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Screening of Chrysin and its Derivatives for their Binding Affinity to Human Histone Deacetylases (Hdacs): A Computer-Aided Approach

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Abstract – Repositioning chrysin and chrysin-analogue as possible human histone deacetylases (PDB ID: 1t64) inhibitor was investigated. The chrysin-analogue CHD1, Chrysin, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7,CHD8, CHD9 and CHD10 was noticed to have docking score of -7.8 kcal/mol, -7.6 kcal/mol, -7.6 kcal/mol, -7.3 kcal/mol, -7.2 kcal/mol, -7.2 kcal/mol, -7.1 kcal/mol, -6.9 kcal/mol, -6.9 kcal/mol,, -6.4 kcal/mol, and -5.7 kcal/mol respectively. The lead molecule (4-(5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride) was noticed to interact with MET 261, PRO 260, TYR 87, GLY 292, TYR 293, LEU 295, ARG 24, PRO 22, ILE 21, LEU 18, TYR 98, TRP 128, TYR 141 and PHE 139. The lead molecule demonstrated good pharmacokinetics and drug-like characteristic. Hence, 4-(5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride is a promising compound that should be further examined as drug candidates

Keywords – Chrysin, Molecular Docking, ADMET, Bioactive Compound, Pharmacophore.

I. INTRODUCTION

Flavonoids are extensive polyphenolic secondary metabolites that are abundant plants and foods such as onions, tea and red wine, fruit, and vegetable [1, 2]. A high amount of flavonoids in human diet is of great benefit to the wellbeing of man. This could be due to its pharmacological uniqueness which includes its anti-oxidant, anti-cancer, anti-viral and anti-inflammatory properties. On the other hand, nitrogen fixation and chemical defences are the vital functions of flavones [3-11]. Meanwhile, there are More than 4,000 types of biologically active flavonoids have been identified, which have been classified as flavonols, flavones, flavanols, flavanones, anthocyanidins and isoflavonoid subclasses [3, 4].

Chrysin, which consists 15-carbon flavone backbone is a wide distributed flavonoid and has been reported to have diverse biological activities such which include anti-oxidant[12, 13], anti-virus [14], anti-diabetogenic activity [15], anti-inflammatory [16] and anti-anxiolytic effect [17] amongst others. Besides the aforementioned characteristics,

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chrysin has demonstrated good inhibitory activities against aromatase and human immunodeficiency virus [18, 19]. Furthermore, chrysin has demonstrated anti-cancer and anti-tyrosinase inhibitory activity [20].

Histone acetylation has been noticed to strongly impact gene transcription [21, 22]. Meanwhile, acetyltransferases (HATs) and histone deacetylases (HDACs) are known to catalyze the addition and elimination of acetyl functional group respectively. The action of these enzymes is often on the amino groups of lysines near the amino termini of histones. The removal of acetyl functional group from histone have been observed to promotes the condensation of chromatin which then results in transcriptional repression [23]. Hence, HDAC is noticed to cause gene silencing. However, some report revealed that HDAC inhibitors (HDIs) could not enhance transcription [24, 25]. Meanwhile, it was found that HDIs inhibit the growth of tumour cells [22]. Hence, HDIs are also used as antitumor agents in oncology.

The computer-aided drug design technique is an effective method for drug design and drug development. The technique saves cost and time required to produce a drug. To enhance the biological activities of chrysin as HDAC inhibitors, pharmacophore modelling technique was used to design chrysin-analogue and applied on HDAC target via molecular docking approach. We report the application of pharmacophore modelling and molecular docking procedure as a tool to unearth molecule with the capacity to function as HDAC inhibitors.

II. Methodology

A. Receptor and ligand preparation

The chemical structure of the chrysin and its analogues were build using ACD/ChemSketch 2018.2.5 Freeware version. The 2D conformation of chrysin-analogues was built by substituting the hydroxyl functional group at position 22 (see Fig 1) with different chemical moiety believed to exhibit pharmacophoric features that can impart biological activity on the target protein. The chemical structures were converted into their 3D forms and thereafter optimized using the MMF94 force field on Avogadro interface [26]. The dock-prep tools on the UCSF Chimera interface were used to prepare the optimized chemical structures before the molecular docking step. The crystallized structure of human histone deacetylases (HDAC8) in complex four structurally diverse hydroxamate inhibitors (Na^{+}, Ca^{2+}, Za^{2+} and trichostatin) having 1.90 Å resolution was retrieved from the Protein Data Bank (https://www.rcsb.org) with ID: 1t64. The structure consists of two distinct chains A and B (a dimer) bounded to small chemical residues (TSN). However, due to computational cost and time, the singular chain A of the protein where the Trichostatin compound (TSN) is bound to was prepared for a molecular docking simulation. Meanwhile, the preparation of the biological target (1t64) was performed on the UCSF Chimera interface[27].

B. Molecular docking

The molecular docking simulation study was assessed by making use of AutoDockVina software [28]. Specific docking of the chrysin-analogues to the active site of human histone deacetylases (HDACs) (ID: 1t64) was achieved by generating a grid box coordinate of the ligand to be substituted on the receptor. The grid box that defines the pocket of 1t64 receptor was designed by making use of AutoDock Vina functionality on UCSF Chimera interface [27]. The grid box size and centre coordinates for the 1t64 were x(61.88, 12.3405), y (69.763, 12.25) and z (11.2344, 11.088) respectively. The chrysin-analogue with the highest binding affinity for the human histone deacetylases (HDACs) (ID: 1t64) was selected for further *in silico* ADME assay.

C. Validation and ADME analysis of lead molecule

The online web server, SWISS-ADME (Adsorption, Distribution, Metabolism and Excretion) was used to predict the drug-likeness, solubility uniqueness and pharmacokinetics characteristics of the lead molecule selected among the analogue (https://www.swissadme.ch).

III. Results and Discussion

A. Molecular docking

The application of computer-aided drug design in drug discovery practices is of great importance. This method often involves molecular docking technique of ligands against a target receptor and can be specific or random, which helps to identify the lead molecule *via* a comparison of the generated docking pose or the predicted docking score. Lower binding energy designates a higher affinity of the ligand for the target molecule. Hence, ligands with the highest binding affinity for the target is selected as a potential drug for further investigation.

