi), PB_Parse (PARSE)) to re-score the docking poses.

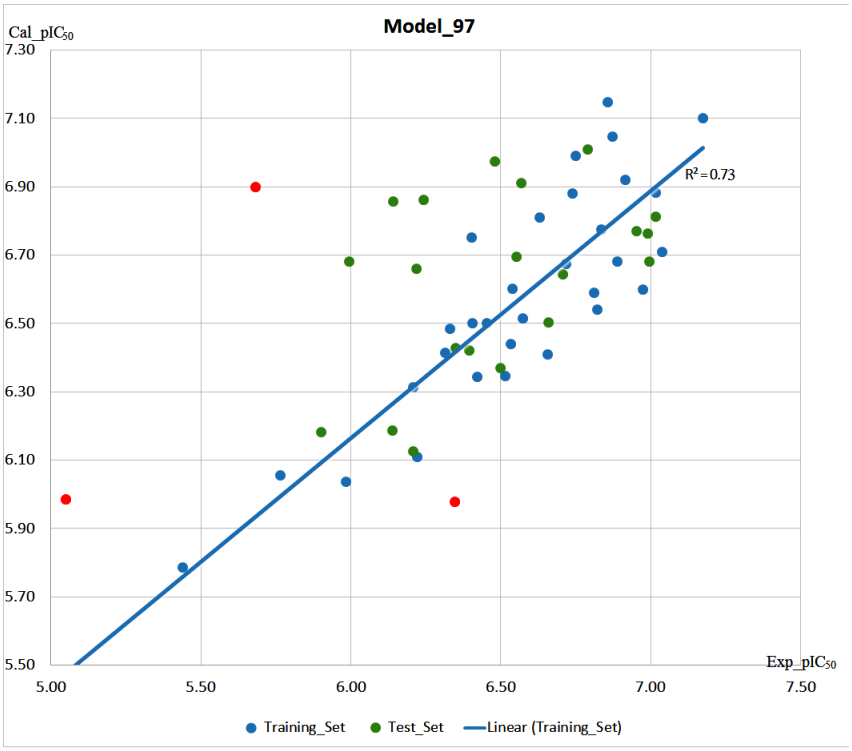


Figure 12: Correlation plot between the experimentally reported pIC_{50} values (X-axis) and the calculated pIC_{50} values (Y-axis) for the training set molecules (blue points) and test set (green points) molecules based on one *Model 97*. Red points represents molecules from the test set with poorly calculated activity.

The computed BFEs were then used to generate 3D-QSAR models. Statistical methods were further used to validate and select models that were deemed good and reliable based on the

coefficient of determination (r^2), cross-validation coefficient (q^2), root mean squared error (rmse) and cross-validated mean square error (qmse) values. Selected models were then used to predict the activity of a new set of molecules that we designed. Our best model could explain ~73 % variation of the reported experimental activity (Figure 12), as well as acceptable rmse, q^2 and qmse of 0.19, 0.66 and 0.22 respectively. Predicted compounds were then synthesized and tested *in vitro* for their *sm*HDAC8 inhibitory activity. The predicted activities of the newly designed molecules were in agreement to the experimentally determined activity. This demonstrates that CADD-based approaches like the use of QSAR methods can speed up the process of identifying novel *sm*HDAC8 inhibitors.

4.2 Application of a Large-Scale Structured-based Virtual Screening to Identify Novel Histone Deacetylase Inhibitors and HIV-1 Latency-Reversing Agents

Our contribution to the search for novel HDACis and/or HIV latency reversal involved a structured-based *VS* process in collaboration with international partners for *in vitro* testing (Section 3.5). The initial *VS* library was a collection of 5,867 unique compounds from 2D similarity search on the PubChem website using 14 known class I HDACis (belinostat, entinostat, givinostat, mocetinostat, oxamflatin, panobinostat, psammaplin A, romidepsin, scriptaid, serpulanine A, thiophenyl benzamide, trichostatin A, valproic acid and vorinostat). A docking protocol able to reproduce the co-crystalized ligand pose with the reported interactions within the active site of PDB ID: 5ICN was used to dock the molecules (Figure 13 and 14). The docking poses/results were clustered using an average rmsd of 1.5 Å and only one representative structure per each cluster was kept based on the docking score. Visual inspection for conserved interactions (such as coordination to the conserved catalytic Zn^{2+} ion and hydrogen bond interactions) led to the selection of 60 compounds as hits.

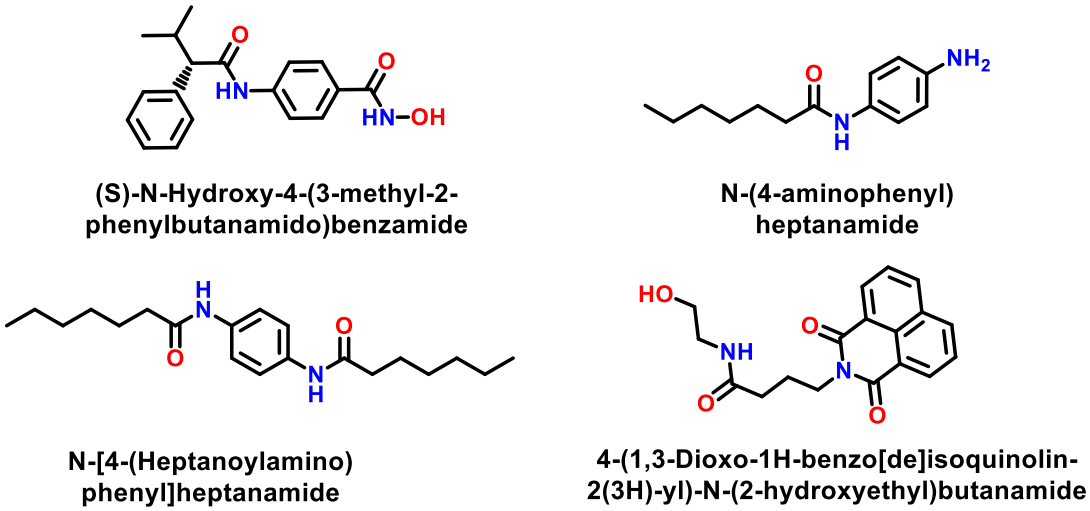


Figure 13: Chemical structures of commercially available screening hits

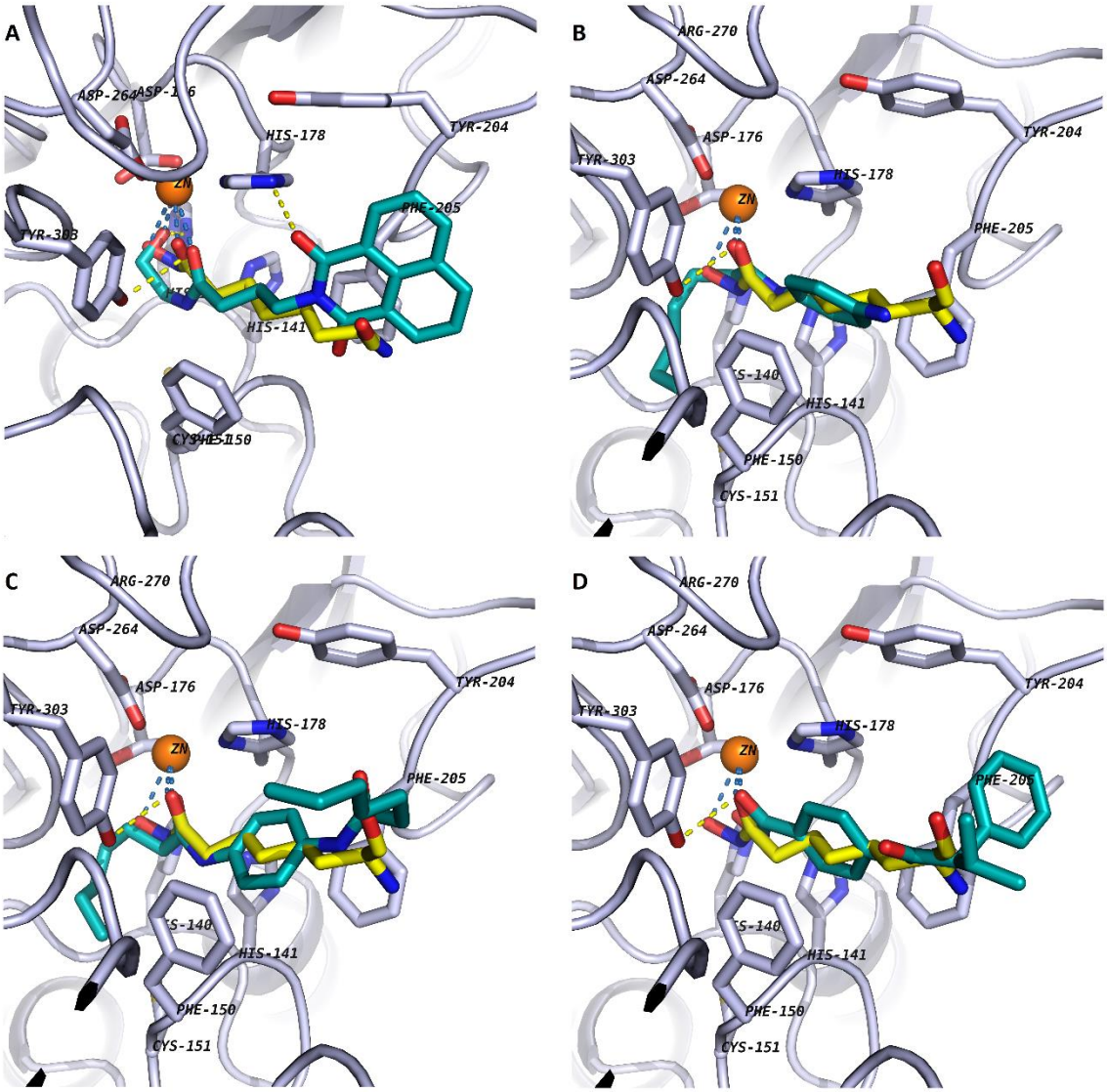


Figure 14: Docking poses of commercially available screening hits: (A) (*S*)-*N*-hydroxy-4-(3-methyl-2-phenylbutanamido)benzamide, (B) *N*-(4-aminophenyl)heptanamide, (C) *N*-[4-(heptanoylamino)phenyl]heptanamide and (D) 4-(1,3-Dioxo-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)-*N*-(2-hydroxyethyl)butanamide. For all the figures, protein backbones are shown as ribbon and amino acid residues within the active site are shown as white sticks while the co-crystallized is depicted as yellow stick. The catalytic zinc ion and conserved water molecule are respectively shown as an orange and red sphere. Coordinations to zinc ion are shown with light blue dashed lines while the hydrogen bond interactions between the ligand and the protein are shown as yellow dashed lines.

Of the 60 suggested molecules, the four compounds depicted in Figure 13 were purchased and tested *in vitro*. Two of the four purchased compounds showed HDAC1 inhibition, (S)-*N*-Hydroxy-4-(3-methyl-2-phenylbutanamido)benzamide demonstrated interesting inhibitory activity against HDAC1 and acted as an HIV LRA too. These results were following the previously published finding of Mates *et al.*[206] and Chen et al.[207] *N*-(4-aminophenyl)heptanamide and *N*-[4- (heptanoylamino)phenyl]heptanamide, on the other hand,

showed limited HDAC1 inhibition, however within the same range of efficacies observed for valproic acid (an established HDACi used in similarly designed assays).

4.3 Contribution to the Development of the African Natural Product Database (ANPDB)

NPs derived from diverse organisms have proven to be a potential starting source for the search of pharmacologically active compounds against several ailments, including cancer, bacterial, parasitic and viral infections. Interestingly, application of CADD methods can facilitate the identification of potential hits from nature for these ailments. For this section, we, first of all, looked into the various CADD methods that can be used to study the biosynthesis and identification of potential hits from nature (Sections 3.6 and 3.7). Our focus was based on NPs from Africa which have till now not received the attention they deserve. A good portion of studies from this area on NPs is linked to neglected tropical diseases (Section 3.8). This, however, confirms the effort made by the locals via folkloric medicine to treat themselves. In this regard, part of my PhD project (Sections 3.9 and 3.10) was intended to show our contribution towards the development of a new database of NPs from African source species which would be of relevance in NP related research studies especially in the area of drug discovery.

Data collected for this purpose was introduced to a wider scientific community electronically via our online platform http://african-compounds.org/anpdb/. Updating the content in our databases is done based on the quantity and/or quality of new information outsourced. Currently, the online version of the African NP database (ANPDB) has ~ 6500 unique molecules after the merging of ~ 4950 and ~ 2000 unique molecules from the Northern African database (NANPDB) and Eastern African database (EANPDB) respectively. The comparison of the chemical structures from the African NP database with one of the World´s biggest available collection of published compounds and their biological activities PubChem revealed a poor overlap. The presence of ~ 3500 unique molecules (more than half of the database) were found in the newly developed African NP database, showing that it covers an uncharted chemical space of natural compounds. Additionally, our online platform is designed with several search methods including search by source species name, compound name, biological activity, compound structure and substructure search etc.

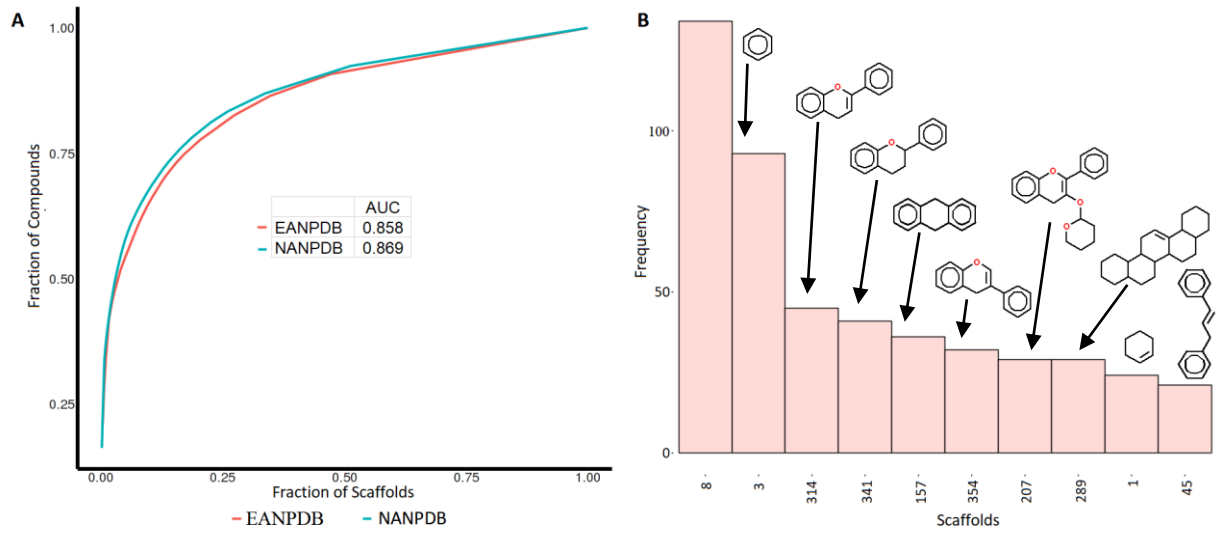


Figure 15: Distribution of scaffold similarity between EANPDB and NANPDB using A) CSR and B) SSE10 with the frequency of some of the corresponding cyclic scaffolds.

Cheminformatics and pharmacoinformatics analysis of the content of the ANPDB was also performed. Such analysis included the comparison of the scaffold diversity between molecules isolated from source species collected from East Africa against those collected from Northern Africa (Figure 15) using the cyclic system recovery (CSR) and scaled shannon entropy (SSE) methods. From the data collected, the most abundant compound classes were terpenoids, flavonoids, quinones, alkaloids and phenolics contributing to about 75% of the total molecules. In the same line, the most investigated biological activity was anti-malarial/anti-plasmodial related evaluations. This observation is quite in line with the fact that malaria and other parasitic diseases remain a serious burden to the people in this region. Therefore, the validation of the traditional methods in treating these diseases via scientific standards goes into confirming the usage of such traditional practices and can help in regulating the quality of the products being consumed.

Furthermore, the distribution of the drug-like properties (such as molecular weight (MW), predicted LogP octanol-water partition coefficient (LogPo/w), the number of hydrogen bond donor/acceptor atoms and Lipinski's "Rule of Five" violation) and *in silico* toxicity predictions for molecules in our collection was also analyzed in comparison to other popular published databases like DrugBank (Figure 16). From the figures presented below, it can be observed that the molecules within the NANPDB and the EANDB were mainly cyclic with relatively similar cyclic scaffold diversity. Also, the content of our databases had a similar distribution of major drug-like properties when compared to the approved drugs (the content of the DrugBank dataset). For example, approximately 85 % of the molecules contained in the analysed datasets had MW less than 500 Da while ~ 80 % of the molecules in the datasets respected the conditions

of donor/acceptor HB in the rule of five. In summary, the molecular enumeration showed that a majority of molecules in the African databases did not show any Lipinski's violation.

In silico toxicity predictions also revealed that most of the compounds in the current version of our database are likely non-toxic and would not interfere with the inhibition of the potassium ion (K^+) channels (encoded by hERG I). Also of interest to us was the fact that very few (about 10 %) of the molecules we present here do have reactive groups and/or toxicophores that can interfere with readouts in assays. This was confirmed after we screened our database collections to see how many molecules contain scaffolds that would be predicted with an endpoint as PAINS. All in all, analysis of the database showed that it contains a significant number of novel natural drug-like molecules.

4.4 Access to the ANPDB

Accessibility to the ANPDB is free of charge online (see Sections 3.9 and 3.10 for more details). The NANPDB and the EANPDB are stored as part of the African NP database hosted at the Albert-Ludwigs-University Freiburg, which can be accessed via http://african-compounds.org/anpdb/. The platform is updated based on the quantity and quality of new information in hand. Curated information from literature sources is grouped into different SQL tables. For each molecule entry, we assigned the same unique ID across all the SQL tables; which is used to link information across the different tables. This platform represents the first database collection of African NPs and the most extensive collection available at the moment. The platform is built with an array of search fields including biological activity, compound name, source species, families and authors/reference. Similarity search and substructure search have also been implemented on the online platform. The structural similarity search uses the tanimoto coefficient of similarity (a number between 0 and 1; with 1 being the highest and referring to an exact match) to measure the 2D similarity between the query molecule and the database entries. The Morgan Fingerprints (Circular Fingerprints) used for the structural similarity search are pre-calculated for all database molecular entries and stored as blob objects in the PostgreSQL-database. For each query structure, calculations are made during the search. The platform also provides users with an option to download the entire content as 2D or 3D .SD files or SMILES. There is also a help page to guide new users through our platform which also answers technical questions that might arise. Thus, our overall vision from this project is to see that some of the promising preliminary results collected from peer-reviewed literature as well as what we have provided can be used to enhance NP driven drug discovery projects from African source species

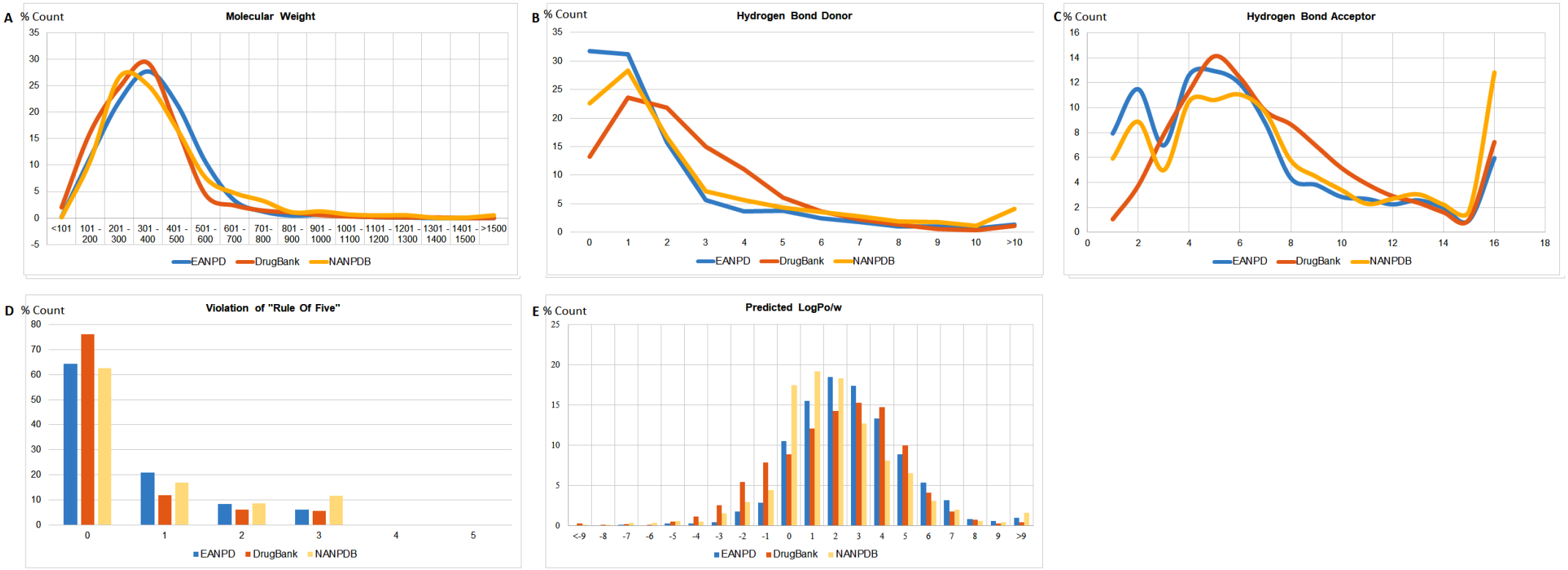


Figure 16: Distribution of drug-like properties between EANPDB, NANPDB and DrugBank. A) Molecular weight, B) Hydrogen bond donor, C) Hydrogen bond acceptor, D) Violation of Lipinski's "Rule of Five" and E) predicted lipophilicity.

4.5 ANPDB as a Digital Source for Novel HDAC Modulators

4.5.1 Virtual Screening of the ANPDB

In the quest to search for HDAC modulators, several NP-based molecules were seen as interesting. Our contribution to propose novel HDACis from the ANPDB started with a structured-based *VS* process followed by the visualization of binding poses and evaluation of their stability. This was done following similar procedures as reported in our previous publications.[55,56,208] For this study, *VS* using the *sm*HDAC8 crystal structure with PDB ID: 5FUE led to the selection of 17 compounds (Figure 17, Table 2). However, due to the difficulty in getting physical samples, unavailability and the high prices of the molecules, only 3 compounds could be obtained for experimental testing (compounds **14**, **15** and **17**)

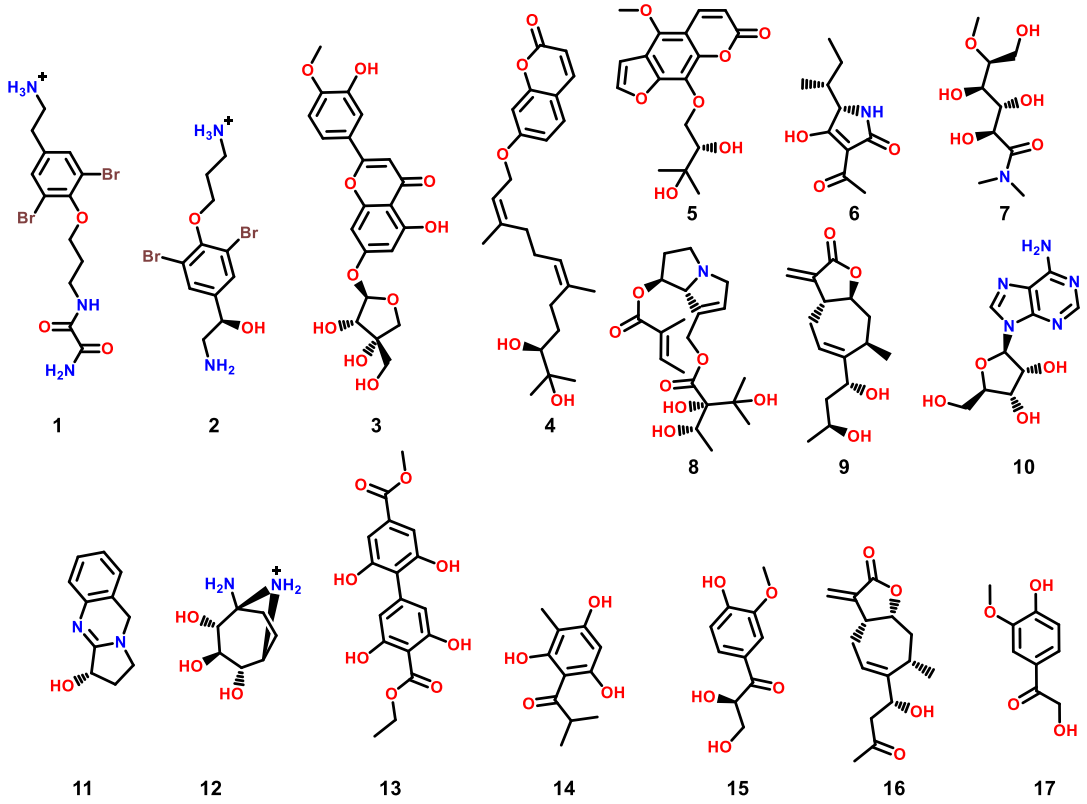


Figure 17: Structure of proposed NP hits from the ANPDB collection.

Table 2: Summary of proposed hits from the ANPDB.

Mol #	Name	Source species	Reported activity	Traditional use of source species
1	moloka'iakitamide	*Pseudoceratina arabica*	antimicrobial activities; parasympatholytic effect	Not reported
2	hydroxymoloka'iamine	*Pseudoceratina arabica*	antimicrobial activities	Not reported
3	diosmetin 7-*O*-beta-D-apiofuranoside	*Phoenix dactylifera*	antidiabetic activity	in the Middle East, it is believed that eating date fruits, particularly in the morning on an empty stomach, can reverse the actions of any toxic material that the subject may have been exposed to. Different parts claimed to be used for the treatment of a broad spectrum
4	karatavicinol	*Ferula sinaica*	Not reported	used as food additives (spice). Extracts of Ferula assa-foetida L. are used as an anti-spasmodic, a diuretic, a vermifuge and an anti-algetic
5	byakangelicol			
6	tenuazonic acid	*Alternaria species*	Not reported	Not reported
7	5-*O*-methyl-D-gluconic acid dimethylamide	*Apis species*		used in folk medicines in many regions of the world for diverse reasons such as the treatment of bacterial and viral infections
8	heliosupine	*Paracaryum rugulosum*	Not reported	Not reported
9	ivalbin	*Pulicaria undulata*	antiinflammatory activity	to treat inflammation, as an insect repellent and as a herbal tea.
10	adenosine	*Oligomeris linifolia*	Not reported	Not reported
11	(+)-vasicine	*Galega battiscombei*	Not reported	Not reported
12	calystegine N1	*Hyoscyamus albus*	Not reported	Not reported
13	4'-ethyl-4-methyl-2,3',5',6-tetrahydroxy[1,1'-biphenyl]-4,4'-dicarboxylate	*Schinus terebenthefolus*	Not reported	Not reported
14	methyl phloroglucinol iB	*Hagenia abyssinica*	Not reported	female flowers are widely used as taenicide against tapeworm.
15	(+)-2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone	*Anastatica hierochuntica*	anticancer activity	treatment of fatigue and uterine haemorrhage in Egyptian folk medicine
16	2-hydroxytomentosin	*Xanthium pungens*	Not reported	Not reported
17	2,4'-dihydroxy-3'-methoxyacetophenone	*Anastatica hierochuntica*	Not reported	treatment of fatigue and uterine haemorrhage in Egyptian folk medicine

Not reported: Information not available on the ANPDB online platform. This was either not provided in the literature source or has not yet been investigated.

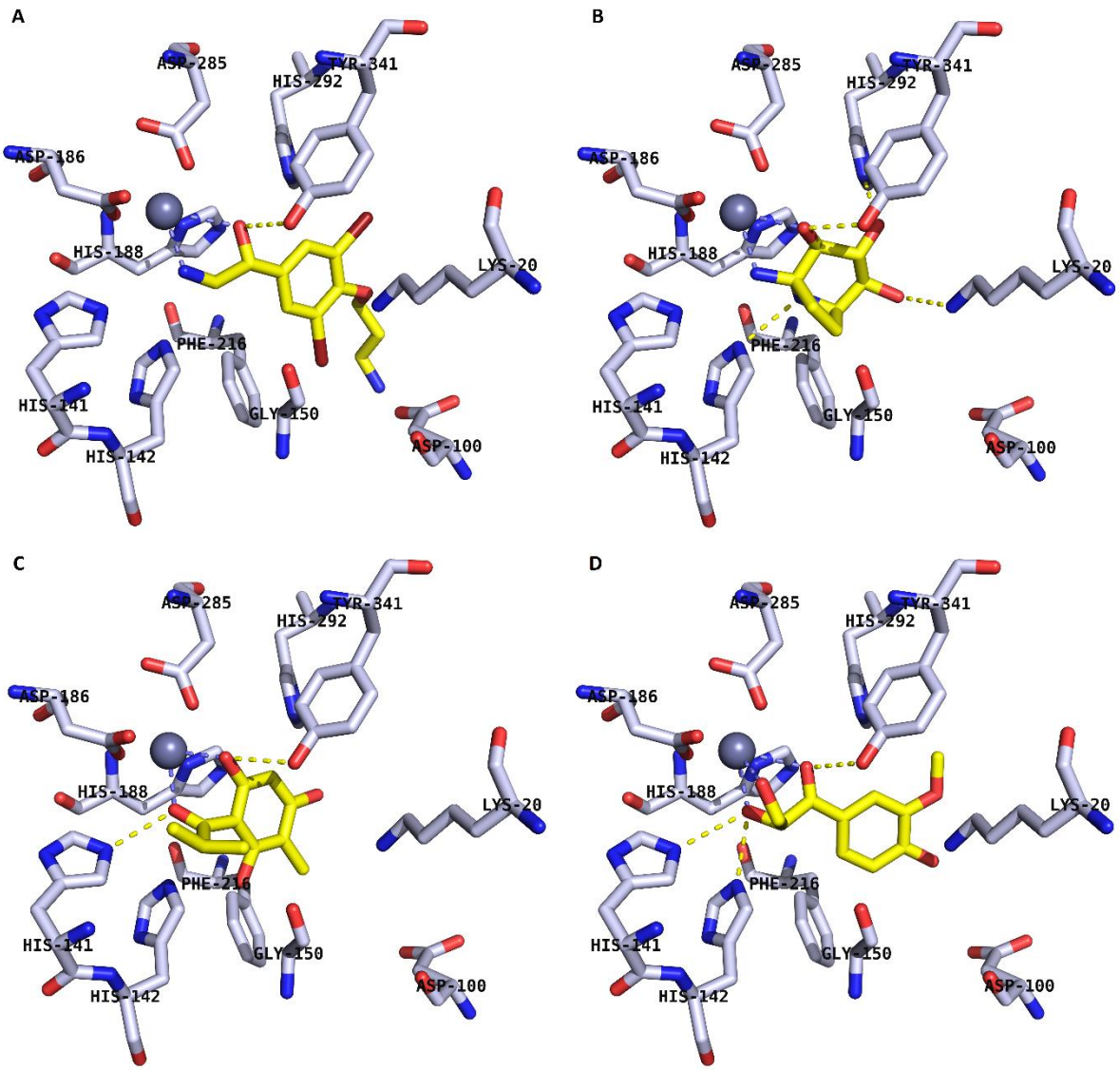


Figure 18: Proposed binding mode of some of the suggested hits in *sm*HDAC8 (PDB ID: 5FUE). A) Compound **2**, B) Compound **12**, C) Compound **14** and D) Compound **15**. For all the figures, coordinations to the catalytic Zn-ion are shown with light blue dashes while yellow dashes represent hydrogen bonds.

4.5.2 Analysis of Binding Mode and Stability of Hits

The predicted binding modes of compounds **2**, **12**, **14** and **15** are shown in Figure 18. Although the compounds are not structurally similar to each other, the predicted binding pose for these compounds within the active site of the *sm*HDAC8 crystal structure (PDB ID: 5FUE) was attractive for further evaluation. Interestingly, coordination to the catalytic Zn-ion alongside reported hydrogen bonds between the compounds and conserved amino acid residue within the binding site were observed. Equally, at least one aromatic contact and/or vdW interaction was additionally observed. For example, analysis of the predicted binding mode of compound **2** (Figure 19 A) showed that it coordinated the catalytic Zn-ion in a bidentate fashion while several hydrogen bonds with conserved amino acids were observed. Concerning compound **14** (Figure

19 B), it was observed that it coordinates the catalytic Zn-ion in a bidentate manner too, as well as forming a π–π interactions with the aromatic ring of His-188. Meanwhile, the commonly observed hydrogen bond interaction between the conserved His-141 and the carbonyl-oxygen atom coordinating the catalytic Zn-ion was also observed.

