

Discovery of A Novel Synthetic Thiazole-benzimidazole Conjugate that Acts as a Potent Pancreatic Lipase Inhibitor using *in silico* and *in vitro* Approaches

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ABSTRACT

Aim/ Background: Pancreatic lipase (PL) inhibition has been suggested to be an effective method for obesity management. In this study, 10 novel thiazole-benzimidazole conjugates were designed. **Materials and Methods:** Conjugates were initially screened via *in silico* experiments, such as ADMET analysis and molecular docking, to identify the most promising PL inhibitors. **Results:** Results revealed that compounds 3, 6 and 8 had the most optimum drug-likeness properties, and the highest binding affinity to PL, with binding energies of -7.7, -7.5 and -8.1 kcal/mol, respectively. Therefore, these promising derivatives were then synthesized and subjected to an *in vitro* PL inhibition assay to validate the results of the *in silico* experiments. The synthetic compounds were fully characterized via FTIR, ¹H-NMR, ¹³C-NMR and LCMS. Results of the enzymatic assay revealed that 3, 6 and 8 inhibited PL potently with high inhibition rates of greater than 80% at the highest tested dose, and demonstrated IC₅₀ values of 68.53, 54.97 and 50.09 μM, respectively. Compound 8 was the most active derivative and displayed comparable activity to orlistat which possessed an IC₅₀ value of 39.18 μM. **Conclusion:** Therefore, we report the discovery of compound 8 as a highly potent PL inhibitor that could act as a lead compound to develop novel anti-obesity agents.

Keywords: Pancreatic lipase, Inhibition, Thiazole-benzimidazole, *in silico*, *in vitro*, Enzyme assay.

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Received: 09-06-2022;

Revised: 13-08-2022;

Accepted: 11-10-2022.

INTRODUCTION

Obesity is one of the most prevalent health concerns globally with 13% of the world's adults being classified as obese in 2016, according to the World Health Organization (WHO). Moreover, more than 4 billion individuals may experience early death as a result of obesity or any of its associated health problems, even though it is a condition that can be prevented.¹ These statistics demonstrate the severe health implications of obesity on the world's population and highlight the need for further research into its prevention and treatment.

Pancreatic lipase (PL) is a key enzyme that hydrolyses triacylglycerides into monoglycerides and free fatty acids that is

either further used for energy production or stored in adipose tissues.² Therefore, PL has been identified as a promising target for regulating lipid absorption, and it is thought that its inhibition would result in the treatment or management of obesity.³⁻⁵ Currently, orlistat is the only PL inhibitory drug that has been approved by the US Food and Drug Administration (FDA) for the treatment of obesity, but it was found to cause serious side-effects that include gastrointestinal problems in addition to liver and kidney damage.⁶⁻⁸ Therefore, more research is needed in order to discover further potent PL inhibitors to be used for the treatment and management of obesity.

Benzimidazole is a chemical moiety that forms an integral part of several clinically approved drugs, such as omeprazole and mebendazole. Moreover, several studies have reported remarkable biological activities for various benzimidazole derivatives and demonstrated their potential use as a potent antioxidant, antimicrobial and anticancer agents.⁹ Benzimidazoles have also been found to exhibit vital PL inhibitory activities.² Thiazole is another heterocyclic chemical moiety that is present in several clinically approved drugs such as bleomycin and ritonavir, while



DOI: 10.5530/001954642131

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several thiazole derivatives have demonstrated potent anticancer, antibacterial, antifungal and anticonvulsant activities.¹⁰ Moreover, it was found that thiazole derivatives inhibited stearoyl-CoA desaturase-1 (SCD1), which is an enzyme involved in obesity.¹¹ However, to the best of our knowledge there were no studies conducted on the effect of thiazole derivatives on PL.

Therefore, due to the high bioactivity demonstrated by both benzimidazole and thiazole derivatives in general, in addition to their potential use as anti-obesity agents as shown by previous studies, we have decided to synthesise thiazole-benzimidazole conjugates as potent inhibitors of pancreatic lipase. We reasoned that fusing both bioactive heterocycles would result in derivatives possessing even higher biological activities due to synergistic effects.

