ORIGINAL ARTICLE

In silico study, design, synthesis, and evaluation of anti-neoplastic activity of hybrid natural antioxidants as histone deacetylase inhibitors

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ABSTRACT

Aim: To design and synthesize hybridized natural antioxidants by following the pharmacophore of the reference Histone Deacetylase (HDAC) Inhibitors with potential to enhance pharmacokinetic parameters and investigate anti-neoplastic activity as a trial to replace the known HDAC inhibitor that suffers from life-threatening adverse effects and poor pharmacokinetics related to hydroxamate functionality.

Materials and Methods: Designing of new natural hybridized molecules, HDAC inhibitor consisting of cap, linker & zinc binding domain. The cap involved (natural antioxidants Thymol, Eugenol, vanillin, and Umbelliferon) linked to the bioactive molecule succinate via an ester, and on the other hand, succinate acts as a zinc zinc-binding group by anion-free carboxylate. Molecular docking analysis, absorption, distribution, metabolism and excretion (ADME) pharmacokinetic prediction, and analysis of the designated new antioxidants hybrid molecules. In addition, organic synthesis, chemical identification, and biological assessment for their antineoplastic activity.

Results: The results of the molecular docking and SWISS ADME for four designated molecules are potentially as HDAC6 inhibitors with chemical synthesis for four molecules and identified by Fourier-Transform Infrared spectroscopy (FTIR), Proton Nuclear Magnetic Resonance spectroscopy (^{1}HNMR), Carbon-13 Nuclear Magnetic Resonance (C13 NMR), Mass, and some physiochemical properties. Anti-neoplastic activity assay in MDA-MB-231 3-(4,5-di methyl thiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT) cell viability assay in breast cancer refers to the designated hybrid molecule-1 as significantly cytotoxic, but molecules-2, molecule-3, and molecule-4 are less significant.

Conclusions: The designated hybrid molecules, especially molecule-1(Thymol-based HDAC inhibitor), exhibit exceptional *in silico* selectivity for HDAC6, the safety of molecule-1, and superior computational pharmacokinetics parameters, making it a building block of a new generation of HDAC inhibitors. The new hybridized molecules of natural origin showed potential anti-neoplastic activities with improved pharmacokinetic parameters compared to SAHA and Tucidinostat.

KEY WORDS: natural hybrid, molecular docking, bioactive molecules, HDAC inhibitor

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INTRODUCTION

Histone Deacetylase (HDAC) inhibitors are a promising backbone for the drug design of cancer, mainly due to their epigenetic modulation in cancer and neurodegenerative and chronic autoimmune diseases. Cardio toxicity mainly QT prolongation, Haematological toxicity like thrombocytopenia, neurotoxicity involving cognitive impairment and peripheral neuropathy. Suberoylanilide hydroxamic acid (SAHA) and belinostat are hydroxamate-based HDAC inhibitors limited clinically due to major adverse effects associated with their drug resistance, poor pharmacokinetics, and lack of selectivity. The leading cause of death worldwide is cancer [1]. A 9.6 million People died in 2018 due to cancer, although extensive cancer drug research and treatment [2]. The growing incidence and prevalence of cancer in the world and an emergent resistance to most drug of cancer drugs associated with the complicated adverse effects related to a lack of selectivity to cancer cells, all these factors are driving force to focus on natural antioxidants and making them as nucleus in design of cancer drug. Antioxidants are functioning in counteracting free radicals and oxidative stress, as well as in fighting cancer both prevention and treatment significant attention for their activity as scaffold nucleus in drug design [3]. Resveratrol and Curcumin are marked antioxidants that can efficiently modify HDAC activity, HDACs [4]. Chemical modification of natural antioxidant molecules via semi-synthetic derivatives have enhanced antiproliferative potential, pharmacokinetic properties and reduced toxicity [5]. Thymol induces oxidative stress-associated mitochondrial damage and both intrinsic and

extrinsic apoptosis in cancer cells. It also suppresses the proliferation of cancer cells. Thymol anticancer effectiveness in several studies may be attributed to its powerful antioxidant activity, cytoprotective action, anti-apoptotic capability, marked anti-inflammatory/ immunomodulatory action, and significant genomic protection from cytotoxic agents to the normal cells [6]. Thymol-Derived Hybrid Molecules Demonstrating Anticancer Efficacy: The investigation of novel therapies employing plant-derived molecules, due to their biocompatibility and non-toxic effects on healthy cells. Therefore, employing them in the production of hybrid therapeutic molecules can mitigate their limitations and represent a viable approach for designing drugs. A variety of Thymol-based hybrid molecules was assessed for their anti-tumour activity against four distinct cancer cell lines ex. MCF-7 & MDA-MB-231. Many of the synthesized compounds exhibited acceptable cytotoxic activity relative to the reference drugs [7]. Eugenol is an aromatic phenolic antioxidant derived from Syzygium aromaticum, possessing promising pharmacological activities including anticancer, antiviral, anti-diabetic, anti-parasitic, anti-inflammatory, and antibacterial activity. This attractive natural molecule inhibits the enzyme cyclooxygenase II (COX-2), lipoxygenase enzyme -5 (5-LOX) and DNA synthesis, increases reactive oxygen species (ROS) production, arrests the cellular cycle, and induces programmed cell death. Numerous semisynthetic pharmaceutical molecules based on Eugenol in their nucleus in recent years, exhibiting enhanced bioactivities and a wide range of mechanisms of action. Eugenol has been investigated for its safety and flexibility in drug design [8]. Vanillin has several pharmacological activities, such as anti-neoplastic activity, antidiabetic characteristics, marked antioxidant activity, antibacterial activity, anti-inflammatory characteristics, cardioprotective agent, and diuretic action. Molecular hybridization strategy is employed for the design of novel drug compounds derived from vanillin [9]. Umbelliferon: promising anticancer benefits in preclinical phases, but their poor solubility and bioavailability limit their application in cancer therapy. A Coumarins derivative is found in Rutaceae and Apiaceae plants. It can reduce cancer cell proliferation and confer considerable cytotoxicity. Furthermore, Coumarins, based on a 2H-1-benzopyran-2-one structure, demonstrate a wide range of therapeutic activities, encompassing anticancer activity. Antiviral, antibacterial activity, anticoagulant ability, blood pressure-lowering action, antioxidant, anti-inflammatory, and neuroprotective capacity, figure (1) [10].

The neglect of toxicity of Umbelliferon from oral administration of within the therapeutic range of 200 mg per kilogram of body weight renders it a compelling framework for the synthesis of active core 7-hydroxy Coumarins-derived molecules in drugs discovery as an innovative platform a multifunctional scaffold for the synthesis of various physiologically active molecules. Umbelliferon is receiving significant attention for its anticancer activities. Preclinical outcomes suggest potential usefulness in the future the therapy of multiple solid tumours, including mouth epithelial cells carcinoma, colorectal carcinoma, cutaneous carcinoma, prostate cancer, breast cancer, and cancer of urinary bladder, as well as malignancies of central nervous system. Umbelliferon, regardless of the type of cancer, activates pathways that facilitate the arrest of cell cycles, apoptotic cascade or restrict both of migration and invasion malignant cells [11]. Succinic acid has selective anti-neoplastic activities via inducing a programmed cell death in cancer cells and its normal metabolic activity in non-cancer cells. The pathway Tricarboxylic Acid (TCA) cycle and its enzymatic constituents depend mainly on succinic acid [12]. Furthermore, succinate-based drugs are used to establish it is potential therapeutic anti-cancer activity and selective capacity of the therapeutic characteristics, as well as improving the pharmacokinetic parameters [13]. The Thymol, Eugenol, vanillin, and Umbelliferon are selected to design hybrid molecules to act as HDAC inhibitors by following the structural pharmacophore of SAHA, figure (2).

Rationale for design

1. Pharmaceutical design of future HDAC inhibitor generation, either to enhance the poor pharmacokinetic characteristics of new classes of HDAC inhibitors[14]or secondly, the efficacy and specificity of the HDAC inhibitors must be improved [15].
2. Hybrid molecules with Anticancer Activity Based on Antioxidants (Thymol, Eugenol, vanillin and Umbelliferon): within limits Lipinski's criteria [7].
3. In oncology, succinic acid has great potential due to its ability to kill cancer cells without harming healthy cells. Succinic acid has been chosen in designated of hybrid molecules.
4. Computational modeling speeds up drug discovery, making it more affordable and faster. To assure the safety and efficacy of possible hypothetical anticancer drugs, computational studies must be validated by experimental testing [16].
5. In designing of Captopril as Angiotensin converting enzyme inhibitor (ACEI) limited it is therapeutic usefulness by adverse effects dry cough (accumulation of bradykinin), skin rash and dysgeusia related to it is sulfhydryl functionality leading cause to the re-introduce of carboxylate as zinc binding group

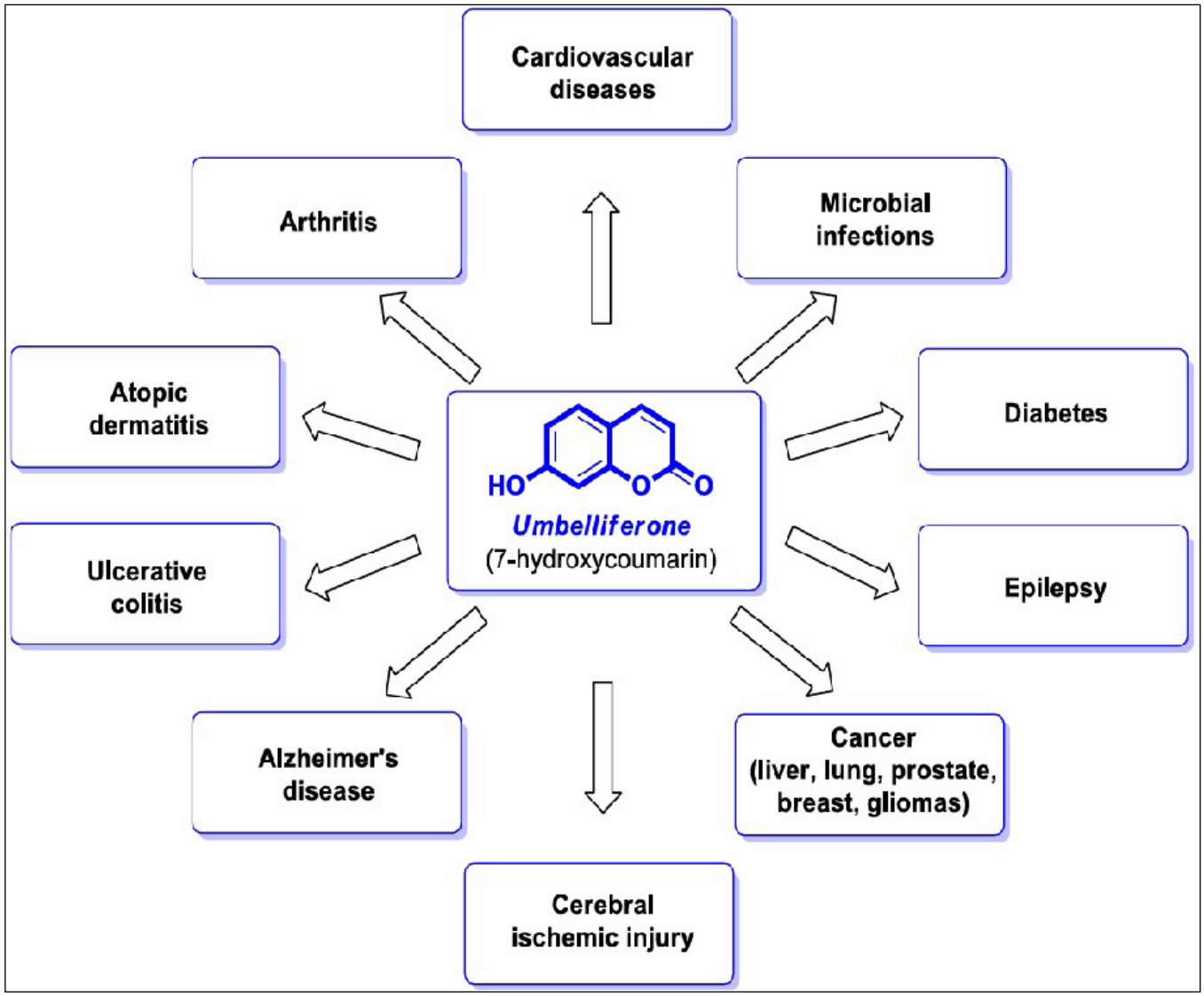

Fig. 1. Umbelliferon in drug design [11]
Source: Own materials

instead of -SH Group in the design of inhibitor as in enalapril and lisinopril [17].