To further understand the nature of docking, ligand interaction of the prospective drug with the receptor or active sites on the target molecules is examined. Meanwhile, intermolecular interactions such van der Waals, conventional hydrogen bonds, Pi Amide stacked, Pi alkyl interactions amongst others are known to influence the binding affinity of a ligand molecule with the active sites of a target. However, hydrogen bond formation and the nature of amino acid residues present within the sphere of the pocket are

commonly used to describe the affinity of the ligand for the target.

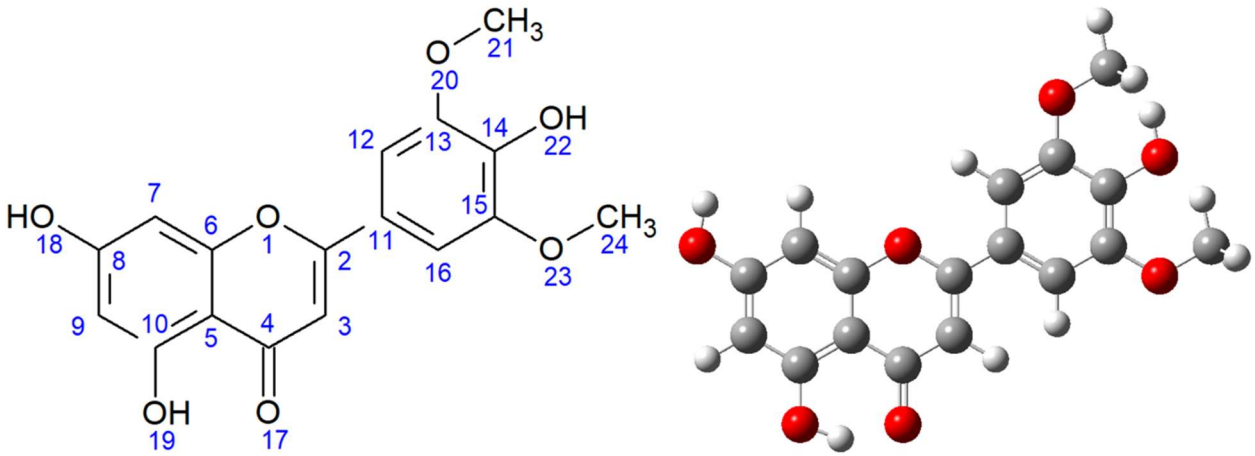


Fig 1: The 2D and 3D chemical structure of chrysin.

In this present study, the bioactive compound (Chrysin) reported to be isolated from the leaves of *Passiflora incarnata* L was used to perform pharmacophore modelling via the substitution of the -OH at position 22 with other functional groups with possible therapeutic implication (see Fig 1). The analogue of chrysin was employed as ligands to investigate their binding affinity with human histone deacetylases (HDACs) as the receptor target protein (see Fig).

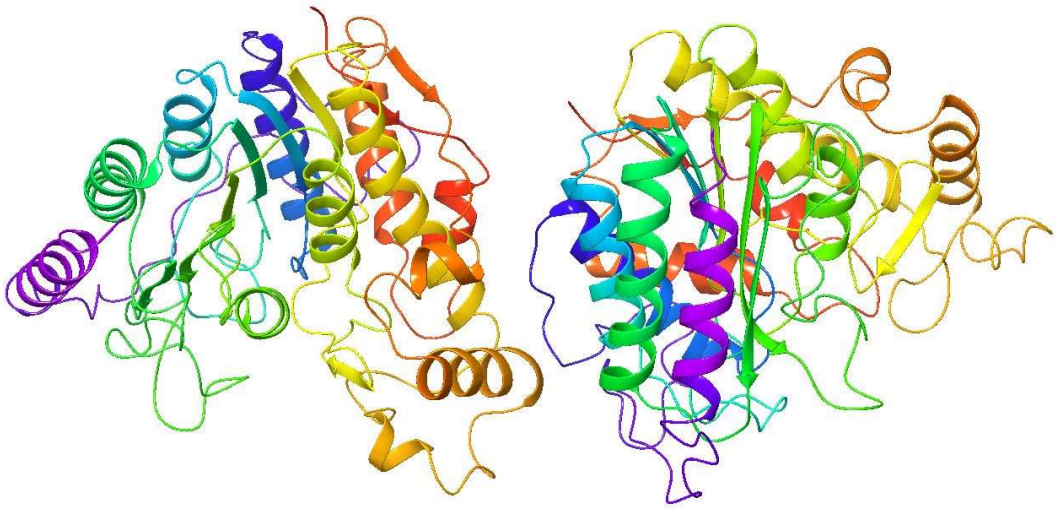
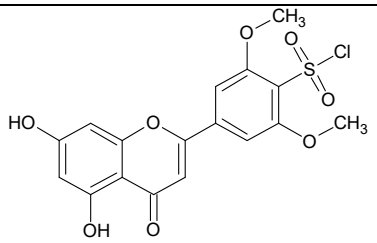
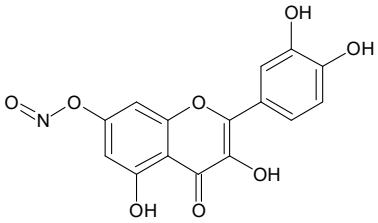
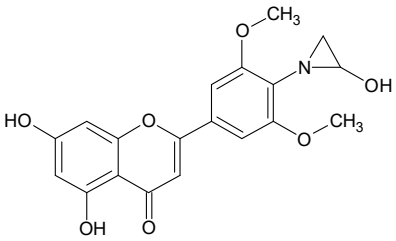
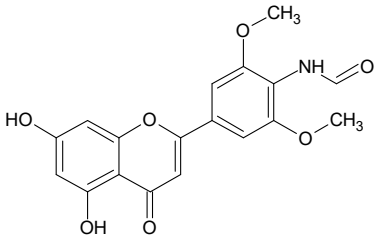
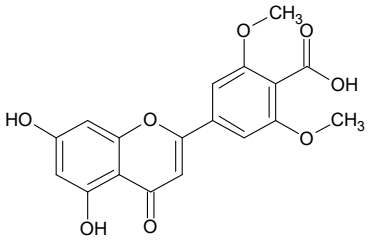
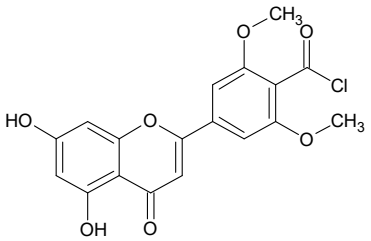
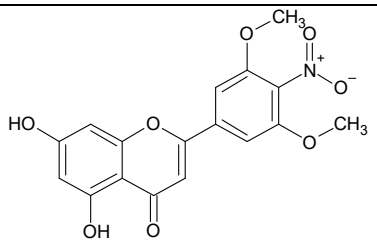
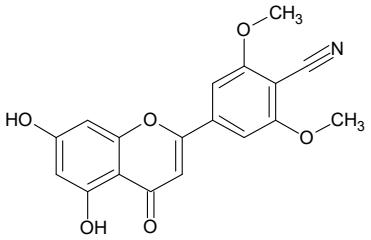
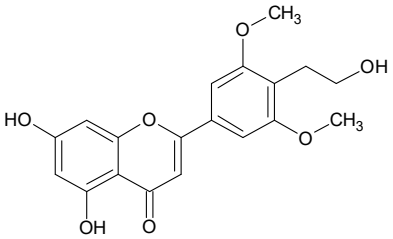
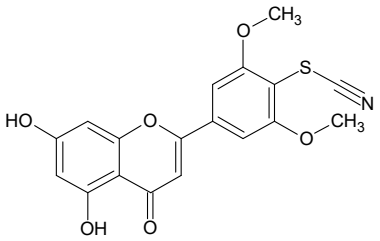
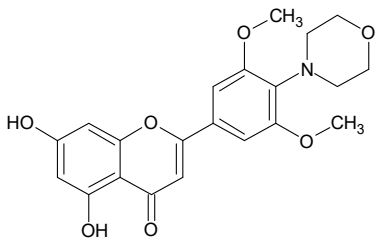


Fig 2: The 3D crystal structure of cancer (PDB ID: 1t64).

Table 1: 2D representation of ligands and their corresponding Glide score (G-Score) value calculated for the related query.

Code	Score *ΔG (Kcal/mol)	Structure
CHD1	-7.8	
Chrysin	-7.6	
CHD2	-7.6	
CHD3	-7.3	
CHD4	-7.2	
CHD5	-7.2	

CHD6	-7.1	
CHD7	-6.9	
CHD8	-6.9	
CHD9	-6.4	
CHD10	-5.7	

The docking study revealed that CHD1, Chrysin, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7,CHD8, CHD9 and CHD10 had a favourable predicted docking score of -7.8 kcal/mol, -7.6 kcal/mol, -7.6 kcal/mol, -7.3 kcal/mol, -7.2 kcal/mol, -7.2 kcal/mol, -7.1 kcal/mol, -6.9 kcal/mol, -6.9 kcal/mol,, -6.4 kcal/mol, and -5.7 kcal/mol respectively. Hence, 4-(5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride (CHD1) was selected as the lead molecule due to its high-affinity interaction for human histone deacetylases (HDACs). Meanwhile, the ligand interaction of the chrysin-analogue with the amino acid residues within the active site of the studied target (ID: 1t64) was displayed in Figs.3.to 8. Meanwhile, the lead molecule (4-(5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride) was observed to interact with fourteen amino acid residues which include: MET 261, PRO 260, TYR 87, GLY 292, TYR 293, LEU 295, ARG 24, PRO 22, ILE 21, LEU 18, TYR 98, TRP 128, TYR 141 and PHE 139. On the contrary, the reference molecule chrysin (5,7-dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-4H-1-benzopyran-4-one) was noticed to interact with ten amino acid residues which include: PRO

260, TYR 87, TYR 293, LEU 295, ARG 24, PRO 22, ILE 21, TYR 87, TRP 126 and PHE 139. The strong affinity of the lead molecule for 1t64 could be attributed to the fact that CHD1 interacted with more amino acid residue (MET 216, GLY 292, TRP 98 and TYR 141) within the hydrophobic pocket of 1t64 than chrysin. It is also envisioned that the heteroatomic nature of the substitute on the lead molecule as well as the non-appearance of steric hindrance on this group could justify the enhanced molecular interactions and the high-affinity CHD1 for the human histone deacetylases.

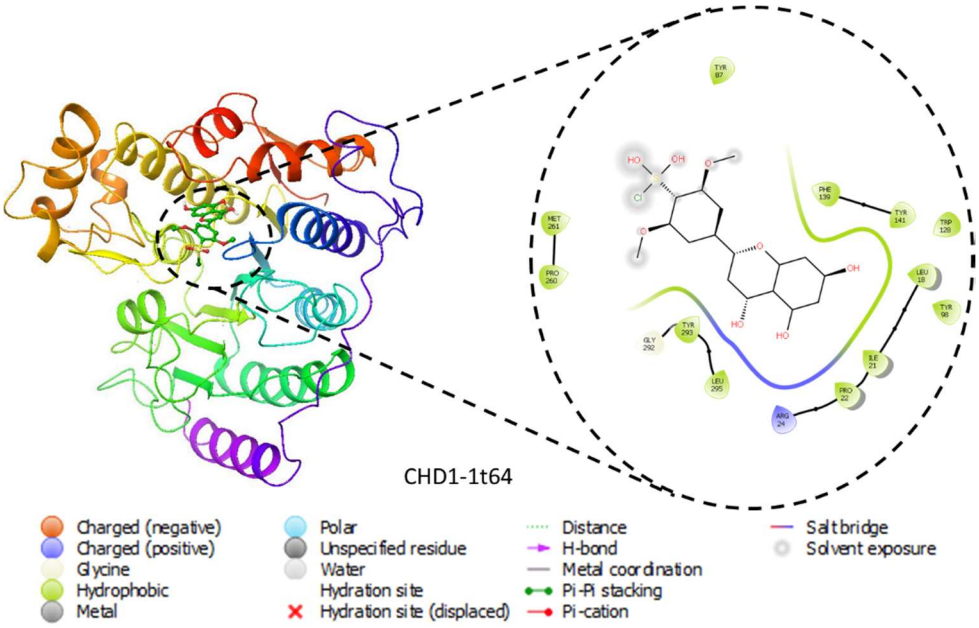


Fig. 3. The 3D X-ray crystal structure of 1t64 complex with CHD1 showing also the binding site region and the residues that constitute this binding site region.