Herein, we designed some novel thiazole-benzimidazole conjugates as potent pancreatic lipase inhibitors that could potentially be developed as anti-obesity drugs. The designed analogs were initially screened *in silico* using the Lipinski rule of five, ADMET analysis, acute toxicity prediction and molecular docking. This *in silico* screening was done to identify the most promising derivatives that could act as potent pancreatic lipase inhibitors. Later on, the promising analogs possessing the most drug-likeness properties were synthesized and characterized, while their biological activity was assayed via an *in vitro* pancreatic lipase enzymatic assay. The study is novel as such derivatives were not investigated before for their pancreatic lipase inhibitory activity and we hope that it would lead to the development of novel anti-obesity drugs.

MATERIALS AND METHODS

General

2-amino-6-chlorobenzimidazole, thiazol-2-amine, ethanol, potassium carbonate (K_2CO_3), *N,N*-dimethylformamide (DMF) and different aromatic aldehydes were obtained from Lab Trading Laboratory, Aurangabad, Maharashtra, India. The pancreatic lipase from porcine (Type-II, Enzyme Commission number: 3.1.1.3, CAS Number: 9001-62-1), *p*-nitrophenyl butyrate (pNPB), and orlistat were purchased from Sigma-Aldrich. Melting point measurements were conducted using the open capillary technique. IR spectra were obtained using an FTIR-4100 (JASCO, Germany) spectrometer, while ¹H NMR at 400 MHz and ¹³C NMR at 100 MHz spectra were obtained *via* JNM-ECZ400S/L1 (JEOL) using tetramethyl silane (TMS) as an internal standard. The chemical shift values (δ) have been reported in parts per million (ppm) relative to TMS. Mass spectra were obtained using mass spectrometer with Single-quadruple detector (SQD-2) (Waters). FTIR, ¹H NMR and ¹³C NMR analyses were performed by Sapala Organics Pvt. Ltd., Hyderabad, Telangana, India.

In silico Studies

The Lipinski rule of five and ADMET properties of the designed analogs was investigated using PubChem,¹² Molinspiration software and SwissADME.¹³ The compounds' toxicity was predicted *via* ProTox-II (http://tox.charite.de/prottox_II).

Molecular Docking was performed using Autodock vina 1.1.2 with PyRx Virtual Screening Tool 0.8 software of Chimera version 1.10.2,¹⁴ in addition to Biovia Discovery studio. The structures of the designed derivatives and orlistat were drawn using ChemDraw Ultra 8.0. Energy minimisation was performed using Universal Force Field (UFF) in PyRx software. The crystal structure of the pancreatic lipase-colipase complex (PDB ID: 1N8S) was obtained from the RCSB Protein Data Bank (<https://www.rcsb.org/>). It was observed that there were two chains i.e. chain A and chain C. Chain A was chosen to be used in the molecular docking investigations. With an exhaustiveness value of 8, the three-dimensional grid box (size_x = 54.2047 Å, size_y = 47.6575 Å, size_z = 81.6381 Å) was modified for molecular docking simulations. The full molecular docking approach was carried out in accordance with the methods outlined in previous studies.¹⁵⁻¹⁸

RESULTS AND DISCUSSION

Results

In silico Drug-likeness and ADMET Properties of the Designed Analogs

We have designed several novel thiazole-benzimidazole conjugates to act as PL inhibitors, which could potentially be further developed into effective anti-obesity drugs as shown in Figure 1 and Scheme 1. However, we decided to screen these designed analogs *in silico* in order to determine the most promising derivatives that would later be synthesized and tested against PL *in vitro*.

Drug-likeness and pharmacokinetic (ADMET) properties are crucial elements in any drug development process. Therefore, in this study, Lipinski's rule of five,¹⁹ and Veber's rules,²⁰ were used to predict if the designed analogs would possess oral bioavailability

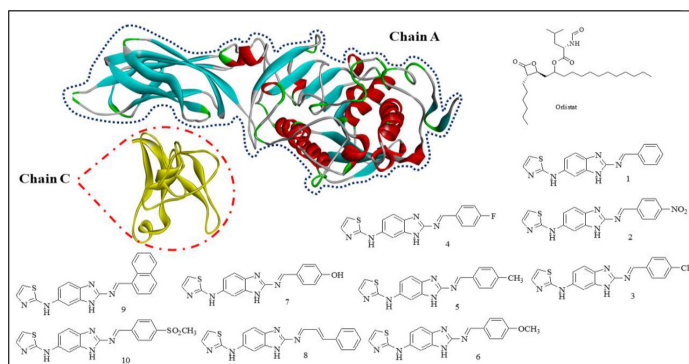


Figure 1: The structures of all the derivatives, orlistat and 3D ribbon view of PL with chain A and chain C.

Table 1: Calculation of Lipinski's rule of five and Veber's rule for orlistat and the designed derivatives.

Compound	Lipinski's rule					Veber's rule		
	Log P	Mol. Wt.	HBA	HBD	Violations	Total polar surface area (Å ²)	No. of rotatable bonds	Violations
Orlistat	7.09	495.73	05	01	01	81.70	24	01
1	3.64	319.38	03	02	00	94.20	04	00
2	1.96	365.39	05	03	00	143.86	05	01
3	4.18	353.83	03	02	00	94.20	04	00
4	3.96	337.37	04	02	00	94.20	04	00
5	3.99	333.41	03	02	00	94.20	04	00
6	3.65	349.41	04	02	00	103.43	05	00
7	3.22	335.38	04	03	00	114.43	04	00
8	4.13	345.42	03	02	00	94.20	05	00
9	4.53	369.44	03	02	00	94.20	04	00
10	3.31	397.47	05	02	00	136.72	05	00

Mol. Wt.: Molecular weight; HBA: Hydrogen bond acceptors; HBD: Hydrogen bond donors.

Table 2: The pharmacokinetics and drug-likeness properties of the designed compounds.

Parameters Orlistat		Compounds											
		1	2	3	4	5	6	7	8	9	10		
Pharmacokinetics	GI absorption	Low	High	Low	High	High	High	High	High	High	High	High	Low
	BBB permeation	No	No	No	No	No	No	No	No	No	No	No	No
	P-gp substrate	Yes	No	Yes	No	No	No	No	No	No	No	Yes	No
Drug-likeness	Ghose	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
	Egan	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
	Muegge	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
	Bioavailability Score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55

as shown in Table 1. Lipinski's rule states that a good drug candidate would be expected to possess a molecular weight fewer than 500 Daltons, a Log P value fewer than 5, no more than 5 hydrogen bond donors (HBD) and 10 hydrogen bond acceptors (HBA). On the other hand, Veber's rule states that good oral bioavailability for a drug may be achieved if it possesses 10 or fewer rotatable bonds (RTB) and a polar surface area (TPSA) that does not exceed 140 Å².²¹

It was found that the derivatives demonstrated drug-likeness properties and mostly obeyed Lipinski's and Veber's rules. The analogs possessed even more drug-likeness properties than the approved drug orlistat, which displayed a violation of Lipinski's rule as it exhibited a Log P value of 7.09 and another violation of Veber's rule due to its possession of a large number of rotatable bonds. Compound 2 was the only designed analog that was

predicted to have less than ideal drug-likeness properties as it exhibited a large TPSA which violated Veber's rule.

Further, *in silico* pharmacokinetics and drug-likeness studies on the designed compounds were summarized in Table 2. The results showed that the designed compounds lacked BBB penetration which is extremely desirable. Moreover, all of them displayed optimum bioavailability scores and also showed high GI absorption except for compounds 2 and 10. It is also crucial to note that only compounds 1, 3, 4, 5, 6, 7 and 8 passed the Ghose, Egan, and Muegge filters but orlistat violated these filters and even exhibited low GI absorption.

The oral bioavailability of the designed compounds was also investigated *in silico*, and the resulting properties were summarized on a bioavailability radar chart as shown in Figure 2. The radar chart contained six physicochemical parameters;

Table 3: The predicted acute toxicity of the designed molecules.

Parameters	Compounds										
	Orlistat	1	2	3	4	5	6	7	8	9	10
LD ₅₀ (mg/kg)	1300	98	670	670	98	98	430	98	670	98	200
Toxicity class	4	3	4	4	3	3	4	3	4	3	3
Prediction accuracy (%)	68.07	54.26	54.26	54.26	54.26	54.26	54.26	54.26	54.26	54.26	54.26
Hepatotoxicity (Probability)	A (0.70)	A (0.68)	A (0.62)	A (0.65)	A (0.69)	A (0.65)	A (0.64)	A (0.65)	A (0.67)	A (0.68)	I (0.52)
Carcinogenicity (Probability)	I (0.55)	I (0.64)	I (0.53)	I (0.61)	I (0.63)	I (0.64)	I (0.53)	I (0.53)	I (0.63)	I (0.64)	A (0.53)
Immunotoxicity (Probability)	I (0.68)	I (0.97)	I (0.96)	I (0.86)	I (0.77)	I (0.97)	I (0.62)	I (0.92)	I (0.82)	I (0.88)	I (0.77)
Mutagenicity (Probability)	I (0.81)	A (0.52)	A (0.66)	I (0.55)	I (0.51)	A (0.57)	A (0.71)	A (0.73)	A (0.53)	A (0.52)	I (0.59)
Cytotoxicity (Probability)	I (0.65)	I (0.75)	I (0.70)	I (0.78)	I (0.77)	I (0.75)	I (0.64)	I (0.62)	I (0.71)	I (0.75)	I (0.80)

Where: I, Inactive; A, Active.

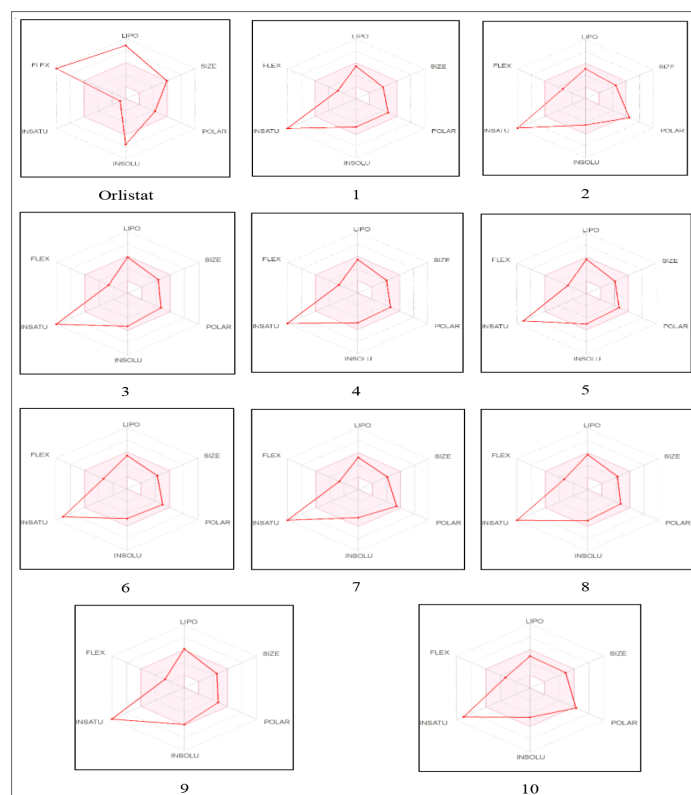


Figure 2: Bioavailability radar charts in which the pink region corresponds to the area that is most appropriate for oral bioavailability, while the solid red line refers to the properties of the designed compounds.

LIPO (Lipophilicity): $-0.7 < \text{XLOGP3} < +5.0$, SIZE: $150 \text{g/mol} < \text{MW} < 500 \text{g/mol}$, POLAR (Polarity): $20 \text{ \AA}^2 < \text{TPSA} < 130 \text{ \AA}^2$, INSOLU (Solubility): $0 < \text{LogS} < 6$, INSATU (Saturation): $0.25 < \text{Fraction of sp}^3 \text{ hybridized carbons} < 1$, FLEX (Flexibility): $0 < \text{Num. of rotatable bonds} < 9$.

Lipophilicity, Size, Polarity, Solubility, Saturation and Flexibility. It was found that the designed molecules generally displayed properties that indicated high oral bioavailability and they only violated one parameter in this regard, which was saturation. On the other hand, orlistat was found to violate three parameters, which further reflects the potential of our designed analogs as anti-obesity drugs.

We have finally decided to assess the toxicity of our designed analogs, and the results have been summarized in Table 3. It was found that all designed molecules fell under toxicity class III ($50 < \text{LD}_{50} \leq 300$) and IV ($300 < \text{LD}_{50} \leq 2000$) with a prediction accuracy of 54.26%. Toxicity class-III indicates that molecules are toxic if swallowed, while class-IV indicates that they are harmful if swallowed. Compounds 1, 4, 5, 7, 9 and 10 fell under toxicity class-III, while compounds 2, 3, 6 and 8 fell under toxicity class-IV. It can be also seen that generally several molecules exhibited hepatotoxicity, carcinogenicity, immune-toxicity, mutagenicity, and cytotoxicity, but with a low probability score indicating that such toxicity might not even occur *in vivo*.

Molecular Docking Studies on the Designed Derivatives

We next conducted molecular docking studies on compounds 3, 6 and 8 to investigate their binding interactions with pancreatic lipase and obtain further information on which designed analogs should proceed to *in vitro* PL activity assay. As stated earlier, compounds 3, 6 and 8 were chosen for further testing as they showed the most optimum *in silico* drug-likeness and ADMET properties.

Results of the molecular docking studies have been summarized in Table 4 whereby the amino acid residues that are predicted to interact with the compounds along with their corresponding bond length, type and category were shown. Molecular interactions and docking poses have been illustrated in Figure 3. Results revealed that compounds 3, 6 and 8 have all exhibited more potent interactions with the target than orlistat with binding affinities of -7.7 kcal/mol , -7.5 kcal/mol and -8.1 kcal/mol , respectively, relative to -4.8 kcal/mol for orlistat as shown in Table 4. The lower binding affinity of orlistat relative to our designed compounds might be due to the lack of conventional hydrogen bonds with the target. On the other hand, compounds 3 and 6 formed conventional hydrogen bonds with ASP328, ASN229 and THR329, while compound 8 interacted with ASP391 and LYS428 *via* conventional hydrogen bonds. It has been also observed that the thiazole group present in our designed analogs

Table 4: Molecular docking results for compounds 3, 6 and 8.

Compounds	Amino acid residue	Bond length (Å)	Bond type	Bond category	Ligand energy (kcal/mol)	Binding affinity (kcal/mol)
3	ASP328	4.98921	Electrostatic	Attractive charge	605.02	-7.7
	ASN229	2.40146	Hydrogen bond	Conventional hydrogen bond		
	ASN229	2.67481				
	ASP328	2.73307				
	THR329	2.34756				
	ASP387	3.44914				
	ARG337	3.55141	Electrostatic	Pi-cation		
	GLU233	4.18373		Pi-anion		
	VAL232	4.28546	Hydrophobic	Alkyl		
	PRO235	4.06198		Pi-alkyl		
	PRO235	3.76975				
6	ASP328	4.93546	Electrostatic	Attractive charge	618.55	-7.5
	ASN229	2.48047	Hydrogen bond	Conventional hydrogen bond		
	ASN229	2.67064				
	ASP328	2.66453				
	THR329	2.3656				
	ASP387	3.41139				
	ARG337	3.52391	Electrostatic	Pi-cation		
	GLU233	4.147		Pi-anion		
	TYR288	5.38844	Hydrophobic	Pi-Pi T-shaped		
	PRO235	4.00192		Pi-alkyl		
	PRO235	3.73814				
8	ASP391	2.10417	Hydrogen bond	Conventional hydrogen bond	628.77	-8.1
	ASP391	2.64973				
	LYS428	3.04895				
	ASP278	4.96885	Electrostatic	Pi-anion		
	GLY393; ASP394	4.1615	Hydrophobic	Amide-Pi stacked		
	VAL426	5.38107		Pi-alkyl		
	VAL426	5.2332				
ARG313	5.36633					
Orlistat	ASP79	2.57799	Electrostatic	Salt bridge; attractive charge	669.66	-4.8
	TRP252	3.23741	Hydrogen bond	Carbon hydrogen bond		
	LYS107	4.31826	Hydrophobic	Alkyl		
	LYS80	4.00092				

seem to be the main source of these hydrogen bonds that formed between our compounds and the target, which might indicate its importance for biological activity.

Therefore, molecular docking studies revealed that compounds 3, 6 and 8 are expected to bind with pancreatic lipase with high affinity and this encouraged us to further test these compounds for their pancreatic lipase inhibitory activity *in vitro*. The

compounds were initially synthesized and then tested for their *in vitro* PL inhibitory activity.

***In vitro* Pancreatic Lipase Inhibitory Activity of Compounds 3, 6 and 8**

Compounds 3, 6 and 8 were tested for their PL inhibitory activity at different concentrations (1 µg/mL – 100 µg/mL) via an *in vitro* enzymatic assay. Results of the assay were summarized in Figure 4

involved initially condensing 2-amino-6-chlorobenzimidazole and thiazol-2-amine under the presence of K_2CO_3 which acted as a base. The resulting thiazole-benzimidazole conjugates were then treated with different substituted/unsubstituted aromatic aldehydes in a Schiff base condensation reaction in the presence of an acid catalyst to form the final derivatives.

Synthetic compounds 3, 6 and 8 were extensively characterized *via* IR, NMR and LCMS. IR spectra of the compounds have generally shown peaks at around 3300 cm^{-1} corresponding to NH stretch, while the peaks at around 1660 cm^{-1} were attributed to the imine (C=N) groups of the compounds. There were also peaks at around 2900 cm^{-1} , 1460 cm^{-1} , 1028 cm^{-1} and 750 cm^{-1} corresponding to aromatic C-H and C-C vibrations. However, the presence of several overlapping peaks corresponding to different chemical groups made it slightly challenging to fully rely on IR for characterizing the synthetic compounds, so further analysis *via* NMR and LCMS was required.

1H NMR spectroscopy confirmed the formation of the synthetic compounds as intended. The 1H NMR spectrum of the intermediate compound, *N*6-(thiazol-2-yl)-1*H*-benzo[*d*]imidazole-2,6-diamine, showed characteristic peaks between 7.3 ppm and 6.6 ppm referring to protons of the thiazole and benzene rings, while the primary amine of the benzimidazole ring was assigned to the singlet peak at 6.562 ppm. However, compounds 3, 6 and 8 1H NMR spectra showed the disappearance of the primary amine proton peak at around 6.5 ppm, and the appearance of a peak at around 7-8 ppm instead, referring to the imine protons (CH=N) which further proved the formation of the intended compounds. Further characteristic peaks such as the singlet for the methoxy protons and doublets of the methylene protons further proved the formation of compounds 6 and 8, respectively. It is also crucial to note that the synthetic compounds' NMR spectra showed that several peaks were duplicated and that is due to a phenomenon known as tautomerism as shown in Figure 5. Tautomerism was also reported previously in other structures containing the benzimidazole moiety, and was

regarded as the main reason behind the appearance of duplicated peaks in the NMR spectra.²²

^{13}C NMR was also used to characterize the compounds and the resulting spectra further proved the formation of the intended synthetic analogs. The spectra showed peaks at around 160 ppm corresponding to the imine carbon atoms of the thiazole and benzimidazole moieties, while the peaks at around 139 ppm and 108 ppm were assigned to the remaining carbon atoms of the thiazole group. The benzene carbon atoms resulted in peaks that ranged between 139 ppm and 90 ppm. There were also characteristic peaks unique to each of the synthetic analogs, such as the peak at around 140 ppm corresponding to the newly formed imine carbon atoms of the analogs and the extra benzene carbon peaks that have been seen in all analogs. Moreover, compound 6 showed a peak at around 55 ppm corresponding to the carbon of the methoxy group, while 8 showed peaks at 135 ppm and 119 ppm corresponding to the compound's (C=C) group.

Finally, LCMS was used to characterize the compounds and the spectra revealed molecular ion peaks with masses that are almost identical to the calculated molecular weight of the synthetic compounds. Therefore, all these different analytical techniques have proven the formation of the intended synthetic compounds and confirmed their structures.

***In vitro* PL Inhibitory Activity of Compounds 3, 6 and 8**

The *in vitro* PL assay of compounds 3, 6 and 8 revealed that they potently inhibited PL in a dose-dependent manner and resulted in more than 80% enzyme inhibition at the highest concentration tested, which reflects their good bioactivity. Compound 8 exhibited the highest inhibitory activity towards PL relative to the other synthetic compounds as can be deduced from its IC_{50} , and it is crucial to note that 8 demonstrated inhibitory activity comparable to that of orlistat which possessed an IC_{50} value of $39.18\text{ }\mu\text{M}$, indicating the compound's potential as a potent anti-obesity drug. Moreover, the *in vitro* enzymatic assay's results are in line with the data obtained from the molecular docking study that was conducted, whereby compound 8 possessed the highest binding affinity to PL and the highest *in vitro* PL inhibitory activity relative to the other synthetic derivatives. This interesting agreement between the *in silico* and *in vitro* assays provides further validation for our ADMET prediction computational studies and molecular docking protocol. Therefore, in the present study, *in silico* and *in vitro* assays were utilised to finally discover compound 8 as a highly potent PL inhibitor that demonstrated comparable activity to orlistat and has great potential to be further developed as a successful anti-obesity drug.

Synthesis

***N*6-(thiazol-2-yl)-1*H*-benzo[*d*]imidazole-2,6-diamine**

A mixture of 2-amino-6-chlorobenzimidazole (3mmol), thiazol-2-amine (3mmol), and K_2CO_3 (3mmol) in 20 mL ethanol and

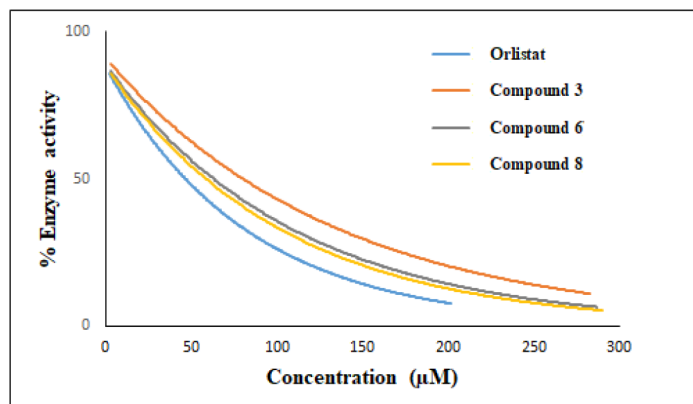


Figure 5: Percentage of enzyme activity vs concentration curves of compounds 3, 6 and 8 in comparison with Orlistat.

5 mL DMF was refluxed. After the reaction was confirmed to have been completed *via* TLC, the surplus solvent was evaporated and the solution was put onto ice. The solution's pH was then corrected from 6 to 8. The resulting solid was obtained *via* vacuum filtration and purified using column chromatography with a solvent system of EtOAc/Petroleum ether at a ratio of 40:60.

Yield: 81%; color: pale yellow; melting point: 189-191°C; R_f value: 0.61; FT-IR (neat, cm⁻¹) ν_{max}: 3328.5 (-NH stretch), 3172 (Ar-NH stretching), 2981.9 (C-H aromatic), 1666 (C=N), 1572.9 (N-H bending), 1464.8 (C-C aromatic), 1028, 756.6 (C-H aromatic); ¹H NMR (400 MHz, CDCl₃, δ (ppm)): 7.354 (d, thiazole C-H), 7.172 (d, thiazole C-H), 6.895-6.645 (m, Ar-C-H), 6.562 (s, NH₂), 4.763 (s, N-H of thiazole), 4.001 (s, N-H of benzimidazole); ¹³C NMR (100 MHz, CDCl₃, δ (ppm)): 159.739 (thiazole N=C-S), 156.921 (benzimidazole N-C=N), 139.802 (thiazole S-C=C-N), 139.478, 138.102, 128.539, 118.173, 116.092, 103.839 (Benzene ring carbon atoms), 108.672 (thiazole S-C=C-N), 89.371, 80.985, 65.782, 24.209. LCMS calculated for [M⁺ - H]: 230.27, found: 230.02.

General method for the synthesis of compounds 3, 6 and 8

3 mmol of an aromatic aldehyde was added to N6-(thiazol-2-yl)-1H-benzo[d]imidazole-2,6-diamine (3mmol) in 20 mL of ethanol, and the mixture was stirred. TLC was used to monitor the completion of the reaction. The resulting crude product was separated, filtered, dried, and recrystallized from the ethanol. The different aromatic aldehydes used were 4-chlorobenzaldehyde for compound 3, 4-methoxybenzaldehyde for compound 6 and cinnamaldehyde for compound 8.

N-(2-((4-chlorobenzylidene)amino)-1H-benzo[d]imidazol-6-yl)thiazol-2-amine (3)

Yield: 68%; color: off white; melting point: 208-210°C; R_f value: 0.63; FT-IR (neat, cm⁻¹) ν_{max}: 3328.5 (-NH stretch), 3172 (Ar-NH stretching), 2929.7 (C-H aromatic), 1673.6 (C=N), 1453.7 (C-C aromatic), 1028.7, 749.2 (C-H aromatic); ¹H NMR (400 MHz, CDCl₃, δ (ppm)): δ 7.854 (d, thiazole C-H), 7.787 (s, CH=N), 7.429 (d, thiazole C-H), 7.241-6.714 (m, Ar-C-H), 4.518 (s, N-H of thiazole), 3.939 (s, N-H of benzimidazole). ¹³C NMR (100 MHz, CDCl₃, δ (ppm)): 160.089 (thiazole N=C-S), 159.769 (benzimidazole N-C=N), 141.109 (Ar-C=N), 139.820 (thiazole S-C=C-N), 138.109, 136.009, 131.571, 129.739, 128.992, 128.087, 127.983, 118.192, 116.327, 103.998, (Benzene ring carbon atoms), 108.182 (thiazole S-C=C-N), 71.821, 53.671. LCMS calculated for [M⁺ - H]: 352.82, found: 352.89

N-(2-((4-methoxybenzylidene)amino)-1H-benzo[d]imidazol-6-yl)thiazol-2-amine (6)

Yield: 73%; color: straw color; melting point: 223-225°C; R_f value: 0.72. FT-IR (neat, cm⁻¹) ν_{max}: 3328.5 (-NH stretch), 3168.2 (Ar-

NH stretching), 2933.4 (C-H aromatic), 1666.1 (C=N), 1464.8 (C-C aromatic), 1282.7 (O-CH₃), 1028.7, 756.6 (C-H aromatic). ¹H NMR (400 MHz, CDCl₃, δ (ppm)): 8.395 (s, CH=N), 7.800 (d, thiazole C-H), 7.600 (d, thiazole C-H), 7.478-7.153 (m, Ar-C-H), 4.098 (s, N-H of thiazole), 2.892 (s, N-H of benzimidazole), 2.424 (s, OCH₃). ¹³C NMR (100 MHz, CDCl₃, δ (ppm)): 163.892 (C-OCH₃), 161.002 (thiazole N=C-S), 159.529 (benzimidazole N-C=N), 139.951 (Ar-C=N), 138.271 (thiazole S-C=C-N), 130.003, 128.102, 126.021, 119.193, 118.362, 114.216, 104.193, 90.972 (Benzene ring carbon atoms), 108.328 (thiazole S-C=C-N), 74.183, 54.829 (C-OCH₃), 29.471. LCMS calculated for [M⁺ + H]: 350.42, found: 350.69

N-(2-((1,2,3-phenylallylidene)amino)-1H-benzo[d]imidazol-6-yl)thiazol-2-amine (8)

Yield: 67%; color: semisolid light yellow; melting point: 178-180°C; R_f value: 0.66. FT-IR (neat, cm⁻¹) ν_{max}: 3324.8 (-NH stretch), 3164.5 (Ar-NH stretching), 2978.1 (C-H aromatic), 1673.6 (C=N), 1453.7 (C-C aromatic), 1084.7, 767.8 (C-H aromatic). ¹H NMR (400 MHz, CDCl₃, δ (ppm)): 9.251 (d, Ar-CH=CH-CH), 8.107 (d, CH=N), 8.002 (dd, