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Design and Structural Investigation of a Series of Prodigiosin and 1,10-Phenanthroline Derivatives as Novel and Highly Potent Anticancer Therapeutic Drugs or Active Pharmaceutical Ingredients

M. Mustafa CETIN[1*], Sümeyye Berfin GÜL[2]

Abstract

Breast cancer is considered as a leading cancer type with the secondary highest possibility of brain metastasis. Most research in breast cancer is currently directed into the mortality of brain metastatic breast cancer. However, there is no effective treatment or anticancer therapeutics specifically for this cancer type. Hence, development of effective and novel anticancer therapeutic drugs/APIs to inhibit HDAC and mTOR, playing very important role on modulating breast cancer progression is an increasing demand. In this study, the structure-activity relationship and *in silico* modeling of a series of prodigiosin and 1,10-phenanthroline derivatives as highly potent anticancer therapeutic drugs/APIs against mTOR and HDAC enzymes have been investigated. Compared to the natural product Ps, 20 of the highly potent ligands, especially **2a**, **6b**, **13** and **13a**, have exhibited very promising binding energies ranging from –9.4 to –7.1 kcal/mol and inhibition constants ranging from 225 to 569 nM against HDAC1 and/or mTOR enzymes. Ligands **2a**, **5**, **6b**, **7b** and **13** in particular show effective dual action against both enzymes. The findings from the *in silico* modeling studies have also been supported with MD simulations and ADMET study with Lipinski's rule of five, providing outstanding therapeutic potential for the breast cancer brain metastasis.

Keywords: Anticancer therapeutic drugs/APIs, breast cancer brain metastases, mTOR, HDAC, 1,10-phenanthroline, prodigiosin.

Bir Dizi Prodigiosin ve 1,10-Fenantrolin Türevlerinin Yeni Nesil Etkin Antikanser Tedavi Edici İlaçlar ya da Aktif Farmasötik Maddeler Olarak Tasarımı ve Yapısal İncelenmesi

Öz

Meme kanseri, beyin metastazı olasılığı en yüksek olan ikinci kanser türü olarak kabul edilmektedir. Bu yüzden meme kanseriyle ilgili araştırmaların çoğu beyin metastatik meme kanserinin mortalitesine yöneliktir. Ancak bu kanser türüne özgü etkili bir tedavi veya antikanser tedavi yöntemi mevcut değildir. Bu nedenle, meme kanseri ilerlemesinin modüle edilmesinde çok önemli rol oynayan HDAC ve mTOR enzimlerini inhibe edecek etkili ve yeni nesil antikanser terapötik ilaçların ve/veya aktif farmasötik maddelerin geliştirilmesi artan bir taleptir. Bu çalışmada, mTOR ve HDAC enzimlerine karşı oldukça güçlü antikanser terapötik ilaçlar ve/veya aktif farmasötik maddeler olarak bir dizi prodigiosin ve 1,10-fenantrolin türevinin yapı-aktivite ilişkisi ve *in silico* modellemesi incelenmiştir. Doğal ürün Ps ile karşılaştırıldığında, bu ligandlardan 20 tanesi, özellikle **2a**, **6b**, **13** ve **13a**, HDAC1 ve/veya mTOR enzimlerine karşı –9.4 ile –7.1 kcal/mol arasında değişen çok umut verici bağlanma enerjileri ve 225 ile 569 nM arasında değişen inhibisyon sabitleri sergilemiştir. Özellikle **2a**, **5**, **6b**, **7b** ve **13** her iki enzime karşı iki taraflı etkinlik (dual action) göstermiştir. *İn silico* modelleme çalışmalarından elde edilen bulgular, MD simülasyonları ve Lipinski'nin beş kuralına göre ADMET çalışmasıyla da desteklenmiş olup, meme kanseri beyin metastazına sahip hastalar için olağanüstü bir terapötik potansiyel sağlamaktadır.

Anahtar Kelimeler: Antikanser tedavi edici ilaçlar/aktif farmasötik maddeler, meme kanseri beyin metastazları, mTOR, HDAC, 1,10-fenantrolin, prodigiosin.

[1,2]Kadir Has University, Faculty of Engineering and Natural Sciences, Istanbul, Türkiye, mustafa.cetin@khas.edu.tr, 20171709010@stu.khas.edu.tr

[*]Sorumlu Yazar/Corresponding Author

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1. Introduction

The attributed deaths of approximately 17% is due to the second leading cause cancer for the world population [WHO, 2020], where breast cancer counts for 25% of all cases [IARC, 2014]. Among these cases, breast cancer is considered to be the most common type of cancer in females, with the second-highest probability of brain metastasis [Forman and Ferlay, 2014; Siegel et al., 2020; Al Shamsi and Alrawi, 2018; Leone and Leone, 2015]. In approximately 10-20% of patients diagnosed with breast cancer, malignant brain metastasis was also found [Pangeni et al., 2015; Engel et al., 2003]. In recent years, this percentage is continuously increasing with the development of diagnostic and prognostic methods [Rostami et al., 2016] in the healthcare system and technology. Most research in breast cancer is currently directed into the mortality of breast cancer brain metastasis with the development of therapeutics and prognostic tools [Kölbl et al., 2015; Lin et al., 2002]. Alternative tools and treatments are investigated due to the development of resistance causing brain metastasis, epigenetic changes and several mutations, and individual differences in response to treatments [Godone et al., 2018]. In particular patients with high brain metastasis risk, there is, however, no effective treatment or this cancer-specific therapeutics. Hence, developing highly effective breast cancer drugs to inhibit the enzymes playing essential roles on the modulation of breast cancer is an ever increasing demand in the field.

In the last decades, many studies focusing on modulation of cancer progression have involved in three highly important enzyme families: (**i**) The phosphatidylinositol-3-kinases (PI3Ks)/AKT [Knight et al., 2006; Porta et al., 2014], (**ii**) the mammalian target of rapamycin (mTOR) [Porta et al., 2014; Maiese, 2016], and (**iii**) histone deacetylases (HDACs) [Seto and Yoshida, 2014]. Inhibition of such enzymes are among the current treatment approaches for the effective treatment of breast cancer and the related brain metastases. The principles in such approaches are based on the direct targeting tumors and proteins with various expression levels by inhibiting the signaling pathways of such enzymes that play a highly important role on the progress of the breast cancer modulation [Godone et al., 2018]. In particular, inhibition of HDAC and mTOR enzymes has appeared to be a high potential strategy [Yao et al., 2021]. Numerous HDAC and mTOR inhibitors have been viewed as highly potent agents that have shown very significant anticancer activities in preclinical studies [Yao et al., 2021; Min et al., 2012; Guo et al., 2020; Bian et al., 2018; Tang et al., 2017; Kawai et al., 2003; Fasolo and Sessa, 2012]. Due to the central roles of mTOR, PI3K/AKT and HDAC as a novel potent anticancer therapy approach [Guo et al., 2020; Bian et al., 2018; Tang et al., 2017], the targeted inhibition of these enzymes and controlling their signaling pathways are very essential to combat with the cancer cells. In particular, HDAC and mTOR inhibitions are very important potent and promising approaches as anticancer therapy. Due by the importance of these two enzymes, most researchers

have, therefore, focused their research [Yao et al., 2021; Min et al., 2012; Guo et al., 2020; Bian et al., 2018; Tang et al., 2017; Kawai et al., 2003; Fasolo and Sessa, 2012; Lu and Liu, 2020] on developing novel inhibitors targeting mTOR and HDAC in order to elucidate a precise mechanism of action for inhibition of the enzymes. In addition, there have been many other novel potent anticancer agent studies [Fricker, 1994; Heffeter et al., 2006; Zhang and Lippard, 2003; Marzano et al., 2002; Ranford et al., 1993; Saha et al., 2004; Zoroddu et al., 1996; Erkkila et al., 1999; Butler et al., 1969; Macleod, 1952; Dwyer et al., 1969; Walsh et al., 2006; Danevčič et al., 2016; Espona-Fiedler et al., 2012; Wang et al., 2016; Müller et al., 2013; de Ruijter et al., 2003; Weichert, 2009; Shouksmith et al., 2019; Senese et al., 2007; Choi et al., 2001; Halkidou et al., 2004; Krusche et al., 2005; Uba and Yelekçi, 2018; Gümüş et al., 2021; Dege et al., 2022] in the field for better understanding of cellular and molecular mechanisms of both HDAC and mTOR inhibition.

The current abovementioned literature clearly states that there have been several designed, synthesized and examined inhibitors for their anticancer and antitumor activities on breast cancer cells, and there is still an urgent demand for more of such novel artificial inhibitors for HDAC and mTOR enzymes and their signaling pathways in particular. A reliable and significant number of such literature reports have highlighted use of 1,10-phenanthroline (PHEN) and its derivatives, and their transition metal complexes (*e.g.*, with copper(Cu)) as anticancer agents with their possible anticancer chemotherapeutic potential [Fricker, 1994; Heffeter et al., 2006; Zhang and Lippard, 2003; Marzano et al., 2002; Ranford et al., 1993; Saha et al., 2004; Zoroddu et al., 1996; Erkkila et al., 1999; Butler et al., 1969; Macleod, 1952; Dwyer et al., 1969; Walsh et al., 2006; Danevčič et al., 2016; Espona-Fiedler et al., 2012; Wang et al., 2016; Müller et al., 2013; de Ruijter et al., 2003; Weichert, 2009; Shouksmith et al., 2019; Senese et al., 2007; Choi et al., 2001; Halkidou et al., 2004; Krusche et al., 2005; Uba and Yelekçi, 2018; Denoyer et al., 2015; Ge et al., 2022; Chang, 2015; Brady et al., 2014; Que et al., 2008; Solomon et al., 1996; Lippard et al., 1994; Hanahan and Weinberg, 2011; Li. 2020; Huang et al., 2021; Hussain et al., 2019; Marzano et al., 2009; Lumme et al., 1984; Molinaro et al., 2020]. Many metal complexes containing PHEN and Schiff bases with a functional group of C=N play very important roles and posses anticancer activity [Zhang et al., 2015; Hindo et al., 2009; Zhang et al., 2012; Zhang et al., 2008; Zuo et al., 2013]. Such ligands and their copper complexes are highly intriguing due to their diverse biological activities, antimycobacterial [Saha et al., 2004], antimicrobial [Zoroddu et al., 1996], antitumor [Ranford et al., 1993], and intercalating agents of DNA [Erkkila et al., 1999]. In addition to the PHEN and its derivatives with metal complexes, a known natural medicine Prodigiosin (Ps) [Walsh et al., 2006] is another structure as a secondary and natural metabolite, which has multiple biological functions, including but not limited to anti-inflammatory, antibacteria, immnosupression and anticancer activity [Danevčič et al., 2016]. Ps has also attracted immense interests with its anticancer function because it is a dual mTOR inhibitor, which has two essential components, mTORC1 and