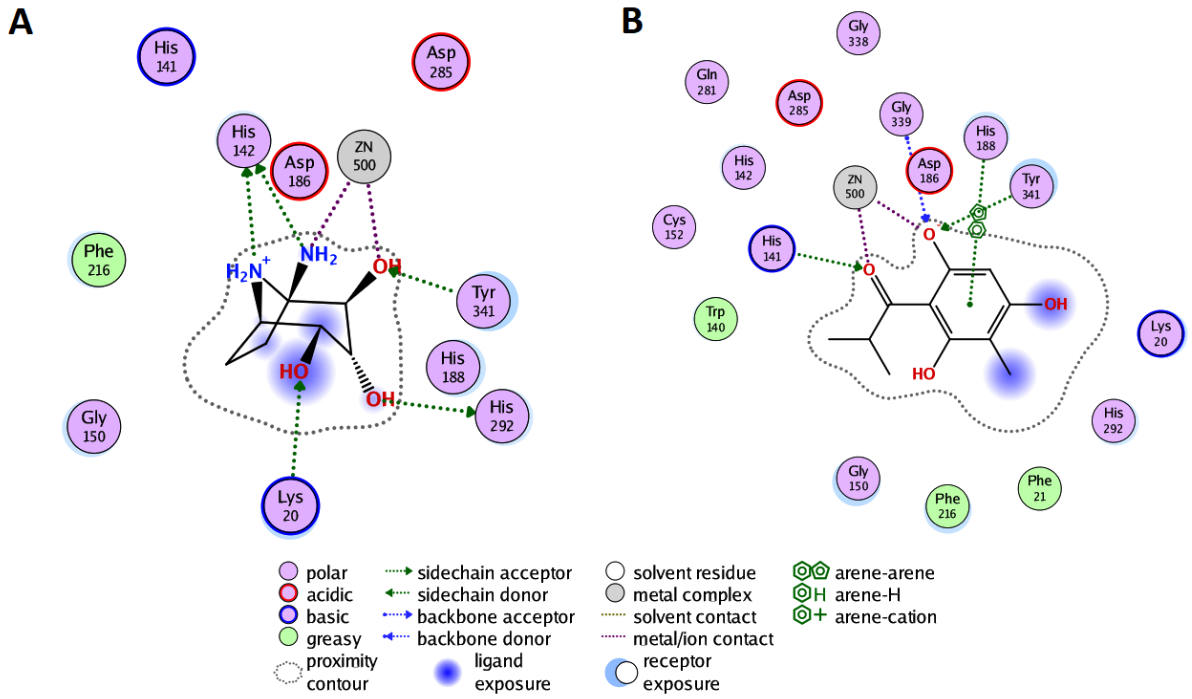


Figure 19: Interaction diagrams for A) compound **2** and B) compound **14.**

The 17 ligands predicted to be strong-binders were selected and short molecular dynamic simulations were performed to check the stability of the interactions between the ligands while observing if they maintain their interactions with the catalytic Zn^{2+} ion throughout the MD simulation. Stability plot after 1 ns MD simulation for compounds **2**, **12**, **14** and **15** are shown in Figure 20. In the analysis of the stability of the compounds, it was observed that during the MD simulation process, most of the compounds maintained their predicted pose and their coordination to the catalytic Zn^{2+} ion. However, for some molecules e.g. compound **15**, the molecule lost its interactions with the catalytic Zn-ion from the very onset of the MD simulation run.

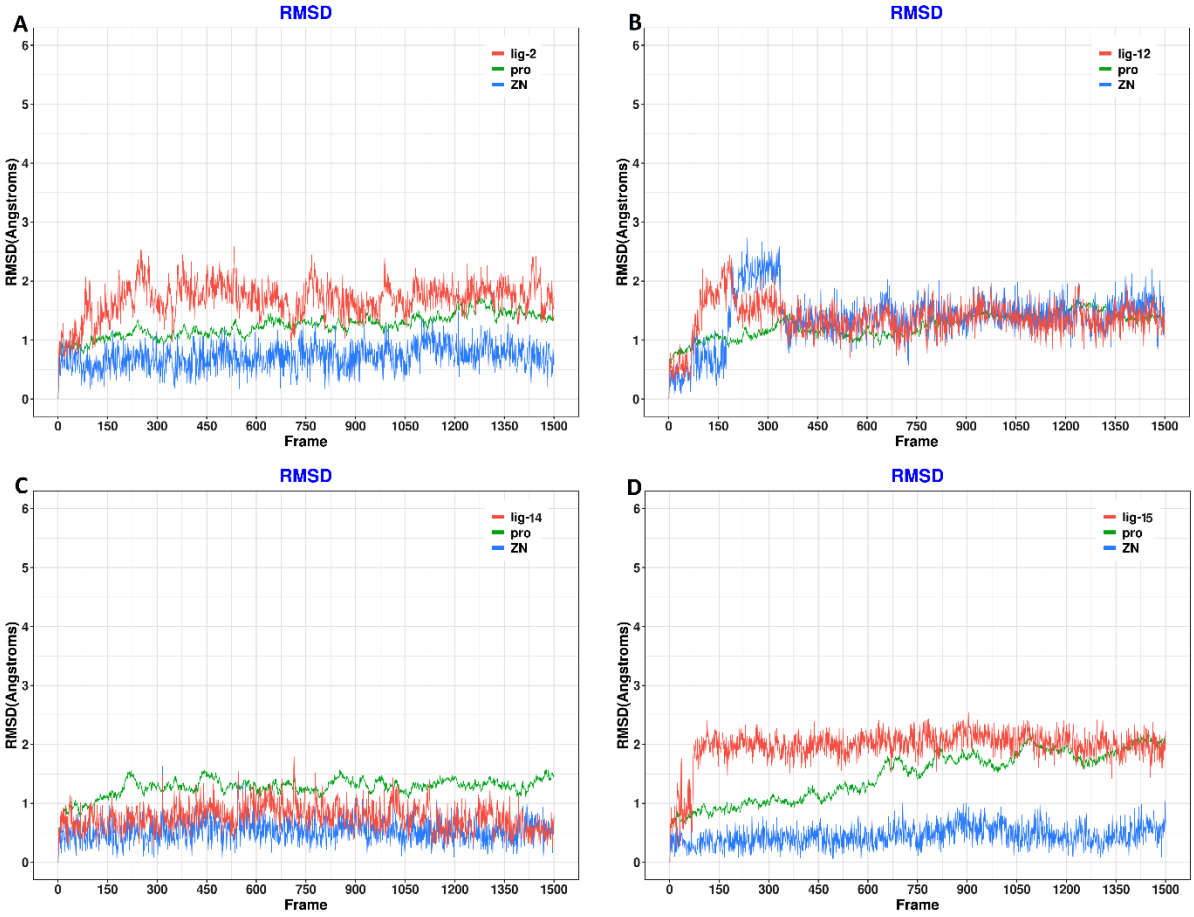


Figure 20: C-alpha rmsd plots for some of the selected hits. A) compound 2, B) compound 12, C) compound 14 and D) compound 15.

4.5.3 *In Vitro* HDAC Inhibitory Assay

In this study, the *sm*HDAC8 inhibitory activity of the 3 purchased compounds (compounds **14**, **15** and **17**) was measured using an enzymatic assay (in house, P. Zeyen). In brief, procedures to express and purify the recombinant *sm*HDAC8 used in this study for inhibition assays are described in a previous publication.[223] Inhibition of *sm*HDAC8 was performed using the commercial Fluor de Lys kit (BML-KI178); a two-step assay based upon the Fluor de Lys®-Green substrate and Fluor de Lys® developer combination (Figure 21). The sequence of peptide used in Fluor de Lys can be either Ac-Arg-His(Ac)-Lys(Ac)-methylcoumarin (for peptide representing p53) or Ac-Lys-Gly-Gly-Ala-Lys(Ac)-methylcoumarin (for peptide representing H4). Incubation of compounds, Fluor de Lys-HDAC8 substrate (50 µM) and enzyme was performed for 90 mins at 37 °C. 50 µL of Developer II (BML-KI176) was subsequently added in the reaction batch and further incubated for 45 mins at 30 °C. Similarly, Trichostatin A (2 µM) was used to stop the reaction while fluorescence was measured in a plate reader (BMG Polarstar).