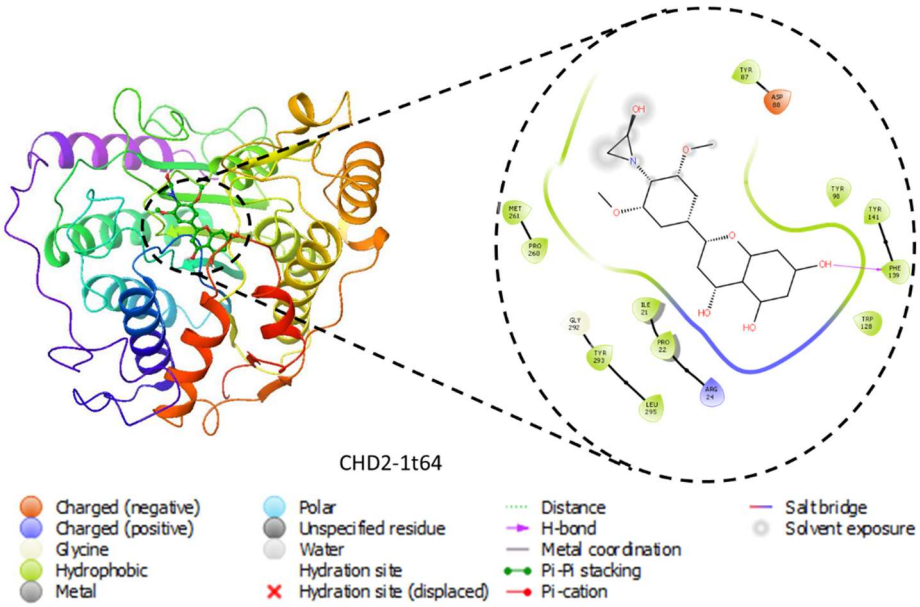


Fig. 4. The 3D X-ray crystal structure of 1t64 complex with CHD2 showing also the binding site region and the residues that constitute this binding site region.

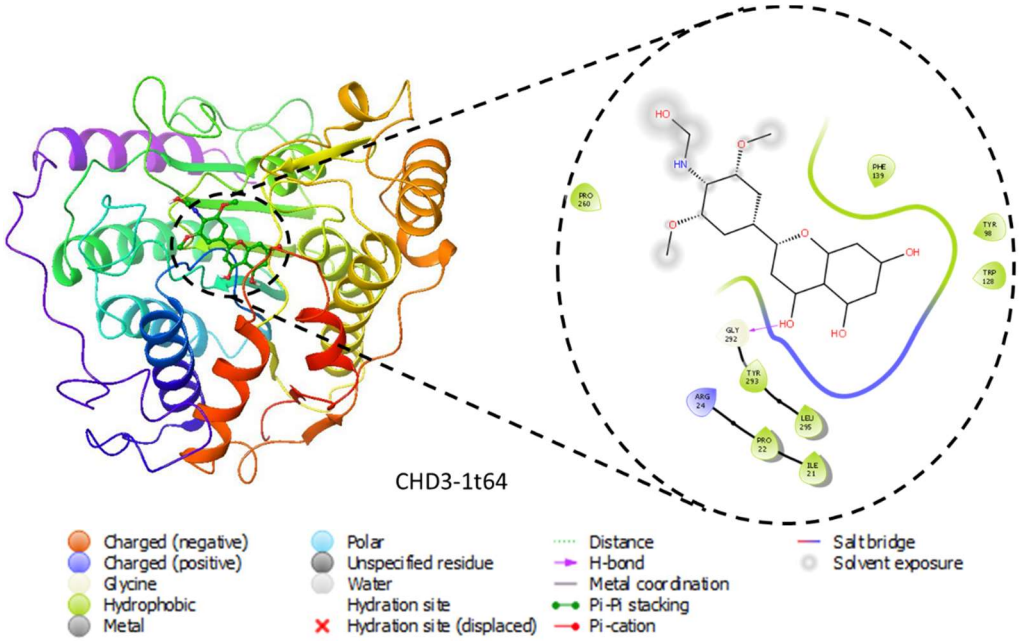


Fig. 5. The 3D X-ray crystal structure of 1t64 complex with CHD3 showing also the binding site region and the residues that constitute this binding site region.

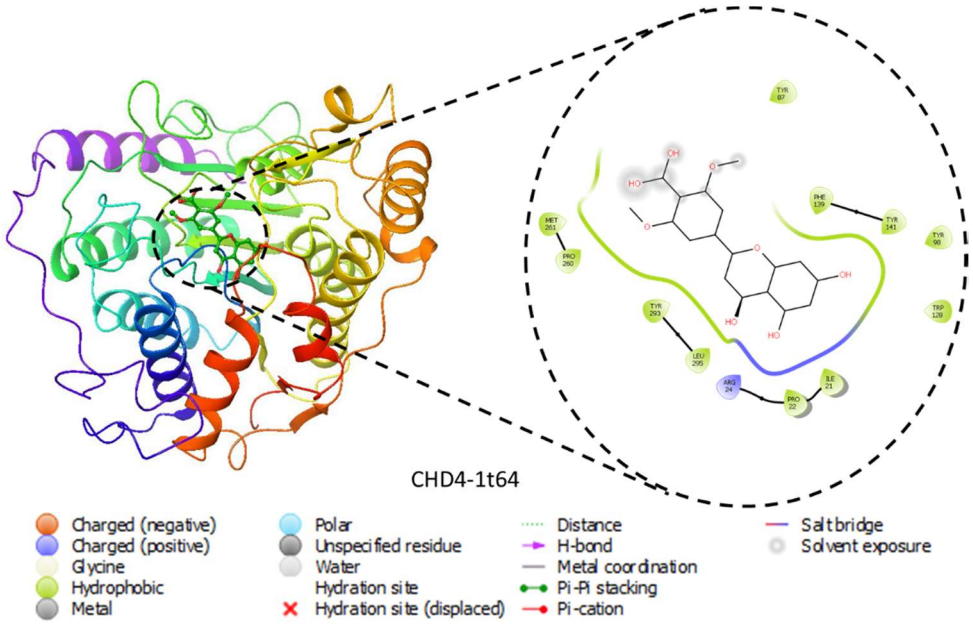


Fig. 6. The 3D X-ray crystal structure of 1t64 complex with CHD4 showing also the binding site region and the residues that constitute this binding site region.

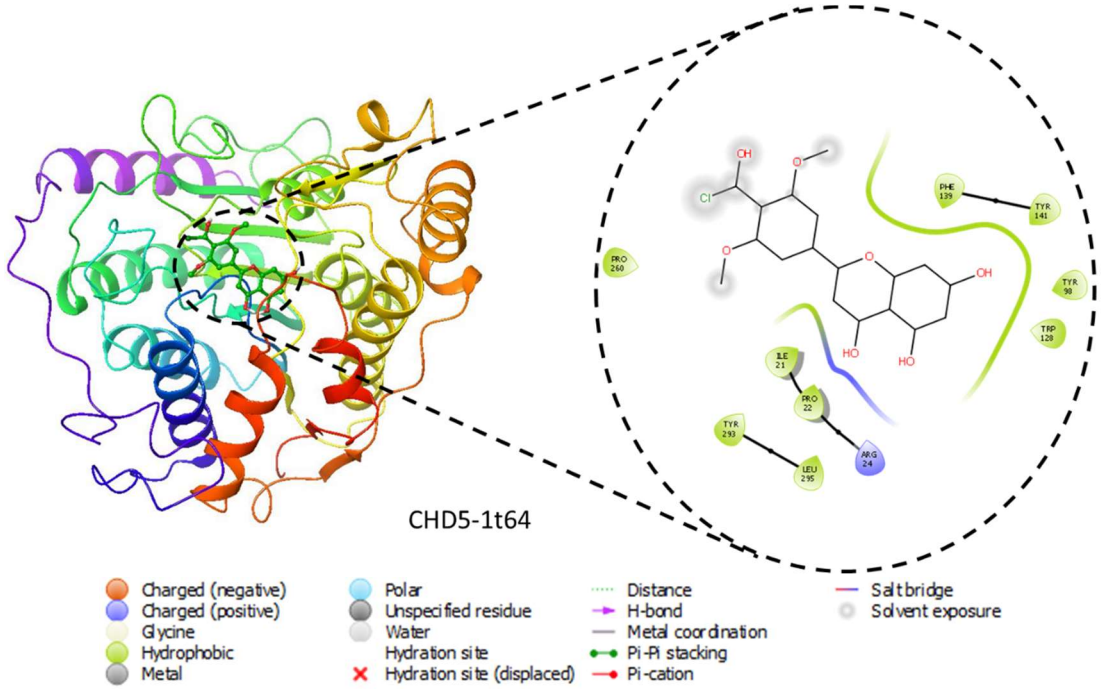


Fig. 7. The 3D X-ray crystal structure of 1t64 complex with CHD5 showing also the binding site region and the residues that constitute this binding site region.

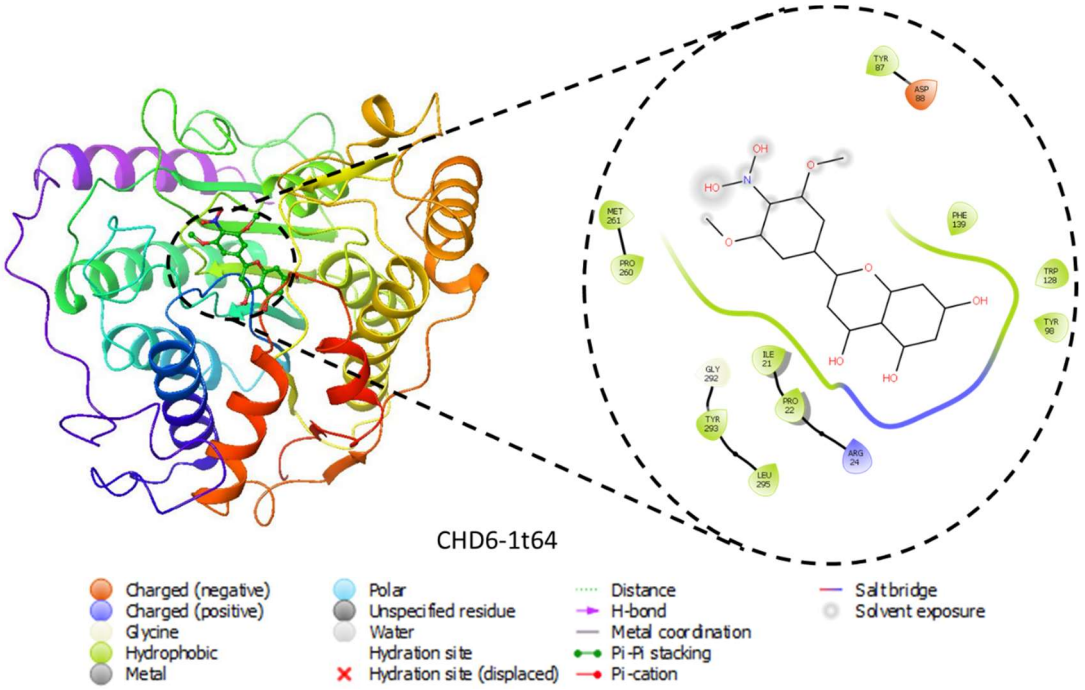


Fig. 8. The 3D X-ray crystal structure of 1t64 complex with CHD6 showing also the binding site region and the residues that constitute this binding site region.

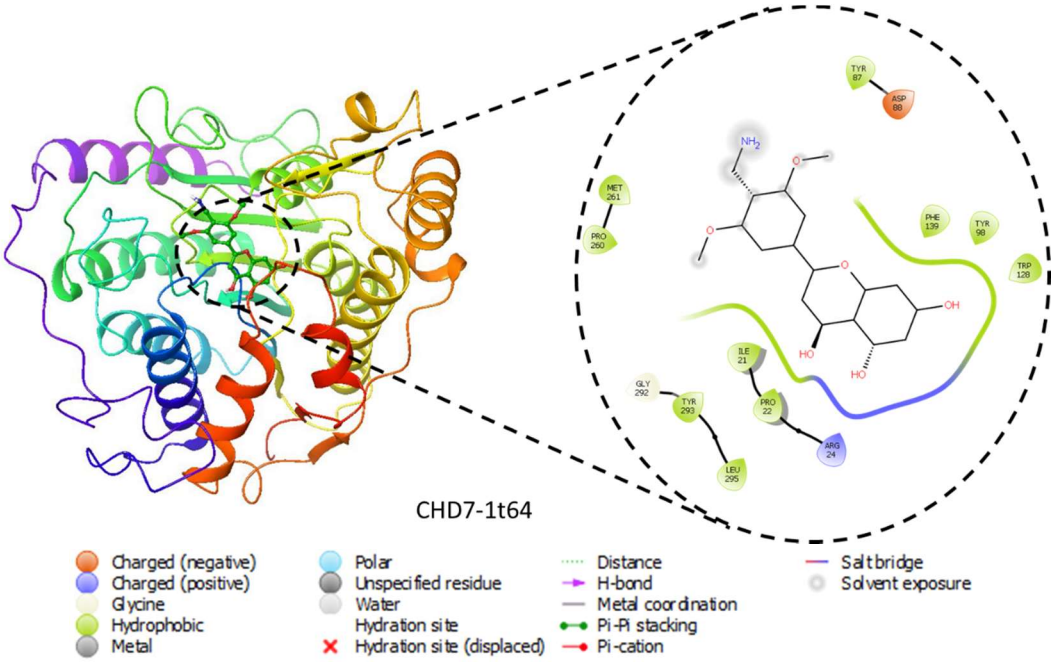


Fig. 9. The 3D X-ray crystal structure of 1t64 complex with CHD7 showing also the binding site region and the residues that constitute this binding site region.