6. HDACs perform many physiological functions, with their dysregulation frequently associated with human diseases, especially cancer [18].
7. Consequently, enhancing HDAC selectivity to reduce off-target toxicity [19].

AIM

The aim of this study is to design and synthesize hybridized natural molecules considering pharmacophore reference HDAC inhibitor data with potential pharmacodynamics and pharmacokinetic candidates and investigate anti-neoplastic activity as a trial to replace the known HDAC inhibitor that suffers from life-threatening adverse effects and poor pharmacokinetics.

MATERIALS AND METHODS

CHEMICAL SYNTHESIS

Thymol, Eugenol, vanillin, and Umbelliferon were purchased from China; the source of the triethylamine was BLD Pharm, China. The solvent used was HPLC grade and without further purification. Thin-layer chromatography (TLC) on Merck silica gel 60F25 reaction progression monitoring under ultraviolet (UV) light via solvent system mobile phase Ethylacetate3: Hexane7 ratio. Identification of the functional Group of compounds by the Fourier-Transform Infrared spectroscopy (FTIR) spectropho-

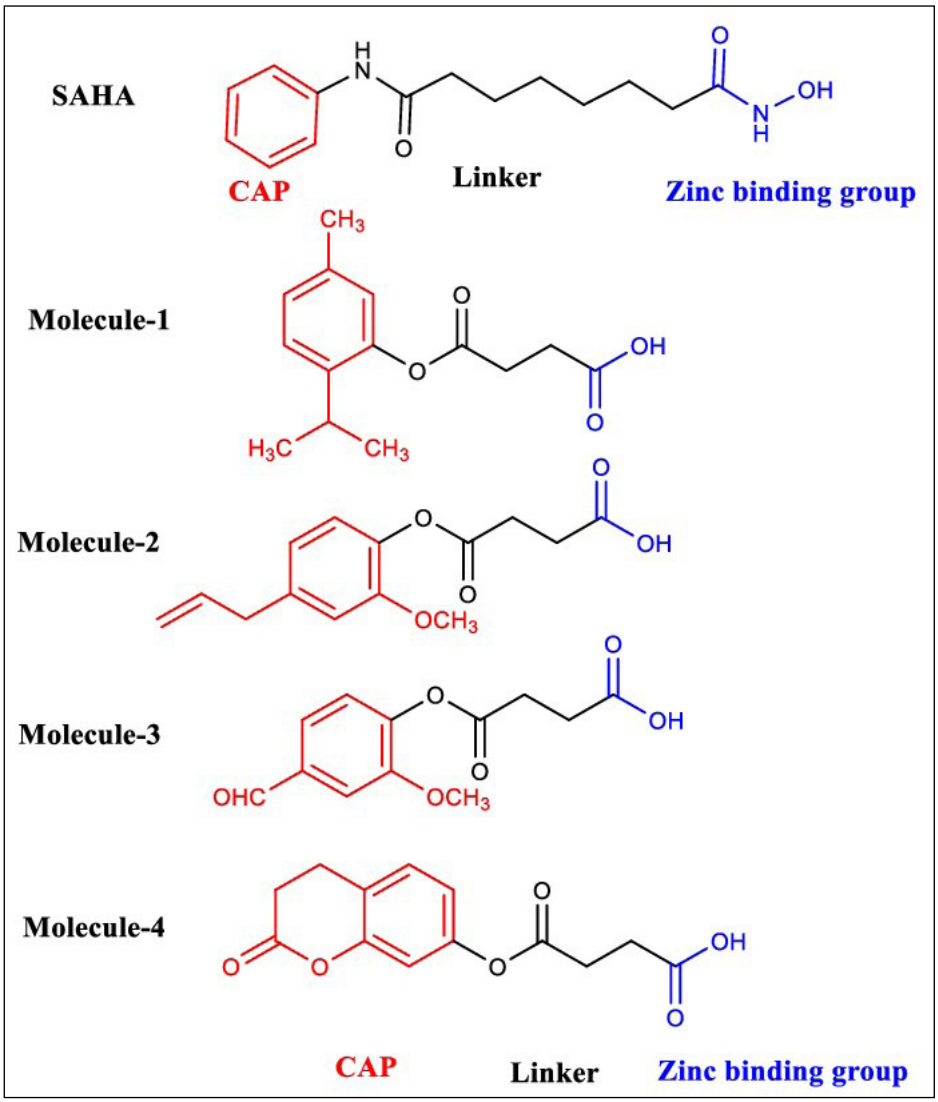

Fig. 2. Designated hybrid molecules compared to the SAHA pharmacophore
Source: Own materials

tometer in University of Kufa, Faculty of Pharmacy, which were recorded on FTIR spectrophotometer, Shimadzu, Japan,$_2$ (Proton Nuclear Magnetic Resonance spectroscopy (^{1}HNMR) spectroscopy 1H-NMR (300 MHz) (Bruker Avance II),$_3$ [Carbon-13 Nuclear Magnetic Resonance (C^{13} NMR) spectroscopy C^{13} NMR (75 MHZ) (Bruker Avance II)].