mTORC2 [Espona-Fiedler et al., 2012]. Therefore, Ps may be another potent candidate as the novel generation anticancer drug due to its unique inhibition mechanisms, and its derivatives have also become one of the most promising anticancer drug classes leading with anticancer and proapoptotic effects in a various cancer cell lines including breast cancer cells in recent years [Montaner et al., 2000; Montaner and Pérez-Tomás, 2001; Díaz-Ruiz et al., 2001; Montaner and Pérez-Tomás, 2003; Soto-Cerrato et al., 2004] even though the structure was first ascertained from a total synthesis in 1960 [Rapoport and Holden, 1960]. Current research efforts are focusing more on synthetic protocols of Ps derivatives due by their expected lower cytotoxicity and anticancer activity. Studying inhibition of HDAC will also be as esssential as mTORs because deregulation of Class I HDAC (HDAC1, 2, 3 and 8) activity has been associated with many cancer types [Müller et al., 2013; de Ruijter et al., 2003; Weichert, 2009; Shouksmith et al., 2019] and its involvement has been [Senese et al., 2007] highly vital in control of mammalian cell proliferation. HDAC1 and its overexpression, in particular, have been related to various types of cancer [Choi et al., 2001; Halkidou et al., 2004; Krusche et al., 2005]. Thus, inhibition of the HDAC1 appears to be a promising therapeutic target for cancer therapy, and this may be regulating the progression of breast cancer and its associated metastasis [Krusche et al., 2005; Uba and Yelekçi, 2018].

Although the current literature has produced many inhibitors against mTOR and HDAC enzymes, there is a very limited number of commercially available common anticancer drugs (*e.g.*, fluorouracil, doxorubicin, carboplatin, and paclitaxel) on the market for the treatment of breast cancer. A major downside to such anticancer drugs is the buildup of resistance toward these drugs by cancer cells. Novel drugs with alternative modes of action are vital to ensure both the effectiveness and worldwide affordability of cancer treatments. As it currently stands, neither PHEN nor Ps or their any derivatives has made any commercial progress to the current anticancer drug market. Even though one of the candidate drugs, obatoclax, passed the phase II clinical trials, its development was unfortunately halted. Thus, alternative PHEN and Ps derivatives have yet to be tested as therapeutic agents for combatting breast cancer. In one of our earlier preliminary studies [Cetin et al., 2022], we found that some Ps and PHEN derivatives with their copper(I) complexes exhibited promising anticancer activities (one of the ligands showed an overwhelming anticancer activity than Ps, the natural drug). When compared ligands with their relevant copper(I) complexes, the complexes were even performed more significant anticancer activities on the selected cell lines. The *in silico* computational modeling studies conducted with the mTOR and HDAC1 enzymes also validated the obtained experimental data. However, the cytotoxicity of some of these ligands and/or their relevant complexes were a little higher than the acceptable cytotoxicity ranges.

In this context, by utilizing the aforementioned state-of-art and employing *in silico* computational modeling studies that are conducted with the mTOR and HDAC1 enzymes considering the *in silico* structure-activity relationship (*in silico* SAR), we, in this work, have focused on

designing, developing and optimizing novel PHEN and Ps derivatives as active pharmaceutical ingredients (APIs), which are expected to inhibit HDAC (especially HDAC1) and/or mTOR enzymes against brain metastatic breast cancer. Such structural and modeling studies will also facilitate to predict the most promising functions/functional groups with the PHEN and Ps core structures (**Figure 1**), binding affinities and binding modes of such desired ligands. With the preliminary results from our earlier work [Cetin et al., 2022], we predict that either PHEN and/or Ps derivatives, including their copper(I) complexes upon complexation, or the combined use of such compounds in different variations, given their numerous modes of cellular actions, could improve the efficacy of anticancer treatments and may also provide great therapeutic potential for the breast cancer patients with brain metastasis risk.

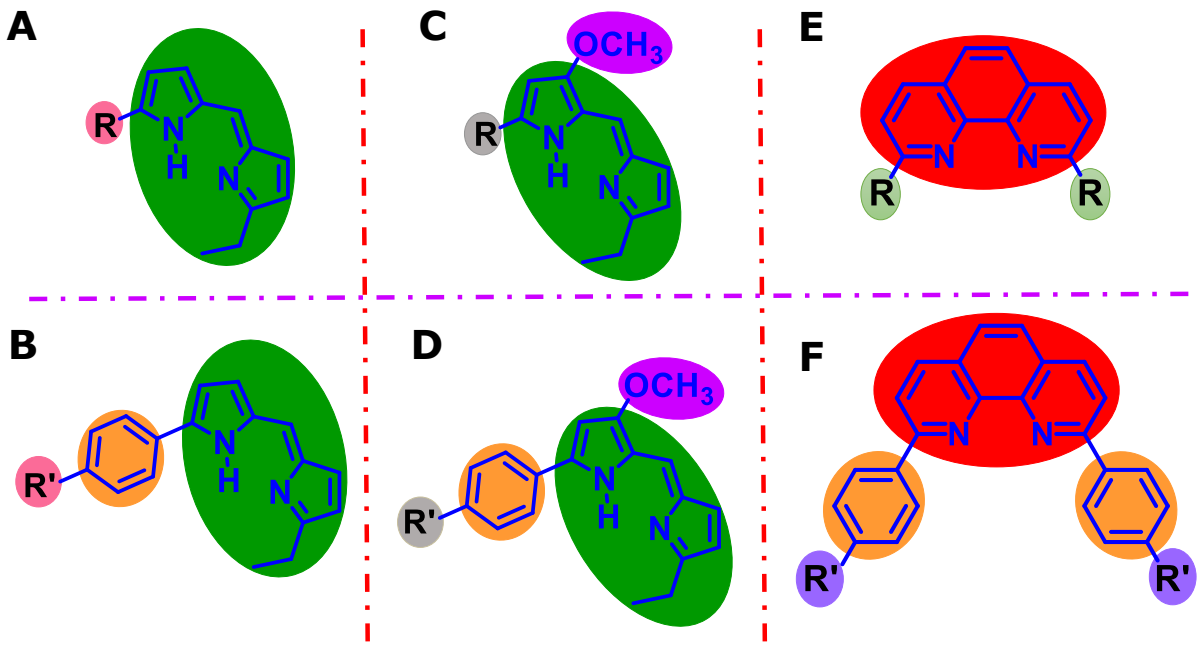


Figure 1. The general structures of Ps- (**A**, **B**, **C** and **D**) and PHEN (**E** and **F**)-based derivatives and their functionalization with different –**R**, –**R'** and/or –**phenyl-R'** groups.

2. Materials and Methods

By aiming to structurally design and develop novel PHEN and Ps derivatives, some *in silico* computational modeling studies that have been carried out with mTOR and HDAC1 enzymes considering the *in silico* SAR details are presented below.

2.1. Molecular Modeling Studies

2.1.1. Enzyme Preparation

The mTOR and HDAC1 crystal structures (selected due by being homosapians enyzmes and having no mutation) were retrieved from protein data bank and used for the protein setup. [(http://www.rcsb.org, (for mTOR pdb code: 4jsv; resolution 3.5 Å) and (for HDAC1, pdb code: 4BKX; resolution 3.0 Å)] [Morris et al., 1998]. Each structure was cleaned of all water molecules and inhibitors as well as all non-interacting ions before being used in the docking studies. One of the two subunits for mTOR and HDAC1 was chosen as the target structure. Geometry of each protein was first optimized using a fast Dreiding-like force field, and subsequently submitted to Discovery Studio's "Clean Geometry" toolset [BIOVIA, 2015] for a more thorough examination. Missing hydrogen atoms were added considering the protonation state of the titratable residues at a pH of 7.4. The dielectric constant was adjusted to 10 and the ionic strength was set to 0.145. The AutoDock Tools (vv. 1.5.7) (ADT) [Morris et al., 2009] graphical user interface program was employed to setup the enzymes for docking.

2.1.2. Ligand Setup

The three-dimensional (3D) structures of ligand molecules were built and minimized using BIOVIA Discovery Studio [BIOVIA, 2015], and optimized at (PM3) level and saved in pdb format. Here, the docking input files of the ligands were also generated using the ADT package. Autodock Vina [Eberhardt et al., 2021] docking program was used for all docking processes. In an earlier literature work [Akdoğan et al.], the comprehensive procedure used for docking methods was reported in details.

2.1.3. Molecular Docking

The data built by using the BIOVIA Discovery Studio [BIOVIA, 2015] was saved in pdb format, and in the meantime missing hydrogen atoms were added considering the protonation state of the titratable residues at a pH of 7.4, and optimization of geometry and minimization processes were employed. Upon preparation of the ligands, molecular docking was performed using the Autodock Vina [Eberhardt et al., 2021] with the parameters (grid center and box dimensions) in **Table 1**. For each ligand, there were 10 runs with the selected enzyme to find out the best binding case.

Table 1. The molecular docking grid center and box dimensions used for Autodock Vina.

Center (Å)	HDAC1	mTOR	Box Dimensions (Å)	HDAC1	mTOR
X	-46.7	-20.7	**X**	25	25
Y	16.3	-29.9	**Y**	25	25
Z	-7.8	-58.3	**Z**	25	25

2.1.4. Molecular Dynamic Simulations

Of the designed and optimized 75 ligands (**Figures 2–4**), six enzyme-ligand complexes (**2a**, **6b** and **13** against the HDAC1 and **6b**, **13** and **13a** against the mTOR) have produced the best results for each virtual screening technique chosen based on their binding energies in order to investigate the structural dynamics and stability of these complexes (**Tables 2–4**). Using the BIOVIA Discovery Studio [BIOVIA, 2015], the six selected enzyme-ligand complexes and the free HDAC1 and mTOR enzymes were prepared [Phillips et al., 2005] for NAMD input file processing. The input files for NAMD were prepared using Charmm-GUI [Jo et al., 2008] that was employed for each enzyme-ligand complex and free HDAC1 and mTOR enzymes. NAMD was used to run unconstrained 50 nanosecond (ns) Molecular Dynamics (MD) simulations on both free enzymes and enzyme-ligand complexes. The validation of the complexes with the free enzymes was examined by comparing the average values of root-mean-square deviations (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg) profiles along the trajectories on the generated graphs.

2.2. Chemistry

2.2.1. Design of Ps-based, Ps and PHEN Derivatives as Novel Ligands

One of the most important studies that needs to be accomplished is to create and design chemical structures that will first exhibit highly promising *in silico* computational modeling results, and then the synthetic possibility with potential anticancer activities. Because not every drug candidate performing anticancer activity can synthetically be obtained, or *vice versa*. Therefore, considering our previous experiences and results from the highly promising preliminary study [Cetin et al., 2022] regarding validation of the computational data for the designed chemical structures and their biological activities with some Ps and PHEN ligands as well as their relevant complexes with HDAC1 and mTOR enzymes, it has been aimed to carry out further studies to create, design and develop novel improved and optimized Ps-based and Ps (**Figures 2** and **3**), and PHEN (**Figure 4**) derivatives by even further enabling the synthesis, isolation, characterization, and *in vitro* biological activity testing of such novel compounds that have never been studied beforehand. In this regard, first

a series of Ps-based derivatives have been designed in order to evaluate the absence of the methoxy group (–OCH_3) and effect of the –phenyl group (–Ph) on one of the pyrrole rings (**1–13** (presence of –Ph, but absence of –OCH_3) and **14–26** (absence of both –Ph and –OCH_3) in the **Figure 2**).

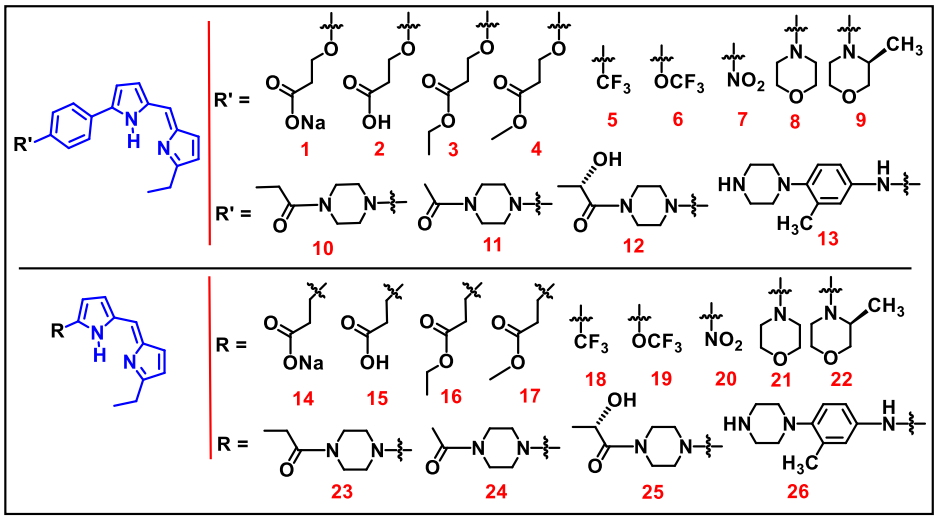


Figure 2. The Ps-based derivatives with various functions introduced into the structure for determining relationships between chemical structures and *in silico* biological activity.

Based on the outcomes of the Ps-based derivatives (**Table 2**), a series of Ps derivatives have further been designed and evaluated in the presence and absence of the –Ph group on the same pyrrole ring that has the –OCH_3 group (**Figure 3**). The obtained results (**Tables 2** and **3**) and discussion of both sets of derivatives are presented in the **Findings and Discussion Section**.

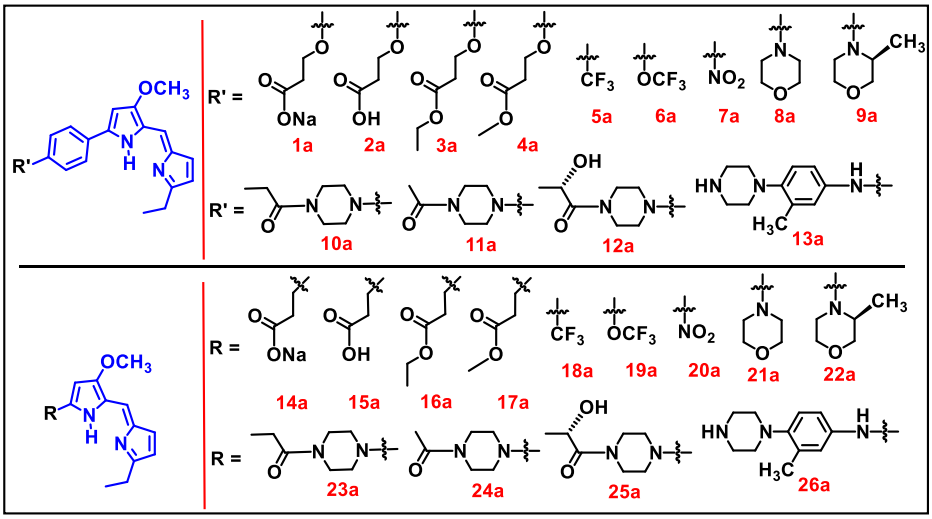


Figure 3. The Ps derivatives with various functions introduced into the structure for determining relationships between chemical structures and *in silico* biological activity.

Upon completion of both Ps-based (**1–26**) and Ps derivatives (**1a–26a**), the PHEN structure has been used to replace the Ps-core structure, conducted similar *in silico* computational modeling studies

for a series of PHEN derivatives (**Figure 4**) and obtained results for both binding energies and inhibition constants of such compounds (**Table 4**).

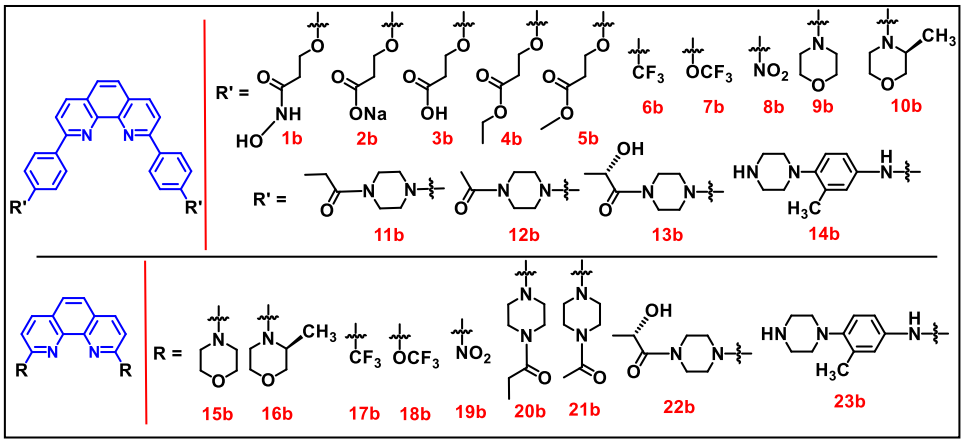


Figure 4. The PHEN-based derivatives with various functions introduced into the structure for determining relationships between chemical structures and *in silico* biological activity.

3. Findings and Discussion

Since not every novel and computationally highly potent and effective therapeutic drug or API candidate showing anticancer activity can be obtained synthetically or most synthetically accessible candidates may not have any anticancer activity, reviewing the literature for the possibility of both synthetic accessibility and anticancer activity is one of the most crucial studies. In the light of this information, a series of Ps-based, Ps and PHEN derivatives (75 derivatives in total, **Figures 2–4**) as therapeutic drug or API candidates have been designed and optimized using *in silico* computational modeling studies. More importantly, some of these derivatives/ligands have exhibited very promising binding energies and inhibition constants (**Tables 2–4**) against HDAC1 and/or mTOR enzymes (**Figure 5**) after forming enzyme-ligand complexes.

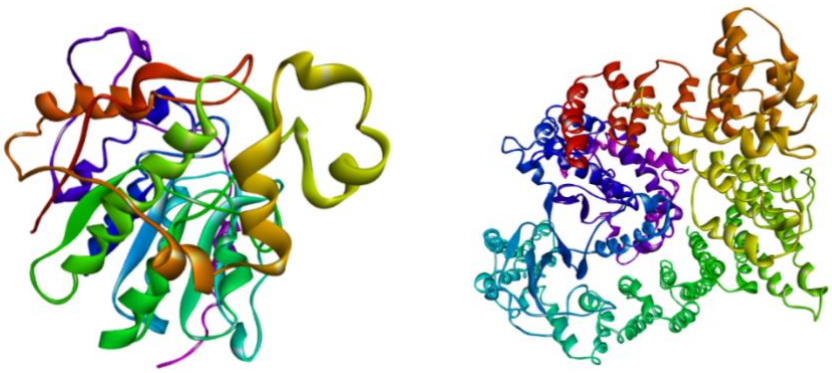


Figure 5. The structural representation of free HDAC1 (left) and mTOR (right) enzymes.

As in general practice for being highly effective, a time-efficient tool of *in silico* computational modeling has initially been utilized to collect initial results for the designed 75 ligands from the docking studies into the active sites of HDAC1 and mTOR enzymes to predict their binding affinities and binding modes by employing the AutoDock Vina docking software [Eberhardt et al., 2021]. Since acquiring the Molecular Dynamics (MD) simulations for each ligand would relatively require longer time intervals, selecting some of the ligands with the best binding energies and inhibition constants have been targeted for the MD simulations after collecting the initial results. Of these 75 ligands, 20 of them (**1**, **1a**, **1b**, **2**, **2a**, **2b**, **3**, **3b**, **5**, **5a**, **6b**, **7**, **7a**, **7b**, **8b**, **12b**, **13**, **13a**, **14b** and **23b**), which are highly potent to inhibit HDAC (especially HDAC1) and/or mTOR enzymes against brain metastatic breast cancer, have exhibited very promising binding energies and inhibition constants (**Tables 2–4**) against at least one or both enzymes, but preferentially both.

Table 2. Calculated binding energies and inhibition constants for Ps [Cetin et al., 2022] and Ps-based derivatives (**Figure 2**) against the mTOR and HDAC1 enzymes.

Compound ID	mTOR		HDAC1	
	Binding Energies (kcal/mol)	Inhibition Constants (nM)	Binding Energies (kcal/mol)	Inhibition Constants (nM)
Ps*	-4.89	258.68 μM	-6.99	7.53 μM
1	-7.1	547	-8.4	336
2	-7.2	547	-8.6	310
3	-6.6	697	-8.1	380
4	-6.9	617	-8.1	380
5	-8.1	380	-8.5	323
6	-7.4	505	-8.0	396
7	-8.0	396	-8.1	380
8	-7.3	525	-7.7	447
9	-7.4	505	-7.7	447
10	-7.4	505	-7.3	525
11	-7.4	505	-7.7	447
12	-7.6	465	-7.7	447
13	-8.2	365	-8.5	324
14	-6.3	786	-7.1	569
15	-6.5	726	-7.1	569
16	-6.1	853	-7.1	569
17	-6.3	786	-7.0	593
18	-6.4	755	-7.3	525
19	-6.6	697	-7.5	485
20	-6.2	819	-7.4	505
21	-6.7	669	-7.4	505
22	-7.1	547	-6.9	617
23	-7.0	593	-7.4	505
24	-7.1	547	-7.4	505
25	-6.8	643	-7.1	569
26	-7.4	505	-7.5	485

*****This data was taken from the previous study [Cetin et al., 2022] for the comparison purpose only.

Table 3. Calculated binding energies and inhibition constants for Ps [Cetin et al., 2022] and Ps derivatives (**Figure 3**) against the mTOR and HDAC1 enzymes.

Compound ID	mTOR		HDAC1	
	Binding Energies (kcal/mol)	**Inhibition Constants (nM)**	**Binding Energies (kcal/mol)**	**Inhibition Constants (nM)**
Ps*	-4.89	258.68 μM	-6.99	7.53 μM
1a	-7.2	547	-8.4	337
2a	-7.1	569	-8.5	324
3a	-6.8	643	-8.0	396
4a	-6.7	669	-8.1	380
5a	-7.9	412	-8.2	365
6a	-7.4	505	-7.5	485
7a	-7.7	447	-7.9	412
8a	-7.0	593	-7.5	485
9a	-7.1	569	-7.9	412
10a	-7.2	547	-7.3	525
11a	-7.3	525	-7.6	465
12a	-7.6	465	-7.7	447
13a	-8.3	351	-8.3	351
14a	-6.4	755	-6.8	643
15a	-6.7	669	-6.8	643
16a	-6.1	853	-6.6	697
17a	-6.4	755	-6.7	669
18a	-6.4	755	-6.7	669
19a	-6.2	819	-6.6	697
20a	-6.2	819	-6.2	819
21a	-6.5	726	-6.7	669
22a	-6.9	617	-6.7	669
23a	-6.6	697	-7.3	525
24a	-6.7	669	-7.3	525
25a	-6.7	669	-7.0	593
26a	-7.1	570	-7.3	525

*****This data was taken from the previous study [Cetin et al., 2022] for the comparison purpose only.

Table 4. Calculated binding energies and inhibition constants for Ps [Cetin et al., 2022] and PHEN derivatives (**Figure 4**) against the mTOR and HDAC1 enzymes.

Compound ID	mTOR		HDAC1	
	Binding Energies (kcal/mol)	Inhibition Constants (nM)	Binding Energies (kcal/mol)	Inhibition Constants (nM)
Ps*	-4.89	258.68 μM	-6.99	7.53 μM
1b	-7.2	547	-9.1	254
2b	-7.9	412	-8.7	299
3b	-8.1	380	-9.1	254
4b	-7.2	547	-7.8	429
5b	-7.3	525	-8.6	311
6b	-9.4	225	-9.4	225
7b	-9.0	264	-9.4	225
8b	-8.9	275	-8.5	324
9b	-8.0	396	-8.4	337
10b	-8.3	351	-7.3	525
11b	-8.3	351	-8.6	311
12b	-8.9	275	-8.7	299
13b	-8.7	299	-6.9	617
14b	-9.2	244	-8.7	299
15b	-6.6	697	-6.8	643
16b	-7.1	569	-7.3	525
17b	-7.7	447	-8.5	324
18b	-7.3	525	-8.5	324
19b	-6.9	617	-7.5	485
20b	-7.1	569	-6.7	669
21b	-7.2	547	-6.7	669
22b	-7.6	465	-7.1	569
23b	-8.9	275	-7.8	429

***This data was taken from the previous study [Cetin et al., 2022] for the comparison purpose only.

As shown in the **Tables 2–4**, the data taken from our previous study [Cetin et al., 2022] presents that the natural product Ps exhibits –4.89 kcal/mol binding affinity and 258.68 μM inhibition constant against mTOR while having –6.99 kcal/mol and 7.53 μM against HDAC1, respectively. Compared to the novel ligands, Ps definitely has lower binding affinity and inhibition constant for the most cases against either one or both enzymes. Among these 75 ligands, **1**, **1a**, **1b**, **2**, **2a**, **2b**, **3**, **3b**, **5**, **5a**, **6b**, **7**, **7a**, **7b**, **8b**, **12b**, **13**, **13a**, **14b**, and **23b** have exhibited highly promising binding energies and inhibition constants (**Tables 2–4**) against at least one or both enzymes. While Ps-based ligands **5**, **7** and **13** on mTOR (average of –8.1 kcal/mol and 380 nM) and **1**, **2**, **5**, and **13** (average of –8.5 kcal/mol and 323 nM) on HDAC1 have exhibited very promising results on at least one of the enzymes, ligands **5** (–8.1 kcal/mol and 380 nM against mTOR and –8.5 kcal/mol and 323 nM against HDAC1) and **13** (–8.2 kcal/mol and 365 nM against mTOR and –8.5 kcal/mol and 324 nM against HDAC1) in particular have showed very effective results (**Table 2**) with dual action against both enzymes. In the case of Ps derivatives, ligands **1a**, **2a**, **5a**, **7a** and **13a** have exhibited higher binding affinities and

inhibition constants (average of –7.6 kcal/mol and 465 nM against mTOR, and average of –8.2 kcal/mol and 371 nM against HDAC1, respectively) against at least one or both enzymes, where ligands **5a** and **13a** have been highly effective on mTOR and **1a**, **2a** and **5a** on HDAC1. However, the ligand **5a** (–7.9 kcal/mol and 412 nM against mTOR and –8.2 kcal/mol and 365 nM against HDAC1) has particularly showed promising binding energy and inhibition constant values (**Table 3**) with dual action against both enzymes. In these two sets of data (**Tables 2** and **3**), the results have showed that presence and absence of the –Ph group on the pyrrole ring without –OCH_3 group (**Figure 2**) have affected the binding affinity and binding mode of the ligands within the same set of structures. Furthermore, simultaneous effectiveness of the ligands, existing of the –OCH_3 group on the same pyrrole ring (**Figure 3**) in the second set of structures (derivatives of the natural product Ps), against both enzymes have changed regarding the binding energy and inhibition constant values (**Table 3**).

Upon completion of the *in silico* computational modeling (**Tables 2** and **3**) for the two sets of ligands in the **Figures 2** and **3**, the core structure of the Ps (**Figures 1C** and **1D** with –OCH_3 group) has been replaced with the PHEN core structure (**Figures 1E** and **1F**) in order to evaluate and compare the effect of the Ps-core and PHEN-core against both mTOR and HDAC1 enzymes. In the case of PHEN derivatives (**Figure 4**), ligands **1b**, **2b**, **3b**, **6b**, **7b**, **8b**, **12b**, **14b** and **23b** have exhibited higher binding affinities and inhibition constants against at least one or both enzymes (average of –8.6 kcal/mol and 322 nM against mTOR, and average of –8.8 kcal/mol and 390 nM against HDAC1, respectively), of which ligands **6b**, **7b**, **8b**, **12b**, **14b** and **23b** have been highly effective on mTOR and **1b**, **2b**, **3b**, **6b**, **7b**, **12b** and **14b** on HDAC1, respectively. The ligands **6b**, **7b**, **12b** and **14b** have showed highly promising binding affinities and inhibition constant values (**Table 4**) with dual action against both enzymes. Comparing with the Ps-based and Ps derivatives, more PHEN derivatives have been exhibiting dual action against both enzymes (4 ligands) than the other two sets of structures (2 ligands in Ps-based and 1 ligand in Ps derivatives).

Our elaborated data from the *in silico* computational modeling studies has directed us to select the top dual-acting candidates with the best binding energy and inhibition constant from the **Tables 2–4** for further MD simulations that relatively require longer time intervals. Thus, ligands **5**, **5a**, **6b**, **7**, **13** and **13a** against mTOR and **1**, **1b**, **2a**, **6b**, **7b** and **13** against HDAC1 have been selected for the MD simulations. Among these ligands, **5**, **5a**, **6b**, **7b** and **13**, are highly important because they are the ones exhibiting dual action (the other dual-acting ligands **12b**, **13a** and **14b** were not utilized for MD simulations due to their lower binding energies and/or inhibition constants relative to the other five dual-acting ligands) against both mTOR and HDAC1 enzymes. From the **Tables 2–4**, the ligands **2a**, **6b** and **13** against HDAC1 and **6b**, **13** and **13a** against mTOR have found to be exhibiting the best results regarding the binding energies and inhibition constants compared to the other ligands. Ligand **6b** in particular shows relatively great binding affinity (ΔG = –9.4 kcal/mol and K_i = 225 nM) against

both HDAC1 and mTOR enzymes. On the other hand, ligands **2a** and **13** have exhibited relatively higher values for binding energies and inhibition constants (ΔG = –8.5 kcal/mol and K_i = 324 nM) against HDAC1 while ligands **13** and **13a** have showed very similar results (ΔG = –8.2 kcal/mol and K_i = 365 nM and ΔG = –8.3 kcal/mol and K_i = 351 nM, respectively) against mTOR. Among these four ligands, **6b** and **13** are highly important due to their dual-acting capacity against both enzymes.

The poses of ligand **6b** in the binding pockets of HDAC1 and mTOR enzymes are presented in the **Figure 6** (**B** and **E** for two-dimensional (2D) and 3D images, respectively) and **Figure 7** (**A** and **B** for 2D and 3D images, respectively) , where two strong hydrogen bonds occur between ligand **6b** and the relevant amino acids (TYR303 and HIS28 amino acids against HDAC1 and the ARG2251 and VAL2240 against mTOR), in which both cases suggest the best inhibition of the enzymes (dual action) with ligand **6b** (potent dual inhibition candidate) among all other ligands. Relative to the ligand **6b**, ligands **2a** and **13** are the other two exhibiting great affinity against HDAC1 (**Figures 6A** and **6C** for 2D and **Figures 6D** and **6F** for 3D images, respectively). In the HDAC1–ligand **13** complex, there are three π-π stacked interactions with the PHE205, HIS178 and PHE150 amino acids (**Figure 6C**) while the HDAC1–ligand **2a** complex exhibits two strong hydrogen bonds occurring between the ligand and the ASP176, TYR303 amino acids (**Figure 6A**). Such strong interactions support that these two ligands **2a** and **13** (second potent dual inhibition candidate) after ligand **6b** can act as highly promising other inhibitors against HDAC1 enzyme.

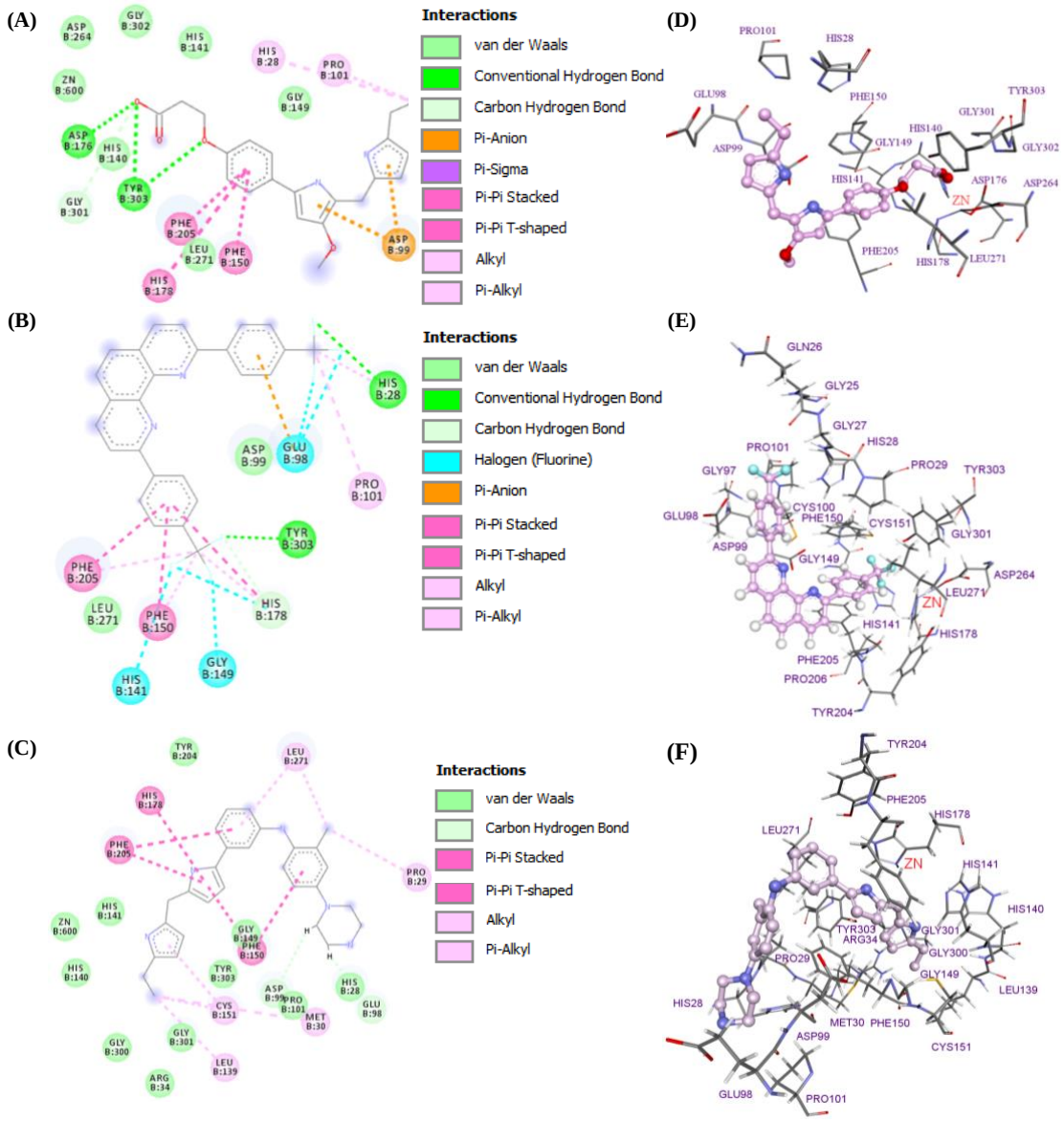


Figure 6. The 2D (**A**, **B** and **C**) and 3D (**D**, **E** and **F**) images generated via molecular docking of the ligands **2a** (**A** with H-bonding lengths of 3.28 and 3.01 & 3.15 Å to ASP176 and TYR303, respectively, and π-π interaction lengths of 5.39, 4.04 and 4.36 Å to HIS178, PHE150 and PHE205, respectively, and **D**), **6b** (**B** with H-bonding lengths of 3.13 and 2.68 Å to HIS28 and TYR303, respectively, and π-π interaction lengths of 4.43 and 3.98 Å to PHE150 and PHE205, respectively, and **E**), and **13** (**C** with π-π interaction lengths of 4.49, 4.46 & 4.90 and 5.92 & 4.45 Å to HIS178, PHE150 and PHE205, respectively, and **F**) with the HDAC1 enzyme. Amino acid side chains are shown as sticks and the ligands as ball and sticks in 3D images.

The ligand **13** is also effective against the mTOR enzyme. In the mTOR–ligand **13** complex (**Figures 7B** and **7E**), there is a strong hydrogen bonding as well as π-π stacking between the ligand and the amino acids the THR2245 and TRP2239, respectively. The ligand **13a** also exhibits great binding affinity against the mTOR enzyme (**Figures 7C** and **7F**), and there is a π-π T-shaped interaction between the ligand and TRP2239 amino acid upon formation of the complex. Although noncovalent interactions like π-π T-shaped are not as strong as hydrogen bonding, such interactions also play an important role in stabilizing molecular structures [Sinnokrot et al., 2004].

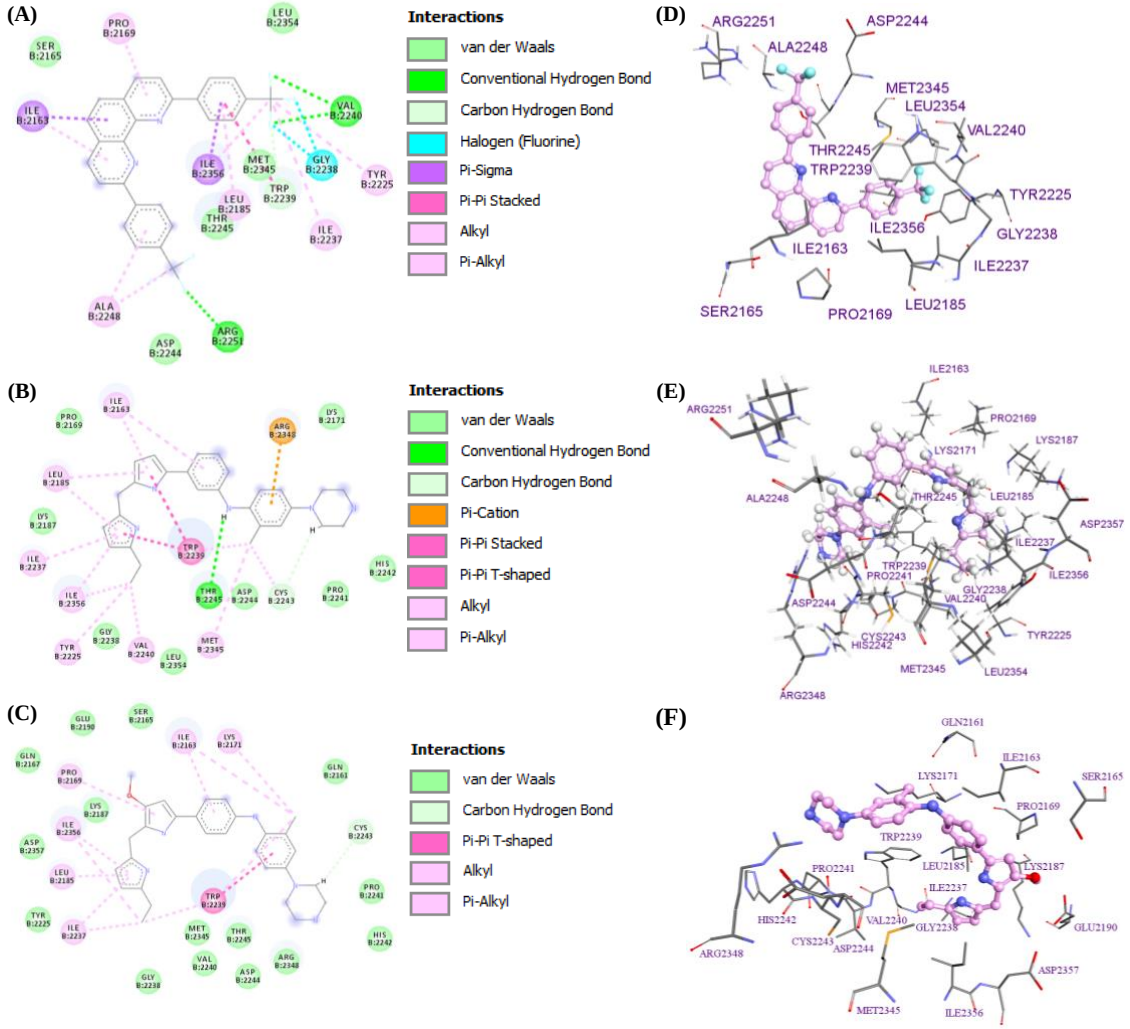


Figure 7. The 2D (**A**, **B** and **C**) and 3D (**D, E** and **F**) images generated via molecular docking of the ligands **6b** (**A** with H-bonding lengths of 2.72 & 2.41 and 2.52 Å to VAL2240 and ARG2251, respectively, and π-π interaction length of 5.00 Å to TRP2239 and **D**), **13** (**B** with H-bonding length of 2.85 Å to THR2245, and π-π interaction lengths of 5.25 & 5.07 Å to TRP2239 and **E**), and **13a** (**C** with π-π interaction length of 5.13 Å to TRP2239 and **F**) with the mTOR enzyme. Amino acid side chains are shown as sticks and the ligands as ball and sticks in 3D images.

Further investigation for various parameters such as, root-mean-square deviations (RMSD) for structural conformations and stability of enzymes upon complex formation, root-mean-square fluctuation (RMSF) for the flexibility/fluctuation of the complex, and radius of gyration (Rg) for the changes in the complex structures and information about overall dimensions (*i.e.*, consistent stability throughout the simulation), MD simulations for both free enzymes and enzyme-ligand complexes with the selected ligands have been conducted, and the parameters have been calculated from the trajectory of MD simulations. The MD simulations of the ligands **2a**, **6b** and **13** against the HDAC1 and **6b**, **13** and **13a** against the mTOR enzymes have also provided highly supporting evidence for the abovefindings.

The combined RMSDs of both the complexes formed by binding of the ligands to free enzymes obtained through MD simulations are presented in **Figures 8** and **9** that provide average RMSD values

of 1.74 and 3.47 Å (average RMSD for only globular proteins is less than 3 Å [Kufareva and Abagyan, 2012]) for free HDAC1 and mTOR enzymes, respectively. The average RMSDs of the HDAC1–ligand **6b** and mTOR–ligand **6b** complexes are of 2.14 and 3.92 Å (in green, **Figures 8** and **9**), respectively, and such higher values compared to the free enzymes suggest the presence of high degree of rotatable bonds or structural flexibilities that cause the ligand **6b** to be unable to attain stability inside the binding pocket of the enzymes in each case, which is assumed to be shallow. The average RMSD values of the the HDAC1–ligand **2a** and HDAC1–ligand **13** complexes are of of 2.09 and 2.20 Å (in blue and red, **Figure 8**), respectively, which are also higher than the RMSD value of the free HDAC1 enzyme. For such large complexes, the obtained values may be more acceptable compared to some simple structures. On the other hand, the average RMSD values of the the mTOR–ligand **13** and mTOR–ligand **13a** complexes are of of 4.13 and 3.42 Å (in blue and red, **Figure 9**), respectively. While the RMSD value for the mTOR–ligand **13** complex is higher than the free mTOR enzyme, this valus is found to be smaller in the case of mTOR–ligand **13a** complex.

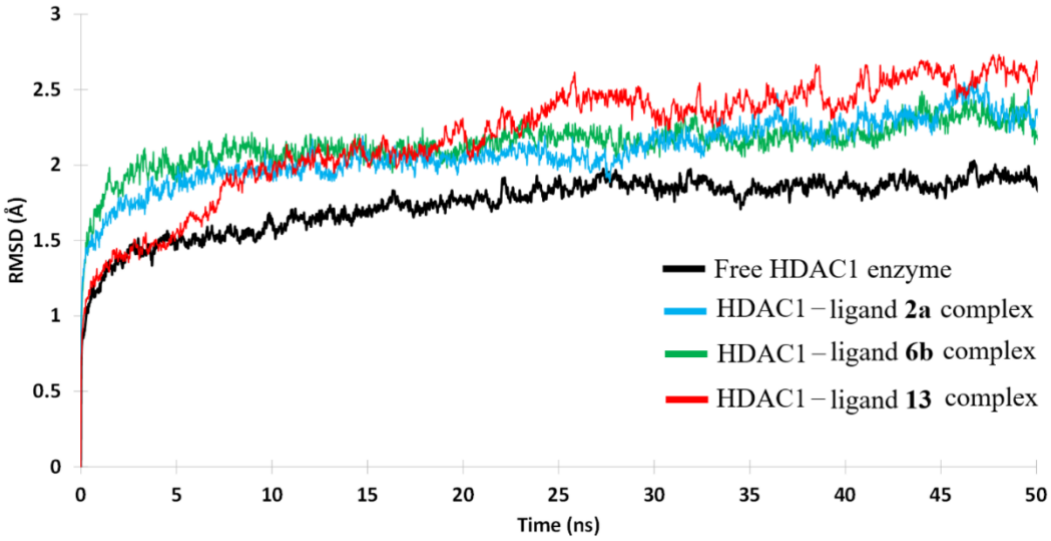


Figure 8. The combined RMSD profiles from the 50ns-MD simulations of both free HDAC1 enzyme (in black) and the complexes formed by binding of the ligands **2a** (in blue), **6b** (in green) and **13** (in red) to HDAC1.

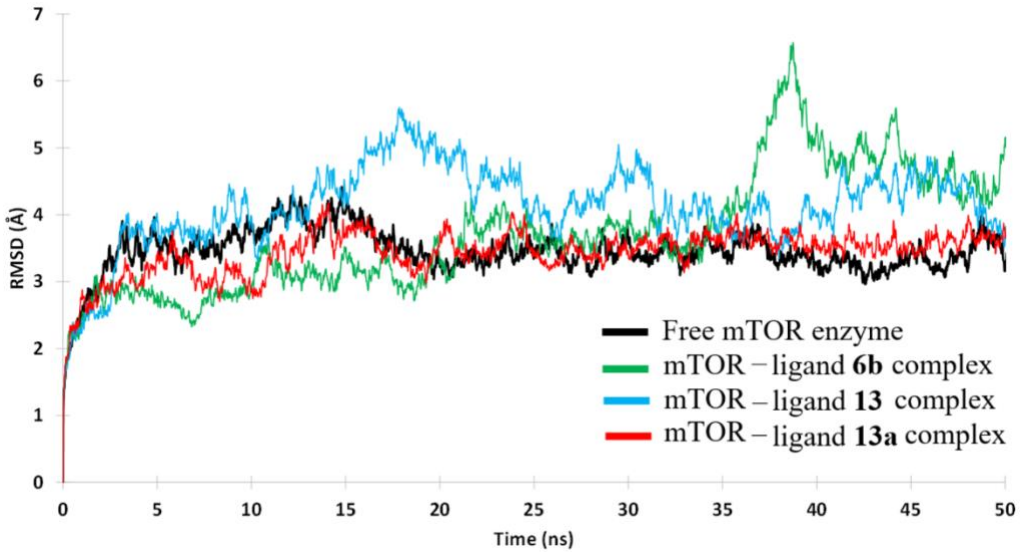


Figure 9. The combined RMSD profiles from the 50ns-MD simulations of both free mTOR enzyme (in black) and the complexes formed by binding of the ligands **6b** (in green), **13** (in blue) and **13a** (in red) to mTOR.

To better understand the deviation of amino acid residues of each complex relative to the reference position, the RMSFs, to which binding energies, ligand binding poses, and interactions entirely depend on [Bhowmick et al., 2020], have been generated through the MD simulations (**Figures 10** and **11**), and collected and evaluated the information regarding the flexibility and dynamics of the complexes and individual fraction of each complex fluctuating from its mean structure (the ratio of fluctuation in the residual level). From the data, the average RMSF value (in black, **Figure 10**) for the free HDAC1 enzyme is of 0.76 Å. In the case of the HDAC1–ligand **6b** complex, the average RMSF value has been found to be 0.78 Å (in green, **Figures 10**). The result for the HDAC1–ligand **6b** complex can be interpretated in the manner of higher level of fluctuation, at which the residues 19, 78 and 91 in the complex produces higher RMSF values than the free enzyme whereas the case is opposite in the residues 201 and 368 of the free enzyme compared to the complex. Therefore, the complex has shown higher fluctuation pattern than the free ligand supported by the greater RMSF values, which indicate that the residues are located in the loop regions with more conformational flexibility. The RMSF values for the other complexes (0.75 Å and 1.00 Å for HDAC1–ligand **2a** and HDAC1–ligand **13**, respectively) have been obtained (in blue and red, **Figure 10**), and it is clearly evident that the residues 0, 19, 77, 198, and 261 from the HDAC1–ligand **13** complex have very sharp peaks while the free enzyme only has residue 368 exhibiting greater intensity. Thus, this is confirming the higher fluctuation pattern for the complex as well. For the HDAC1–ligand **2a** complex, however, only residue 201 gives sharp peak for the complex while residue 368 does for the free enzyme. In the case of the RMSF values for the complexes of the ligands with the mTOR enzyme, the mTOR–ligand **6b** complex has an average RMSF value of 1.73 Å (in green, **Figure 11**) while the free mTOR enzyme exhibits 1.61 Å (in black, **Figure 11**). Similar results

and predictions can be speculated for the mTOR–ligand **13** (1.82 Å) and mTOR–ligand **13a** complexes (1.84 Å) as well. The results also support that the free mTOR enzyme has shown lower fluctuation pattern than the complexes demonstrating that the restricted movements during the simulation. Thus, the residues located in the loop regions in the complexes have greater RMSF values that are corresponding to higher level fluctuations and more conformational flexibility rather than more constrained dynamics [Pereira et al., 2021].

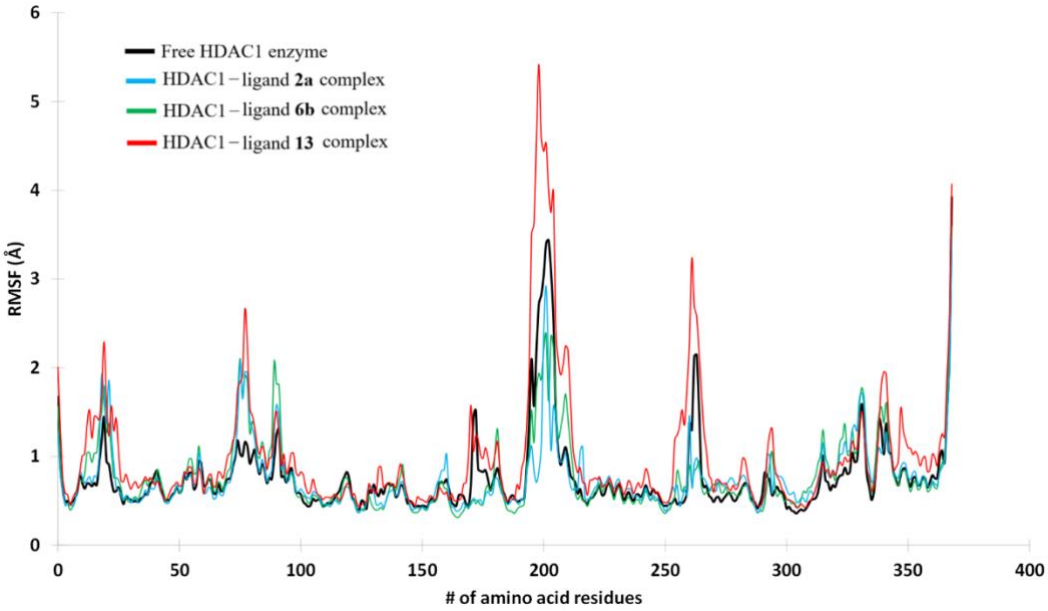


Figure 10. The combined RMSF profiles of both free HDAC1 enzyme (in black) and the complexes formed by binding of the ligands **2a** (in blue), **6b** (in green) and **13** (in red) to HDAC1.

The difference between the values (~0.01–0.02 Å for HDAC1-ligand complexes and ~0.1–0.2 Å for mTOR-ligand complexes) may be neglected because this much differences (a little more stable association in the case of mTOR complexes) may not cause any significant changes on the enzyme structures. However, the results provide clear evidence of small changes in the conformational state of the enzymes, and more importantly, our study is focusing more on the functions of ligands on the enzyme inhibitions rather than the small structural changes.

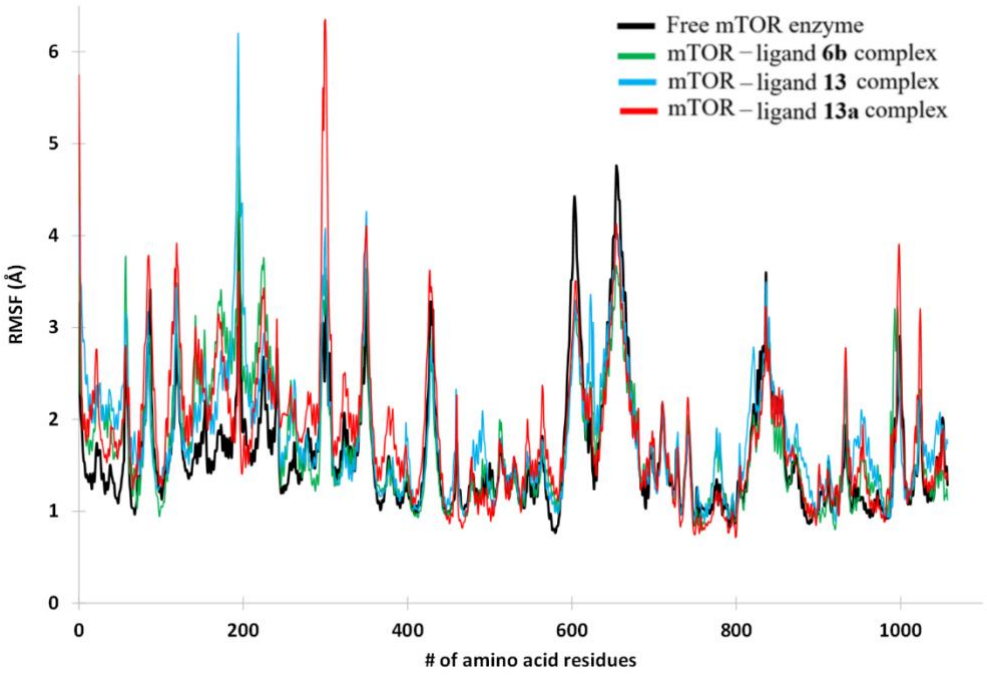


Figure 11. The combined RMSF profiles of both free mTOR enzyme (in black) and the complexes formed by binding of the ligands **6b** (in green), **13** (in blue) and **13a** (in red) to mTOR.

In order to collect more information about overall dimensions, investigate the changes of the complex structures, and further analyze the compactness and rigidity of the enzyme-ligand complexes, the mass-weighted root-mean-square distance of a group of atoms from their typical center of mass (also known as radius of gyration (Rg) parameter [Lobanov et al., 2008]) has been calculated (**Figures 12** and **13**). From such values, consistency of the complex and consistent stability throughout the MD simulations can be predicted (the larger variations from the Rg indicates the inconsistency of the complexes [Bhowmick et al., 2020]). The free enzymes HDAC1 and mTOR have exhibited average Rg values of 1.376 and 1.383 nm (in black, **Figures 12** and **13**), respectively. In the case of HDAC1–ligand **6b** and mTOR–ligand **6b** complexes, the average Rg values are of 1.378 and 1.381 nm, respectively, and such values are slighly lower than the values exhibited by the free enzymes. This means the complexes have very smaller differences (almost negligible) than the free enzymes, referring to a little or almost no conformational changes throughout the MD simulations. A slight decrease in the average Rg values in each case elucidates higher compactnesses of the complexes, thereby suggesting higher stability and lower flexibility.

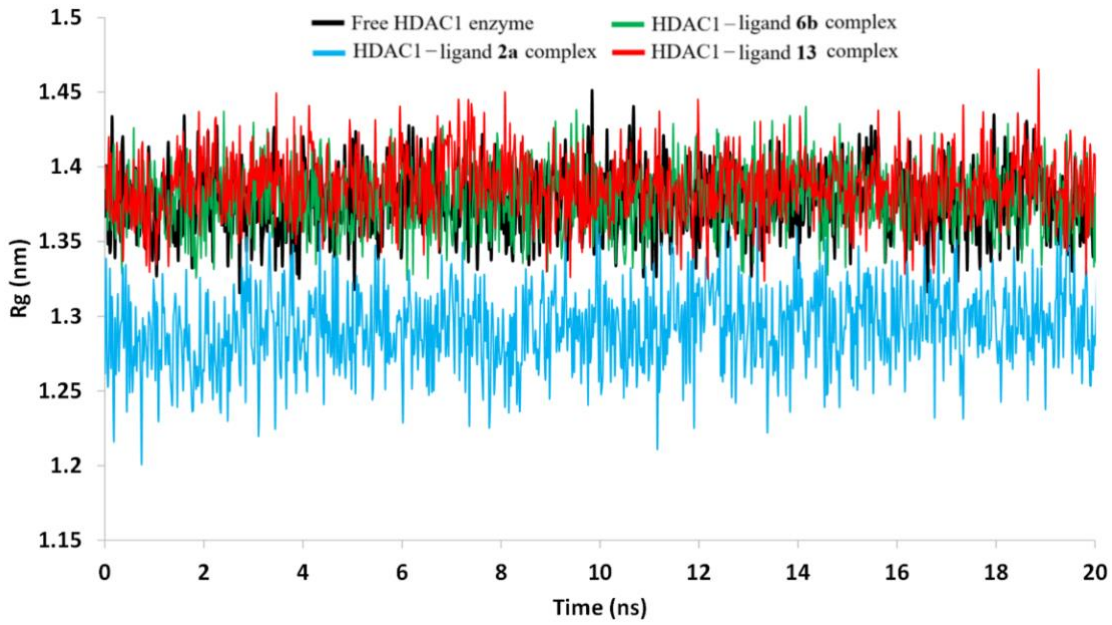


Figure 12. The combined Rg profiles of both free HDAC1 enzyme (in black) and the complexes formed by binding of the ligands **2a** (in green), **6b** (in blue) and **13** (in red) to HDAC1.

For the average Rg values of the HDAC1–ligand **13** (1.389 nm, in red, **Figure 12**) and mTOR–ligand **13** (1.381 nm, in blue, **Figure 13**) complexes, the free HDAC1 enzyme exhibits lower Rg value than its pertinent complex with the ligand **13**, while mTOR–ligand **13** complex has lower average Rg value compared to its free enzyme mTOR. Although the smaller average Rg values denotes the higher compactness and stability with lower flexibility, the differences between the average Rg values of the free enzymes and complexes are still negligible. The HDAC1–ligand **2a** (1.293 nm, in blue, **Figure 12**) and mTOR–ligand **13a** (1.381 nm, in red, **Figure 13**) complexes also have lower average Rg values than their pertinent free enzymes, which suggest the higher compactness and stability with lower flexibility in these complexes as well.

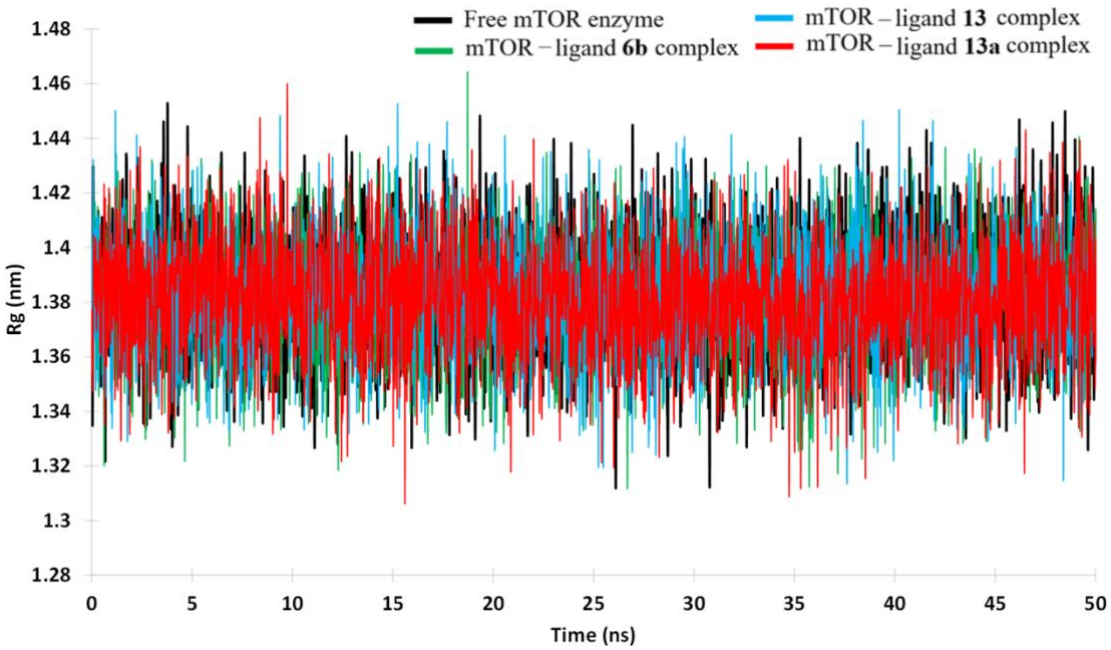


Figure 13. The combined Rg profiles of both free mTOR enzyme (in black) and the complexes formed by binding of the ligands **6b** (in green), **13** (in blue) and **13a** (in red) to mTOR.