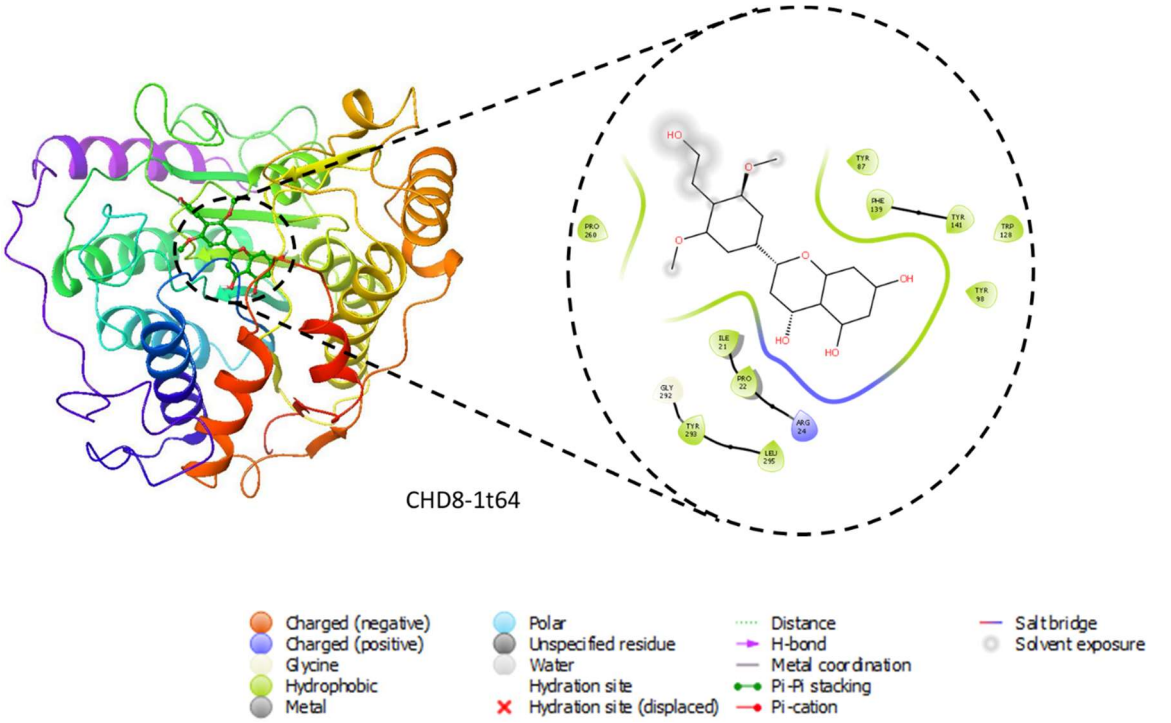


Fig. 10. The 3D X-ray crystal structure of 1t64 complex with CHD8 showing also the binding site region and the residues that constitute this binding site region.

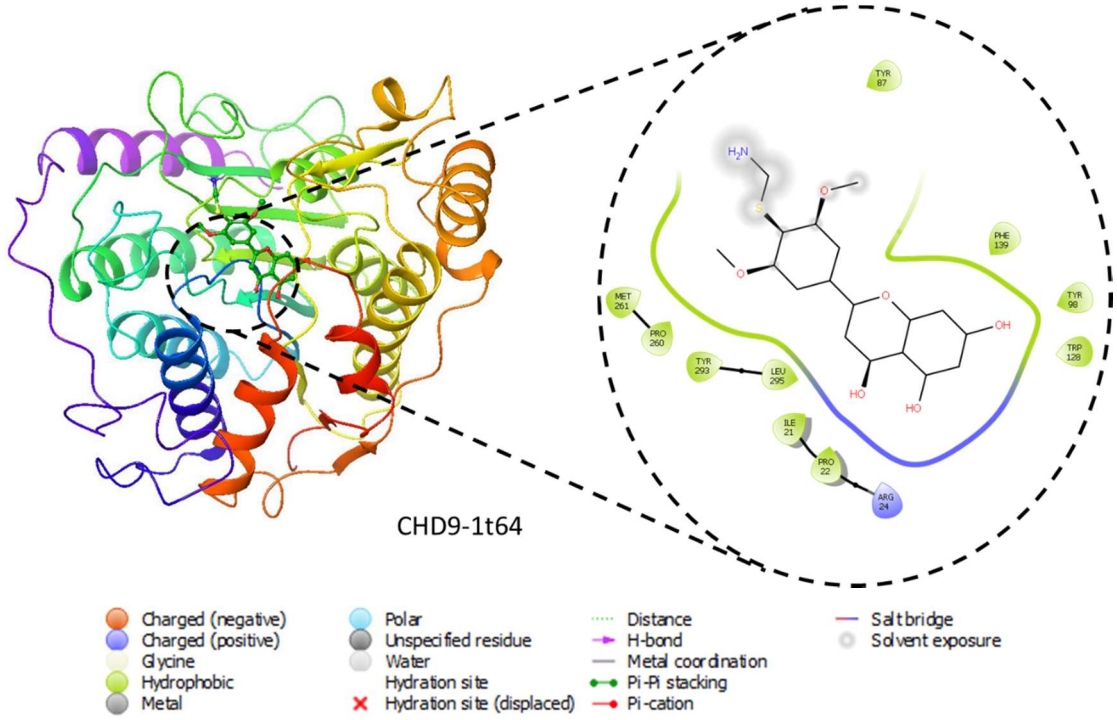


Fig. 11. The 3D X-ray crystal structure of 1t64 complex with CHD9 showing also the binding site region and the residues that constitute this binding site region.