COMPOUND -1

Thymol: (0.75 gm., 5 mmol) was dissolved in 20 ml of dry chloroform, then triethylamine (0.7 ml, 5 mmol), 4-Dimethylaminopyridine (DMAP) (0.1gm) was added dropwise. The resulting mixture had been stirred at room temperature for 10 minutes and then succinic anhydride (0.5 gm., 5 mmol) was poured. The reaction mixture was stirred at room temperature for a period of 24 hours at 25^{0}C. After finishing the reaction, the solvent was evaporated under declined pressure to get the final product as colourless oily substances, furthermore, dissolved in dry chloroform again and re-distilled. Re-crystalized using petroleum ether 85%. FT-IR (cm-1): 1749 (ester group), 3500 (-OH carboxylic acid), 1143 (ether group), 3062 (C-H aromatic group), 1570 (C=C bending aromatic). ^{1}H NMR (300 MHz, Dimethyl sulfoxide (DMSO)-d6) δ 9.48 (s, ^{1}H, -COOH), 6.98-6.54 (m, 3H, Ar-H), 2.37(s, 3H, -O-CH3), 2.97 (m, 2H, CO-CH2-), 2.46 (m, 2H, CH2-COOH). ^{13}C NMR (75 MHz, DMSO-d6) δ 175.38 carboxylic carbon, 174 (carbonyl carbon), 154.70, 135.69, 131.60, 126.02, 119.99 (aromatic carbon), 23.05, 21.12 (-CH3), 26.48 (Carbonyl-CH2-), 31.25 (-CH2-Carboxyl) [20].

COMPOUND-2

Eugenol: (0.82 gm., 5 mmol) density 1.066 gm./cm3 which corresponds to 0.76 ml liquid was dissolved in 20 ml of dry chloroform. Then, triethylamine (0.7 ml, 5 mmol), DMAP (0.1 gm.) was added dropwise. The mixture had been stirred at room temperature for 10 min and then succinic anhydride (0.5 gm., 5 mmol) was poured. The reaction mixture was stirred at room temperature for 48 hours at 25^{0}C. After finishing the reaction, the solvent was evaporated under

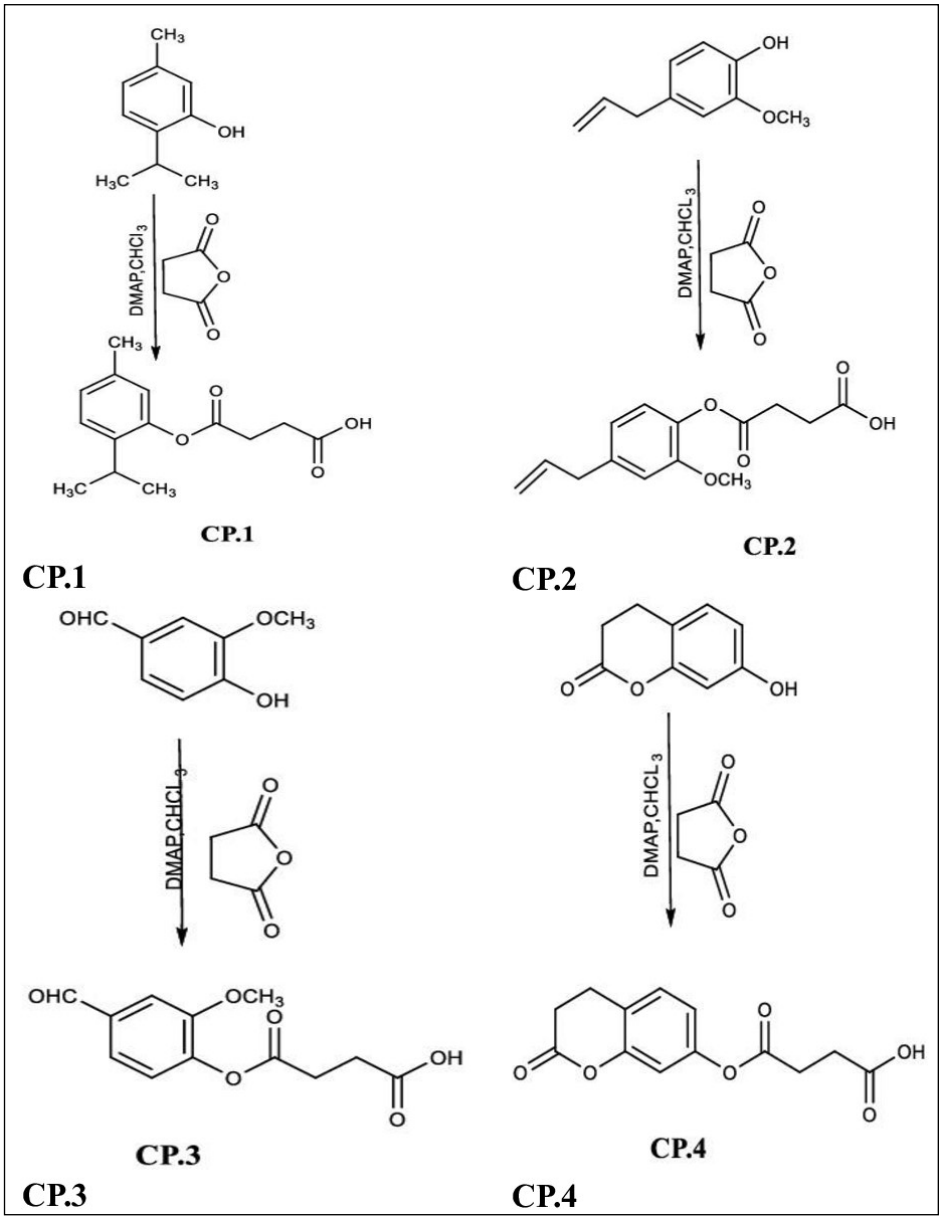

Fig. 3. Chemical synthesis scheme
Source: Own materials

lower pressure to get the final product as colourless oily substances; furthermore, dissolved in dry chloroform again and re-distilled. Re-crystalized using petroleum ether 89%. FT-IR (cm-1): 1720 (ester group), 3431 (-OH carboxylic acid), 1163 (ether group), 3070 (C-H aromatic group), 1516 (C=C bending aromatic). 1H NMR (300 MHz, DMSO-d6) δ 10.56 (s, 1H, -COOH), 7.01-6.01 (m, 3H, Ar-H), 5.98(=CH2) 5.09.37 (s, 3H, -O-CH3), 2.91 (m, 2H, CO-CH2-), 2.44 (m, 2H, CH2-COOH). 13C NMR (75 MHz, DMSO-d6) δ 175.27 carboxylic carbon, 172.80 (carbonyl carbon), 155.40, 147.93, 145.22, 138.63, 120.96, 11583, 112.99 (aromatic carbon), 55.94 (-OCH3), 29.84 (Carbonyl-CH2-), 29.57 (-CH2-Carboxyl).