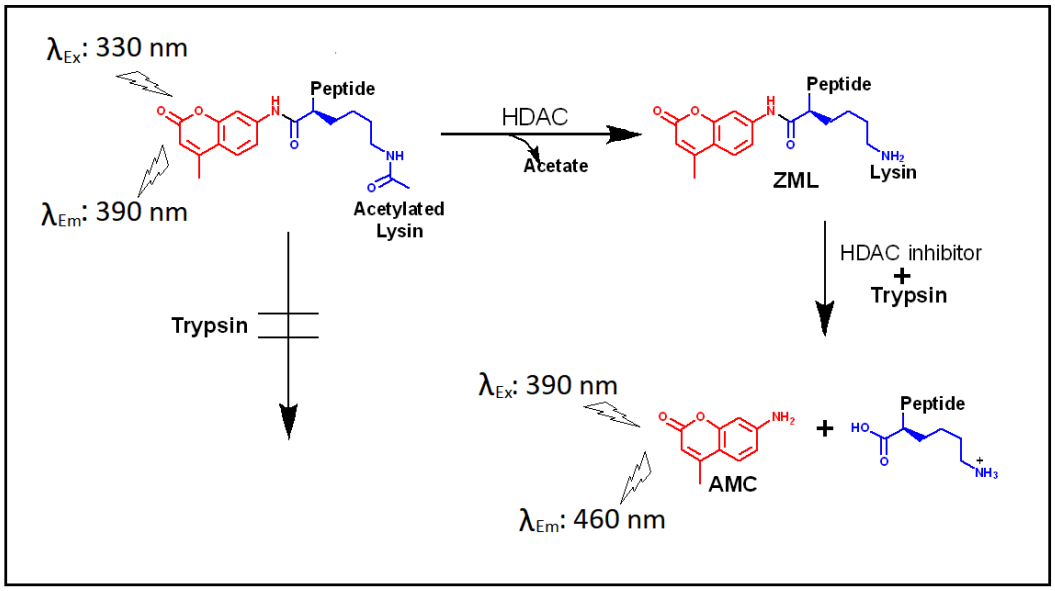


Figure 21: Schematic representation of the detection principle of the trypsin assay.

Based on the performed *in silico* results, it was expected that the purchased compounds are modulators of *sm*HDAC8. However, all the molecules showed only very weak inhibitory activity against *sm*HDAC8 being 25-40 % inhibition even at a concentration of 100 µM (Table 3). While no significant inhibition was observed for the compounds **14**, **15** and **17**, it is still evident that they could bind to HDAC. The inhibitory activity of the tested molecules are low and may be due to several causes such as the small molecular sizes, tautomeric transformations, etc. Hence, the search of novel NP-inspired molecules as modulators of HDACs *via* the testing of the other proposed hits would be a desirable approach.

Table 3: Summary of *in vitro* inhibitory activities of the purchased NPs against *sm*HDAC8

Compound/Concentration	***sm*HDAC8 Inhibition [%]**		
	100 µM	**10 µM**	**1 µM**
Compound **14**	23.5	16.0	13.9
Compound **15**	34.1	7.8	9.8
Compound **17**	39.9	26.3	9.7

5. General Conclusion and Perspectives

"A man is not finished when he is defeated. He is finished when he quits."

~Richard M. Nixon.

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The spread of diseases has urged the dawning of new strategies; permitting researchers to delve into projects that can lead to the proposal of new candidate drugs. Computational methods applied in drug discovery have proven to be worthy in the entire drug discovery and development pipeline. In the studies reported herein, we were able to demonstrate that the application of various computational methods coupled to *in vitro* screening permitted us to identify novel inhibitors of HDACs. These HDACs are well recognized and validated targets to treat several diseases including cancer, parasitic and viral infections. Firstly, the generated protocols were able to identify novel HDACis and correctly predict their binding pose within the active site of the antiparasitic target *sm*HDAC8. Generally, key interactions that were reported were observed. Nevertheless, novel HDACis with a novel zinc-binding motif (reversed hydroxamate moiety) could be identified that had not been previously explored. Furthermore, CADD methods such as QSAR models led to computer-guided optimization of benzhydroxamates as *sm*HDAC8 inhibitors. However, problems with data size, sample size and chemical space were observed. Thus, the results from the QSAR prediction clearly showed that accurate prediction was only possible for highly similar compounds. Nevertheless, predictive models were able to explain ~ 73 % variation in the reported biological activity of the molecules. Our findings, therefore contributes to the global picture of using CADD methods to predict the activity of new molecules with inhibitory activity against *sm*HDAC8. Future work in this line would include a larger sample size with the current results presented herein being a basis for that sample size generation.

Secondly, a *VS* procedure was carried out to identify novel HDAC1 inhibitors. HDAC1 have been recognized to reactivate latent proviral cells and can be very useful for the complete eradication of HIV when such HDAC1 inhibitors are used simultaneously with cART. The performed *VS* permitted us to suggest novel HDAC1 inhibitors. Thus, the reported approaches can be used, in principle, to identify additional, novel and selective HDAC modulators with other functional groups/pharmacophores which can serve as starting points to design more potent inhibitors. Such synthetic compounds can be used not only in the treatment of schistosomiasis or HIV/AIDS, but also for cancer, neurodegenerative diseases and other pathological conditions. Finally, the current work was, however, not limited to synthetic HDAC modulators. In this era of growing interest in NPs and pseudo-natural compounds coupled to the plentiful information and investment on NPs, huge data has been made available online. Nonetheless, to satisfy the needs of *in silico* methods in identifying potential hits with NP based origin, NP-based compounds information must be made accessible in the form of libraries with well characterized molecules. Novel databases of African NPs were developed and overcame

the challenges in the process of data collection for NPs isolated from African sources. The ANPDB, although not exhaustive, represents the largest collection of its kind for the African continent so far. Hopefully, the new and freely accessible information (http://african-compounds.org/anpdb/) provided for natural products isolated from African sources can be successfully translated into a pool of hit identification by the scientific community. Also, the major merit of the current African NP collection is seen with the presence of ~ 3500 molecules that are not found in PubChem. One of the perspectives would be to follow-up the submission process of these molecules to PubChem. While this process would help in improving the visibility of the current African collection, it would also call for more scientific collaboration or works on these molecules. Such collaboration could involve the investigation of modes of action and alternative biological activities, incorporation of more computed molecular descriptors, experimental data leading to the characterization of the NPs, possible biosynthetic pathways for metabolites included in the collection. However, this part of the study was additionally limited due to the problematic access to data for outsourcing/curation to construct the ANPDB.

Albeit this challenge, several scientific collaborations were established, providing access to information not available in online journal databases. Even so, availability of physical samples for molecules included in the ANPDB, likewise, most available NP databases, for biological testing is still a limitation. Notwithstanding, current efforts to provide NP samples are being targeted through novel and enhanced techniques such as genomics-based approach, design and total or semi-synthetic chemical synthesis of NPs. From the work, plans to create collaborative support with end users of our platform to access services like on-demand sourcing and extraction are being put in place. Nevertheless, successful identification of hits from the developed database has already been reported, such as the identification of Sirtuin inhibitors and the proposing of potential RNA-polymerase inhibitor for SARs-CoV-2 via the use of CADD methods.[209,210] Preliminary screening of the database suggested that there are plenty of putative HDAC modulators. In the current study, three compounds identified from the ANPDB and purchased, showed only a very weak inhibition of *sm*HDAC8 in µM concentrations. However, identifying low nM molecules as hits from *in silico VS* projects remains a bottleneck. Optimization of these primary hits with low molecular weights from the screening campaigns to leads with even potent activities is important. Further *in vitro* screening of the other suggested molecules against different HDAC isoforms will be carried out in future work. Additionally, known ZBGs like tropolone and thiol are present in the current collection and can serve as a starting point for substructure search as a means to suggest hit molecules for *in vitro* screening.