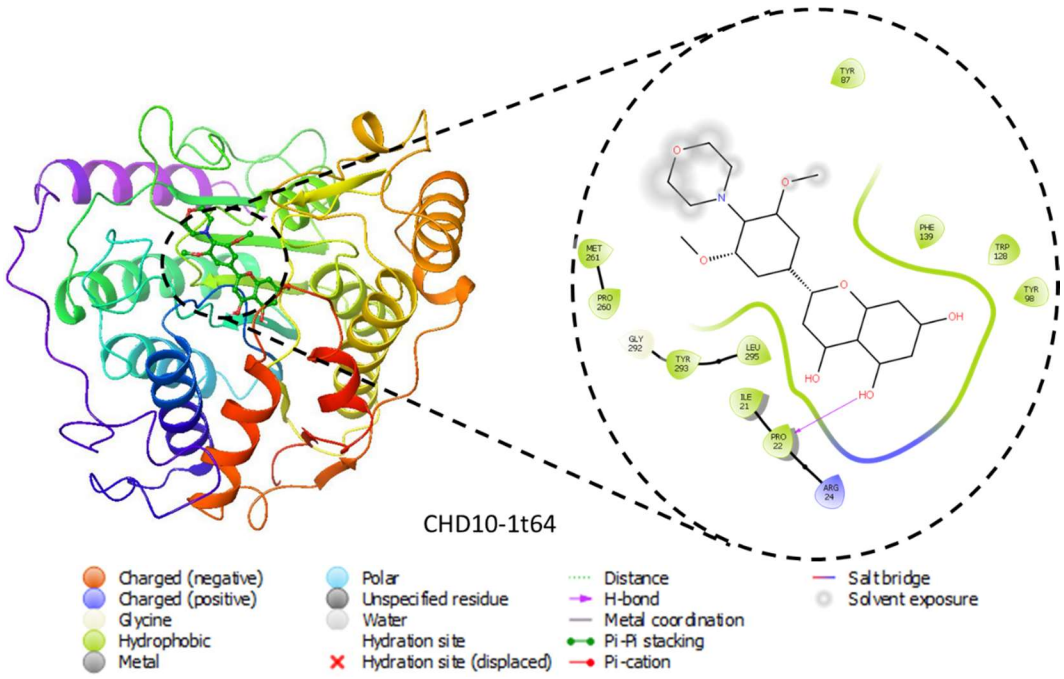


Fig. 12. The 3D X-ray crystal structure of 1t64 complex with CHD10 showing also the binding site region and the residues that constitute this binding site region.

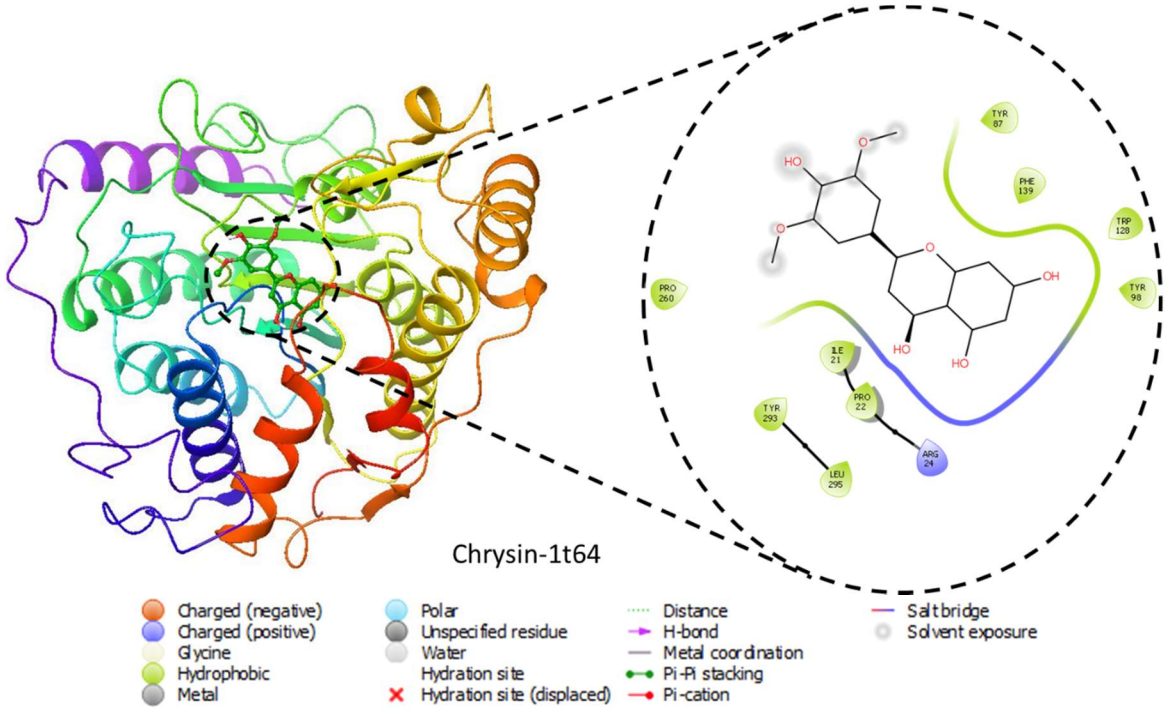


Fig. 13. The 3D X-ray crystal structure of 1t64 complex with chrysin showing also the binding site region and the residues that constitute this binding site region.

ADME assessment of potential human histone deacetylases (HDACs) inhibitors

The bioavailability radar of 5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride was used to observe the physicochemical space of the lead molecule employed for the pharmacokinetics and drug-likeness predictions (see Fig 14).