COMPOUND-3

Vanillin: (0.76 gm., 5 mmol) was synthesized in the same procedure as Compound 2, to get the final product as red oily substance. Furthermore, dissolve in dry chloroform again and redistill. Re-crystalized using petroleum ether 74%. FT-IR (cm-1): 1720 (ester group), 3433 (-OH carboxylic acid), 1163 (ether group), 3020 (C-H aromatic group), 1516 (C=C bending aromatic). 1H NMR (300 MHz, DMSO-d6) δ 11.88 (s, 1H, -COOH), 9.89(s, 1H) (-CHO), 7.03.98-6.56 (m, 3H, Ar-H), 3.75 (s, 3H, -O-CH3), 2.93 (m, 2H, CO-CH2-), 2.44 (m, 2H, CH2-COOH). 13C NMR (75 MHz, DMSO-d6) δ 191.38 (-CHO), 175.33 (carboxylic carbon), 172.86 (carbonyl carbon), 154.02, 148.72, 128.66, 127.71, 12664,115.88 (aromatic carbon), 55.94 (-OCH3), 29.59 (Carbonyl-CH2-), 29.88 (-CH2-Carboxyl).

COMPOUND-4

Umbelliferon: (0.81 gm., 5 mmol) was synthesized in the same procedure as Compound 2, to get the final product as a yellow oily substance. Furthermore, dissolve in dry chloroform again

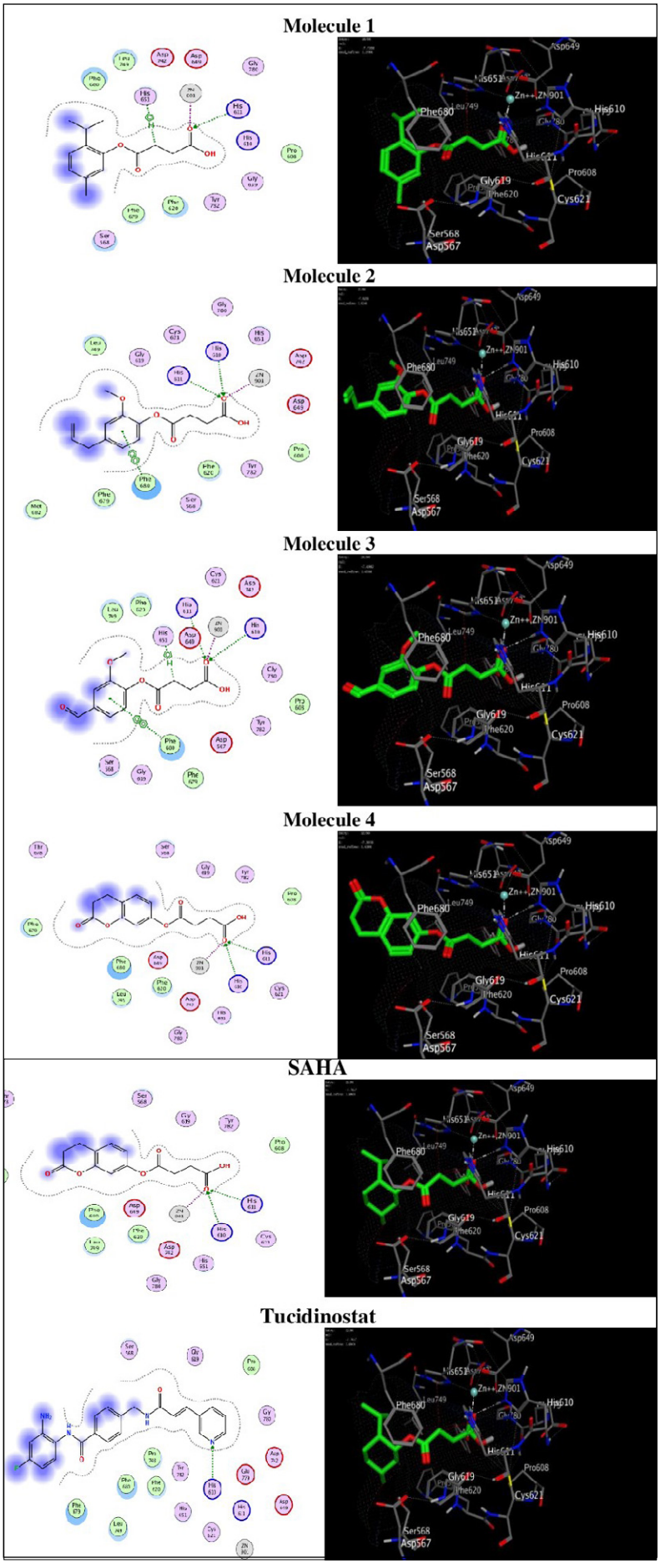

Fig. 4. 2-D and 3-D pose of the four designated hybrid molecules in the active site of PDB 5EDU HDAC6, and two references, ASHA and Tucidinostat
Source: Own materials