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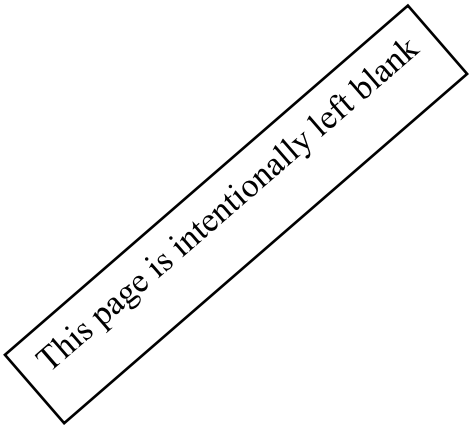
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Appendix

Curriculum Vitae

"I tried and failed. I tried again and again and succeeded"

~Epitaph from Gail Borden's gravestone.

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PERSONAL INFORMATION

VERANSO CONRAD SIMOBEN

https://www.researchgate.net/profile/Conrad-Veranso-Simoben
https://scholar.google.com/citations?user=qZYGPzIAAAAJ&hl=en
https://www.linkedin.com/in/veranso-conrad-simoben-phd-msc-amrsc-32899aa1/

Languages| English (Near native), French (Near native), German (Intermediate), Lamnso (Native), Pidgin (Very good)

EDUCATION AND TRAINING

October, 2016 – December, 2020
PhD Studies
Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg

October 2018 - January 2019
Programming with Python (Intensive online Course)
Code Academy, United States

April 2016 – September 2016
Deutsch Language (B1 Level)
Göethe Institute, Göttingen-Germany

October 2012 – July 2015
Master of Science (M.Sc.) Degree in Chemistry
University of Buea, Cameroon

October 2008 – November 2011
Bachelor of Science (B.Sc.) Degree in Chemistry, Minor in Pharmaceutical Chemistry
University of Buea, Cameroon (Second Class Honours Upper Division)

WORK EXPERIENCE

October 2016-November 2020
PhD Student and Research Assistant at the Institute of Pharmacy, Martin-Luther-University, Halle-Wittenberg.

2015/2016 Academic Year
Full-time chemistry teacher at St. Anne High School Limbe, Cameroon

November 2013 – May 2016
Researcher at the Chemical and Bioactivity Information Centre (CBIC), Department of Chemistry, University of Buea, Cameroon.

July – August 2010
Intern student at Mountain Pharmacy, Buea, Cameroon.

ADDITIONAL INFORMATION

Honours and awards

1. Travel Grants (GBP 800) for PhD Students and Early Career Scientists (Turned Down) Funding agency: Royal Society of Chemistry (RSC), Grant reference: T19-1715

2. Royal Society of Chemistry (RSC), Travel and accommodation funding to attend and give a flash presentation PACN Congress 2019 - Healthcare: From discovery to delivery 05/11/2019 to 07/11/2019, United Nations Conference Centre, Ethiopia.

3. Award: To most committed international student (500€ awarded by the DAAD through the International Office) at the Martin Luther Universität Halle-Wittenberg for the Academic year 2017/2018, Oct **2018**

4. Award: Student fellowship (500€) to attend the XXVIII edition of ESMEC School held from July 1 to 5, 2018 in Urbino, Italy, Jul 2018 Award: Student bursary (650€) to attend the 11th International Conference on Chemical Structures (ICCS-

2018) May 27-31, 2018 at Noordwijkerhout, The Netherlands, May **2018**

5. Award: Digital badge from INASP (http://moodle.inasp.info): AuthorAID Proposal Writing and Research Writing Online Course (April to June 2017) - Merit Grade, Jun **2017**

6. DAAD Scholarship: Fellowship award for long research stays for doctoral candidates (Full PhD research in Germany), The German Academic Exchange Service (DAAD, April 01, **2016** - September 30, **2019**)

Scientific Contributions

Book Chapters

1. **Conrad Veranso Simoben**, Fidele Ntie-Kang, Dina Robaa, Wolfgang Sippl. "Case studies on computer-based identification of natural products as lead molecules. Physical Sciences Reviews **2020**; (published online ahead of print), Physical Sciences Reviews, 2020, https://doi.org/10.1515/psr-2018-0119.

2. **Conrad Veranso Simoben**, Fidele Ntie-Kang: Chapter 5: *African medicinal plants: an untapped reservoir of potential anticancer agents*. In Cancer Preventive and Therapeutic Compounds: Gift From Mother Nature, Edited by Sahdeo Prasad, Amit K Tyagi, **2017**; pp 85-104; Bentham Science. eISBN: 978-1-68108-491-6

3. Aurélien F. A. Moumbock, **Conrad Veranso Simoben**, Ludger Wessjohann, Wolfgang Sippl, Stefan Günther, Fidele Ntie-Kang: Chapter 10: Computational studies and biosynthesis of natural products with promising anticancer properties. In Phytochemistry - Natural Products and Cancer, Edited by Farid A. Badria, **2017**; InTech Open., ISBN: 978-953-51-5277-4

Publication List

1. Jelena Melesina, **Conrad Veranso Simoben**, Lucas Praetorius, Emre F. Bülbül, Dina Robaa, Wolfgang Sippl. Strategies to Design Selective HDAC Inhibitors. *ChemMedChem* **2021**; https://doi.org/10.1002/cmdc.202000934

2. Smith B. Babiaka, **Conrad Veranso Simoben**, Kennedy O. Abuga, James A. Mbah, Rajshekhar, Karpoormath, Dennis Ongarora, Hannington Mugo, Elvis Monya, Fidelis Cho-Ngwa, Wolfgang Sippl, E. Joel Loveridge, Fidele Ntie-Kang, Compounds with Anti-Onchocercal Activity from Voacanga africana Stapf (Apocynaceae): Identification and Molecular Modeling. *Molecules* **2021**; 26 (1), 70 https://doi.org/10.3390/molecules26010070

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2. **Conrad Veranso Simoben** et al., Challenges involed in in silico based natural product drug discovery (In preparation)

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Memberships

1. Royal Society of Chemistry (RSC), since, **2014**
2. Pan African Chemistry Network (PACN) since, **2014**
3. Alumni Network Subsahara Africa (ANSA e.V.) (post@ansa-ev.org) since, **2016**
4. International Union of Pure and Applied Chemistry (IUPAC), since, **2017**
5. Board member for the Alumni Network Subsahara Africa (ANSA e.V.) (post@ansa-ev.org) since, **2018**

Halle (Saale)
23. 03. 2021

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Declaration of Authorship

Selbstständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertationsschrift selbständig und ohne fremde Hilfe angefertigt, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und die aus ihnen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe. Die Arbeit wurde ausschließlich der Mathematisch-Naturwissenschaftlichen Fakultät der Martin-Luther-Universität Halle-Wittenberg vorgelegt und an keiner anderen Universität oder Hochschule weder im In- und Ausland zur Erlangung des Doktorgrades eingereicht.

Halle (Saale), den 23. 03. 2021

Conrad Simoben, Veranso

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I hereby declare that I am the sole author of this thesis and that I have not used any sources other than those listed in the bibliography and identified as references. I further declare that I have not submitted this thesis at any other institution in order to obtain a degree.

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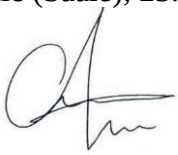

Conrad Simoben, Veranso