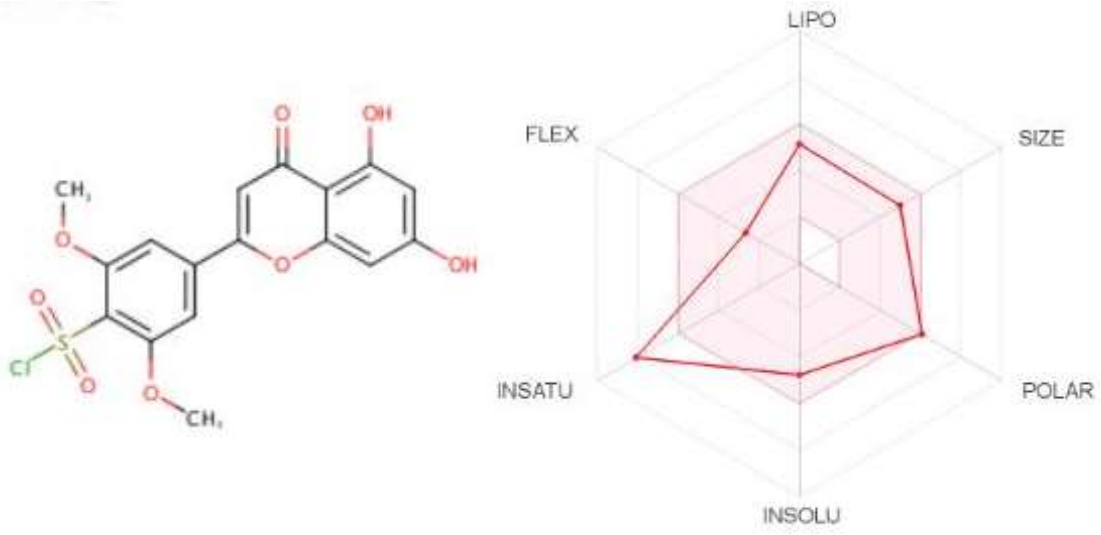


Fig. 14. The bioavailability radar of CHD1 using Swiss ADME predictor.

The ADME prediction of the 5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride was performed by making use of SWISSADME online webserver. The physiochemical properties of the 5,7-

dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride include: 27 heavy atoms, 8 hydrogen bond acceptors, 2 hydrogen bond donors, molar refractivity of 98.03and topological polar surface area (TPSA) of the molecule is found to be 131.65 $Å^2$. Meanwhile, the lipophilicity of the molecule includes: iLOGP is 2.63, XLOGP3 is 3.49, WLOGP is 3.90, MLOGP is 0.05, SILICOS-IT is 2.57 and Consensus P0/W is 2.53. Pharmacokinetic data were acquired and displayed using the molecule falling in egg's yolk model. As shown in Fig 15, no blood-brain barrier permeant was noticed, also the gastrointestinal absorption (GI) was low. Table 2, showed that the water solubility of 5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride was moderate. The lead molecule was noticed to inhibit CYP1A2, CYP2C19 and CYP3A4 cytochromes but was observed not to interfere with CYP2D6 and CYP2D9 isoenzymes. Hence, the activity of 5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride will need further evaluation.

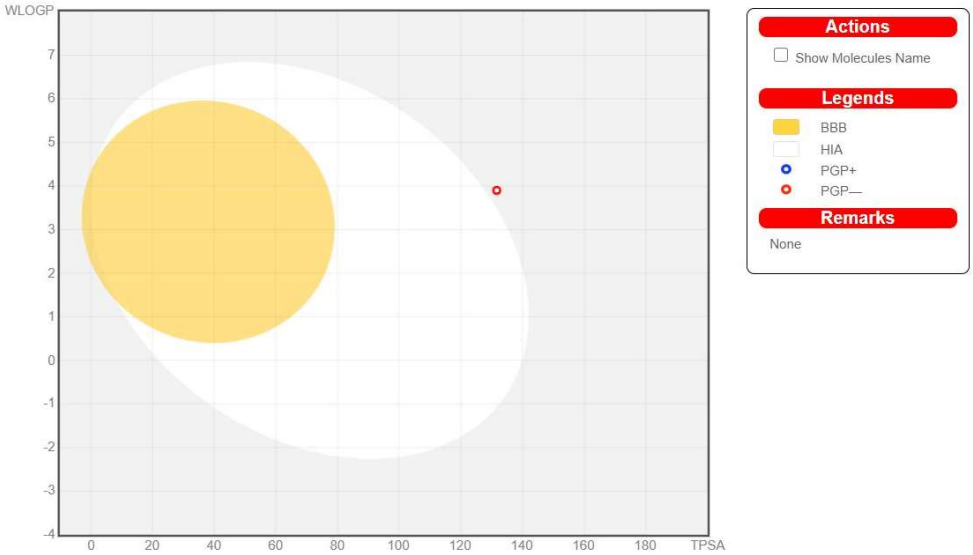


Fig. 15. Molecule falling in egg's yolk prediction CHD1.

Table 2: Water solubility of CHD1.

Log *S* (ESOL)		-4.77
	Solubility	$6.97e^{-03}$ mg ml^{-1}; $1.69e^{-05}$ mol ml^{-1}
	Class	Moderately soluble
Log *S* (Ali)		-5.94
	Solubility	$4.77e^{+04}$ mg ml^{-1}; $1.15e^{-6}$ mol ml^{-1}
	Class	Moderately soluble
Log *S* (SILICOS-IT)		-5.69
	Solubility	$8.51e^{-04}$ $mgml^{-1}$; $2.06e^{-06}$ mol ml^{-1}
	Class	Moderately soluble

Table 3. Pharmacokinetics of CHD1.

GI adsorption	Low
BBB permeant	No
P-gp substrate	No

CYP 1A2	Yes
CYP2C19	No
CYP2C9	Yes
CYP2D6	No
CYP3A4	Yes
Log Kp (skin permeation)	-6.34 cm s^{-1}

As listed in Table 4, the lead molecule was noticed to obeys Lipinski's Rules with no violation. It also obeys drug-likeness score rules such as Ghose, Veber and Muegge and with 0.55 Bioavailability score. On the contrary, Egan score rule was not obeyed with a violation.

Table 4. Druglikeness of CHD1.

Lipinski	Yes, 0 violation
Ghose	Yes
Veber	Yes
Egan	No. 1 violation TPSA >131.6
Muegge	Yes
Bioavailability score	0.55

IV. Conclusion

The main objective of the present study was to use computational techniques such as pharmacophore modelling, molecular docking and ADME prediction to identify effective inhibitor for human histone deacetylases which may, in turn, demonstrate great potential as a prospective antitumor agent. A comparison of the docking score and ligand-receptor interactions showed that CHD1 (5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride) had a better affinity for the target. Hence, have demonstrated good tendency to inhibit human histone deacetylases. Besides, the binding affinity of the lead molecule for the target, the ADME prediction also projected the lead molecule as a chemical compound with druggable characteristics. The study concludes that pharmacophore modelling of phytochemicals is essential to enhancing bioactivity of secondary metabolite sourced from folk medicine. Hence, the *in vitro* and *in vivo* assay of 5,7-dihydroxy-4-oxo-4H-1-benzopyran-2-yl)-2,6-dimethoxybenzene-1-sulfonyl chloride may serve as a path to the discovery of novel antitumor agent.

Acknowledgement

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Declaration of Interest

The authors declare no conflict of interest.

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