Table 1. S-score & RMSD value of all the molecules are listed

mseq	S-score	RMSD _refine
Molecule 1	-7.73931	1.238553
	-7.72422	1.145665
	-7.53628	0.962826
	-7.52902	1.148029
	-6.8746	1.708983
Molecule 2	-7.62932	1.624763
	-7.54466	1.542013
	-7.44754	1.247373
	-7.31624	0.713143
	-7.07375	1.551248
Molecule 3	-7.49815	1.659777
	-7.30356	1.556132
	-7.25448	1.176839
	-7.20749	1.192163
	-6.90244	2.53427
Molecule 4	-7.41243	2.453249
	-7.30557	1.416625
	-7.215	1.468781
	-7.04577	1.428322
	-7.00273	2.629645
SAHA	-8.84678	1.968135
	-8.83205	1.627789
	-8.63910	2.2169015
	-8.49357	1.8273996
	-8.45259	2.5052261
Tucidinostat	-7.02955	1.8229722
	-6.95895	1.7007053
	-6.84878	2.3356028
	-6.83148	2.2330139
	-6.78206	2.1497548

Source: Own materials

and redistill. Re-crystalized using petroleum ether 88%. FT-IR (cm-1): 1724 (ester group), 3439 (-OH carboxylic acid), 1170 (ether group), 3070 (C-H aromatic group), 1510 (C=C bending aromatic). 1H NMR (300 MHz, DMSO-d6) δ 11.97 (s, 1H, -COOH), 7.95-6.66 (m, 3H, Ar-H), 6.64 (cyclic carbon proton), 2.91 (m, CO-CH2-), 2.45 (m, 2H, CH2-COOH). 13C NMR (75 MHz, DMSO-d6) δ 175.41 carboxylic carbon, 172.84 (carbonyl carbon), 162.30 (cyclic carbon), 156.02, 155.37, 145.02, 130.09, 127.71, 115.00 (aromatic carbon), 29.65 (Carbonyl-CH2-), 29.98 (-CH2-Carboxyl), the chemical synthesis scheme is shown in figure (3).

BIOLOGICAL EVALUATION

In Sharif University of Technology, MDA-MB-231 (cell line for human breast cancer) was purchased from the Iranian National Bank for Cell of (Pasteur Institute, Iran. Cells were developed in RPMI-1640 medium (Gibco) with standard supplement 10% phosphate-buffered saline (PBS) (Gibco) with effective antibiotics 100 U/ml and 100 µg/ml of penicillin and streptomycin, respectively. Cells were maintained C under humidified air containing 5% CO_2 at 37°C and were passed on trypsin/EDTA (Gibco) and phosphate-buffered saline combination (PBS) solution.

RESULTS AND DISCUSSION

MOLECULAR DOCKING OUTCOMES

The docking score (S) and root-mean-square deviation (RMSD) value of all the molecules are listed and given in table (1).

Table 2. Pharmacokinetic parameters of designated molecules and two references

Molecule	Molecule 1	Molecule 2	Molecule 3	Molecule 4	Molecule 5 (SAHA)	Molecule 6 (Tucidinostat)
Molecular weight (MW)	250.29	264.27	252.22	264.23	264.32	390.41
#Rotatable bonds	6	8	7	5	10	8
#Hydrogen-bond acceptors	4	5	6	6	3	4
#Hydrogen-bond donors	1	1	1	1	3	3
Molecular Refractivity (MR)	68.87	69.92	61.21	63.68	73.33	110.01
Topological Polar Surface Area (TPSA)	63.6	72.83	89.9	89.9	78.43	97.11
Implicit log P (iLOGP)	2.29	2.46	1.68	1.66	1.84	2.51
eXtended LOGP version 3 (XLOGP3)	2.6	2.15	0.43	0.83	1.86	2.3
Gastrointestinal absorption	High	High	High	High	High	High
Blood–Brain Barrier (BBB) permeant	Yes	Yes	No	No	No	No
P-glycoprotein (Pgp) substrate	No	No	No	No	No	Yes
CYP1A2 inhibitor	No	Yes	No	No	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	Yes
CYP2C9 inhibitor	No	No	No	No	No	Yes
CYP2D6 inhibitor	No	No	No	No	No	Yes
CYP3A4 inhibitor	No	No	No	No	No	Yes
Lipinski #violations	0	0	0	0	0	0
Ghose #violations	0	0	0	0	0	0
Veber #violations	0	0	0	0	0	0
Egan #violations	0	0	0	0	0	0
Muegge #violations	0	0	0	0	0	0
Bioavailability Score	0.85	0.85	0.56	0.56	0.55	0.55
PAINS #alerts	0	0	0	0	0	0
Brenk #alerts	1	2	2	1	2	2
Leadlikeness #violations	0	1	0	0	1	2

Source: Own materials

A Two-dimension (2-D) and three-dimension 3-D pose for the four designated hybrid molecules in the active site of Protein Data Bank (PDB) 5EDU HDAC6, and two references, SAHA and Tucidinostat are given in figure (4), while Pharmacokinetic parameters of designated molecules and two references is given in table (2).

ACCORDING TO THE KINETICS RESULTS, WE ANALYSED THE DATA OBTAINED IN TWO WAYS:

Firstly: the BOILED-Egg, figure (5). The plotting of WLOGP versus TPSA, arranges the position of any molecule in the white area, referring to the ability of the drug molecule to undergo passive absorption from the gastrointestinal tract, while the yellow area refers to the ability of the drug molecule to brain. The blue color point indicates the drug molecule is a suitable substrate for Pgp -glycoprotein, while the red point color refers to the drug molecule free from efflux by Pgp –glycoprotein [21]. Neither Tucidinostat nor SAHA crosses the references cross blood-brain barrier. Only designated hybridized molecule-1(Thymol-based HDAC inhibitor) and (Eugenol-based inhibitor) cross the blood–brain barrier (BBB) permeant and reach to the brain.

The Biological assay (MTT) of antineoplastic activity, where IC50% for M1 (37.95), M2 (93.72), M3 (540.81) and M4 (115.98) are shown in figure (6).

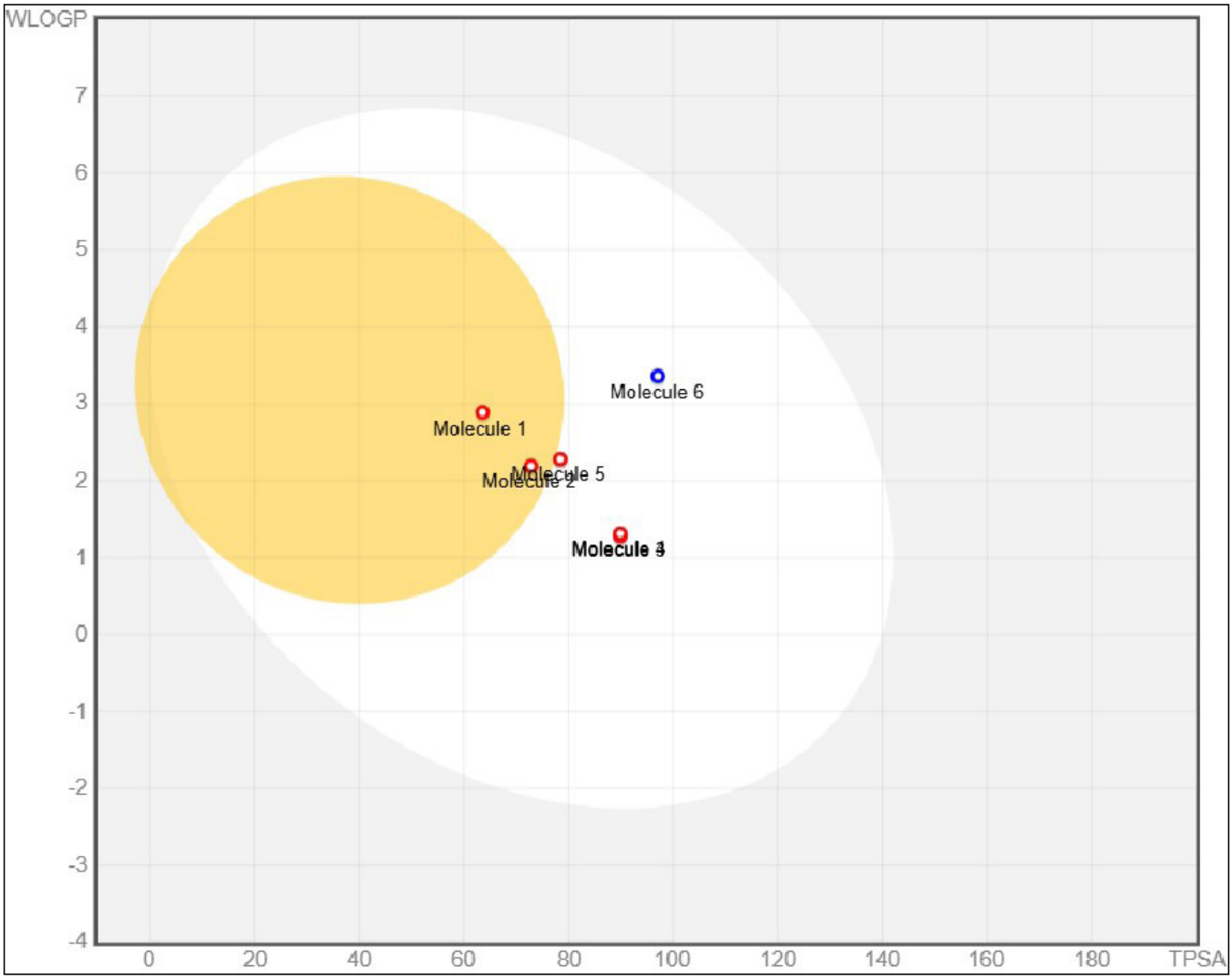

Fig. 5. BOILED-Egg -SWISS-ADME for four designated hybrid molecules and two references, where M1 = Thymol-based inhibitor M2=Eugenol-based inhibitor, M3 = based inhibitor, M4 = Umbelliferon-based inhibitor, M5=SAHA referance-1and M6=Tucidinostat -reference-2
Source: Own materials

FLUORESCENT ASSAY

The concentrations for Thymol-based inhibitor, Eugenol-based inhibitor, Vanillin-based inhibitor, and Umbelliferon-based inhibitor are shown in figure (7)

SAHA is the FDA-approved pan HDAC inhibitor associated with cardiotoxicity QT prolongation, cardiac arrhythmia, haematological toxicity including thrombocytopenia, severe anaemia, hepatotoxicity and neuron toxicity including impaired cognitive function, seizure, and peripheral neuropathy, leading to the limitation in clinical use and reduced patient compliance. Furthermore, the poor pharmacokinetic characteristics of SAHA. The major adverse effects of SAHA and its poor pharmacokinetic profile are caused by the hydroxamate functionality. By applying pharmaceutical isosteric to design new molecules with an isosteric entity of hydroxamate to it is carboxylate that resembles in steric and electronic environments and has zinc chelation activity [22]. Valproic acid is an HDAC synthetic-based carboxylate inhibitor, but also associated with adverse effects, but acceptable pharmacokinetics parameters, so drug designing of natural molecules (Thymol, Eugenol, vanillin and Umbelliferon) with succinate to yield natural hybrid molecules with structural similarity to HDAC inhibitors. The computer aid drug design involve molecular docking program (MOE), in-silico pharmacokinetics analysis, protein data bank sources are the leading way to design new potential chemical molecules and their drug likeness candidate by verification with two the references drug, our designated molecules exhibited comparable inhibitory activity relative to the (hydroxamate based inhibitor) SAHA and (benzamine based inhibitor) Tucidinostat as references drugs. The design of specific hybridized molecules to establish the specific requirements of HDAC enzyme inhibition.

Fig. 6. Biological assay (MTT) of anti-neoplastic activity. Where IC50% for M1 (37.95), M2 (93.72), M3 (540.81) and M4 (115.98)
M1=Thymol-based inhibitor, M2=Eugenol-based inhibitor, M3=Vanillin-based inhibitor and M4=Umbelliferon-based inhibitor
Source: Own materials

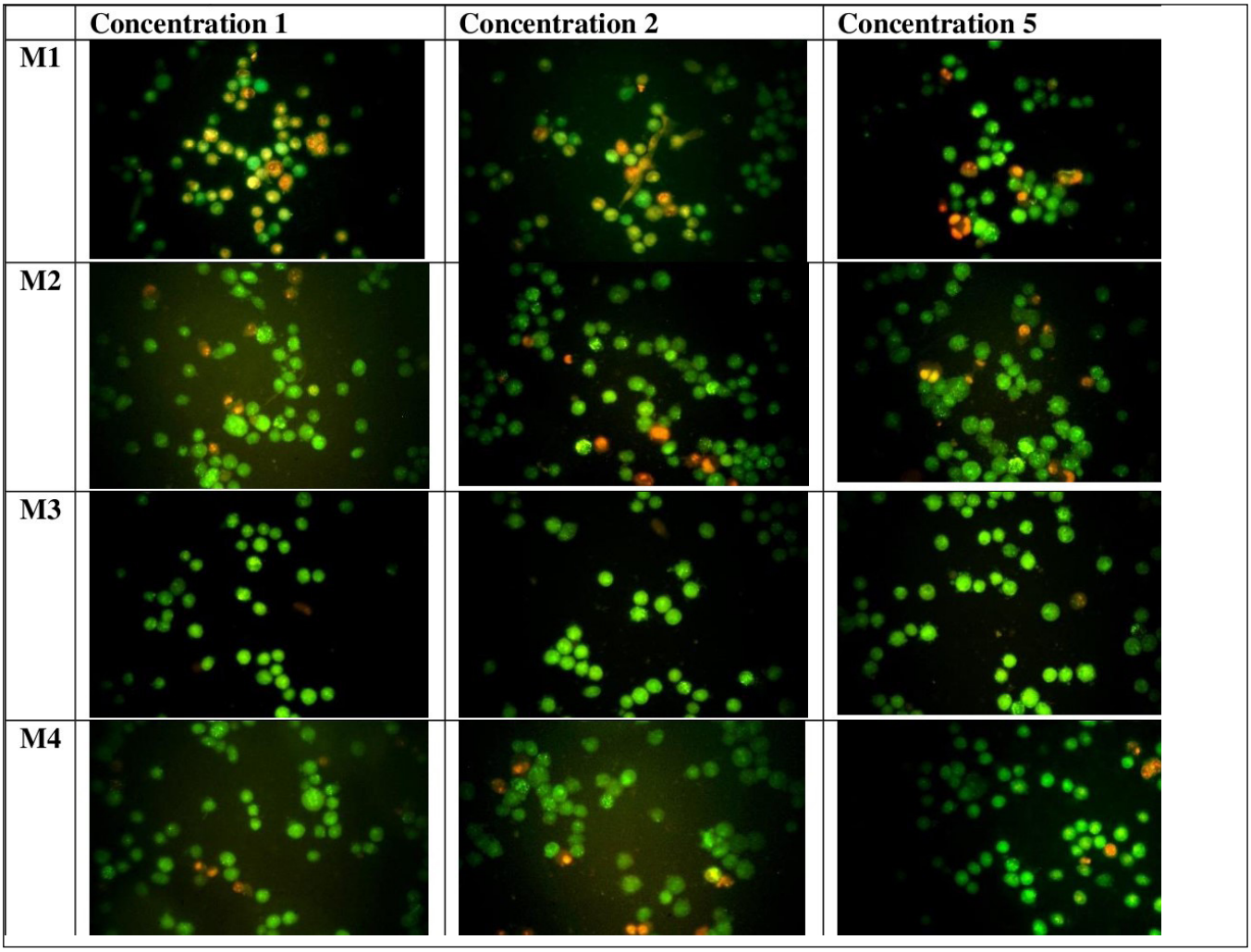

Fig. 7. concentrations for Thymol-based inhibitor, Eugenol-based inhibitor, Vanillin-based inhibitor, and Umbelliferon-based inhibitor
Source: Own materials

Analysis of molecular docking of active site of HDAC6 (5EDU) PDB shows score (S-value), molecule-1(Thymol-based HDAC inhibitor) exhibited (-7.7) the lowest S-value, establishing it as the most effective ligand among our selected candidates for inhibiting HDAC6 activity & than the two references, SAHA (-8.8) &Tucidinostat (-7.029), regarding all acceptable RMSD values below 2. S-score & RMSD value of all the molecules. The binding of hybrid designated molecules and two references to HDAC6 (5EDU). Molecule-1 exhibited the highest binding affinity among the proposed inhibitors analysed, based on Lipinski's rule of five and the BOILED-egg technique, yet it was not considered a therapeutic drug according to Lipinski's criterion and the BOILED-egg approach.

CONCLUSIONS

Our study elucidates, in comparison to two of the references, firstly the hydroxamate-based inhibitor SAHA and secondly the benzamide-based inhibitor Tucidinostat, the binding characteristics of established designated hybrid molecules as HDAC6 inhibitors. The molecular docking analysis refers to the hybridized molecules exhibiting zinc binding activity at the active site of the enzyme HDAC6. Designated hybridized molecules have an exceptional zinc-binding capacity as to that of the SAHA HDAC inhibitor. The *in silico* ADME pharmacokinetics of molecules (1, 2, 3 and 4) exhibit good dynamics scores, and the computational kinetic parameters are superior to the reference molecules (SAHA and Tucidinostat). In summary, we introduced a new class of HDAC6 inhibitors. The compounds exhibit exceptional selectivity for HDAC6, especially Molecule-1 Thymol-based HDAC inhibitor, which is superior in drug-receptor binding (S score in molecular docking and no substrate to Pgp, a Pgp-glycoprotein with computationally validated crossing of the blood brain barrier. As a result, molecule-1 (Thymol-based HDAC inhibitor) has a higher cytotoxic effect than other designated hybrid molecules. The biosafety

of molecule-1 and excellent *in silico* pharmacokinetics parameters make it a building unit of the new generation of HDAC6 inhibitors. The new hybridized molecules of natural origin showed potential anti-neoplastic activities with improved pharmacokinetic parameters compared to SAHA and Tucidinostat.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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