Since the chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET, one of the most essential parts of computational drug design for the assessment of pharmacokinetics of a drug and/or an API) play key roles in drug discovery and development, an ADMET study by using a free web tool, SwissADME (with Lipinski's rule of five during the preclinical phase of drug discovery: Molecular Weight (MW) < 500, Number of Hydrogen Bond Donors ≤ 5, Number of Hydrogen Bond Acceptors ≤ 10, Calculated Log p ≤ 5, and Polar Surface Area (PSA) < 140 $Å^2$) [Daina et al., 2017], has been investigated (**Table 5**) for the most effective 20 ligands (**1**, **1a**, **1b**, **2**, **2a**, **2b**, **3**, **3b**, **5**, **5a**, **6b**, **7**, **7a**, **7b**, **8b**, **12b**, **13**, **13a**, **14b** and **23b**). The Lipinski's rule of five states that if a drug and/or an API violates more than two of the abovementioned criteria, it is considered impermeable or badly absorbed [Daina et al., 2017].

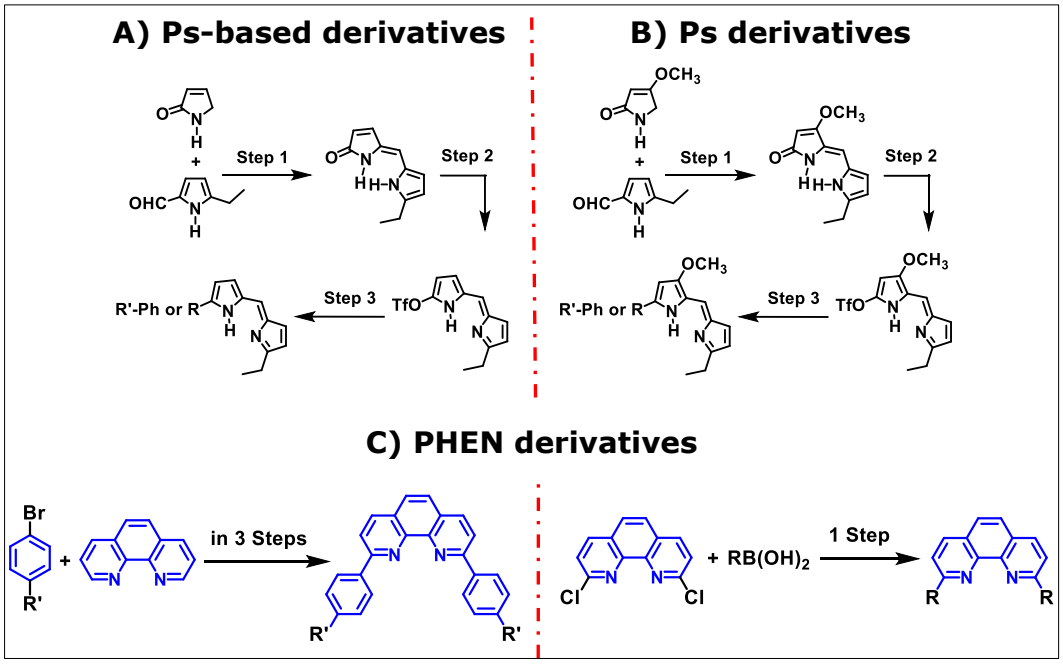
Table 5. The ADMET study for the selected 20 ligands using a free web tool, SwissADME (with Lipinski's rule of five) [Daina et al., 2017].

ADMET ANALYSIS					
Ligand Name	**Molecular Weight (**g/mol**)**	**Log P**	**H Bond Donor**	**H Bond Acceptor**	**Total Polar Surface Area (Å^2)**
1	353.41	1.93	3	5	77.92
1a	383.44	1.87	3	6	87.15
1b	538.55	3.29	4	8	142.90
2	336.38	3.23	2	4	74.68
2a	366.41	3.23	2	5	83.91
2b	508.52	4.37	2	8	118.84
3	364.44	3.98	1	4	63.68
3b	508.52	4.37	2	8	118.84
5	316.32	4.65	1	4	28.15
5a	346.35	4.59	1	5	37.38
6b	468.39	7.17	0	8	25.78
7	293.3.2	3.00	1	3	73.97
7a	323.35	3.01	1	4	83.20
7b	500.39	6.79	0	10	44.27
8b	422.39	4.10	0	6	117.42
12b	584.71	4.11	0	4	72.88
13	437.58	4.51	3	2	55.45
13a	467.61	4.52	3	3	64.68
14b	710.91	6.74	4	4	80.38
23b	558.72	4.28	4	4	80.38

None of our abovelisted ligands violate more than two of the Lipinski's rule of five criteria, which provides a very promising and higher tendency for all ligands to pharmacologically be active (great API potents). Of the ligands, 12 of them (**1**, **1a**, **2**, **2a**, **3**, **5**, **5a**, **7**, **7a**, **8b**, **13** and **13a**) completely meet all the criteria of Lipinski's rule of five. As stated above, the ligands **2a**, **6b** and **13** against the HDAC1 and **6b**, **13** and **13a** against the mTOR enzymes have exhibited very promising binding energies and inhibition constants from the docking processes with supporting MD simulations for the

respective complexes. Except for the ligand **6b** (Log P = 7.17), ligands **2a**, **13** and **13a** also meet all the Lipinski's rule of five criteria.

Considering all the above findings with the supported literature details, synthetic possibility of the ligands have also been investigated and some general synthetic protocols utilizing and/or modifying the literature [Cetin et al., 2022; Dietrich-Buchecker and Sauvage, 1990; Zhong et al., 2010; Kang et al., 2014; Cetin, 2017; Cetin et al., 2017; Cetin et al., 2020; Schmittel et al., 1997; Kohler et al., 2017; Kohler et al., 2016; Hayes et al., 2018; Hayes et al., 2018] have been proposed (**Scheme 1**: **(A)** for the Ps-based derivatives from the **Figures 1A** and **1B**, and **Table 2**; **(B)** for the Ps derivatives from the **Figures 1C** and **1D**, and **Table 3**; and **(C)** for the PHEN-based derivatives from the **Figures 1E** and **1F**, and **Table 4**) in order to further obtain such ligands. Upon synthesis of the each promising ligand, their copper(I) complexes may also be obtained by mixing 2:1 ratio of the relevant ligand and $[Cu(CH_3CN)_4]PF_6$ salt in the 1:1 mixture of dichloromethane and acetonitrile solvents. Such copper(I) complexes will also be further investigated.



Scheme 1. The proposed general synthetic routes for the syntheses of (**A**) Ps-based, (**B**) Ps, and (**C**) PHEN derivatives from either literature or adapted procedures [Cetin et al., 2022; Dietrich-Buchecker and Sauvage, 1990; Zhong et al., 2010; Kang et al., 2014; Cetin, 2017; Cetin et al., 2017; Cetin et al., 2020; Schmittel et al., 1997; Kohler et al., 2017; Kohler et al., 2016; Hayes et al., 2018; Hayes et al., 2018].

4. Conclusions and Recommendations

In conclusion, the SAR and *in silico* modeling of a series of Ps and PHEN derivatives (75 in total) as highly potent new anticancer therapeutic drug/API candidates against mTOR and HDAC enzymes have been investigated. Some of the designed and optimized ligands via *in silico* computational modeling (**1**, **1a**, **1b**, **2**, **2a**, **2b**, **3**, **3b**, **5**, **5a**, **6b**, **7**, **7a**, **7b**, **8b**, **12b**, **13**, **13a**, **14b** and **23b**) have exhibited very promising binding energies and inhibition constants against HDAC1 and/or mTOR enzymes after formation of the relative enzyme-ligand complexes. Such ligands have been found to be highly potent to inhibit either one or both enzymes against brain metastatic breast cancer, preferentially against both for the dual action purposes. Compared to the natural product Ps, such proposed structures have exhibited effectiveness on inhibition of enzymes at nM level with very promising binding energies. While the ligands **6b** and **7b** have been showing very promising dual action against both of the enzymes from the PHEN derivatives, **2a**, **5** and **13** are the effective ones exhibiting dual action from the Ps-based and Ps derivatives. The further MD simulations for the selected ligands (**2a**, **6b** and **13** against HDAC1 and **6b**, **13** and **13a** against mTOR), exhibiting the best results considering the binding energies and inhibition constants, have also been conducted, and the parameters have been calculated from the trajectory of the 50ns-MD simulations, which have provided highly supporting evidence regarding structural conformations, stability of the enzyme backbone upon complex formation, consistent stability throughout the simulation, flexibility/fluctuation of the complexes, changes in complex structure and information about overall dimensions of complexes. All the complexes against both enzymes have exhibited higher RMSD values, referring to the presence of high degree of rotatable bonds or structural flexibilities that cause the ligands to be unable to attain stability inside the binding pocket of the enzymes, which are assumed to be shallow. When RMSF values are compared, the free enzymes have exhibited lower RMSFs than their relevant ligand-enzyme complexes with the selected ligands. Thus, the results have shown that the complexes have higher level of fluctuation patterns than the free enzymes, indicating that the residues are located in the loop regions with more conformational flexibility in the complexes rather than more constrained dynamics. The outcomes from the RMSFs support that the free enzymes demonstrate restricted movements during the simulations as well. According to the calculated average Rg values, very small differences in the compared values for the free enzymes and complexes refer to a little or almost no conformational changes throughout the MD simulations in all cases. Slight decreases in the values have elucidated higher compactnesses of the structures, thereby suggesting higher stability and lower flexibility. The ADMET study using SwissADME (with Lipinski's rule of five) have provided that none of the selected ligands have violated more than two of the Lipinski's rule of five criteria, proving a highly promising tendency for the ligands to be pharmacologically

active. Thus, we have acquired some compounds through *in silico* computational modeling that may provide great therapeutic potential for the breast cancer patients who are at a large brain metastasis risk. Considering highly promising outcomes of this work, further syntheses of some of the selected compounds are still under study in our laboratories.

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Authors' Contributions

MMC created, designed and optimized the 75 ligands, and SBG conducted molecular modeling and computational studies. Both MMC and SBG wrote, reviewed and edited the main text. SBG run the bioactivity evaluation against mTOR and HDAC enzymes via molecular modeling and computational studies, and MMC supervised these studies. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Statement of Conflicts of Interest

The authors declare that they have no known competing financial interest and/or conflict of interest or personal relationships that could appeared to influence the work reported in this paper.

Statement of Research and Publication Ethics

The authors declare that all the rules required to be followed within the scope of "Higher Education Institutions Scientific Research and Publication Ethics Directive" have been complied with in all processes of the article, that The Black Sea Journal of Science and the editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than The Black Sea Journal of Science.

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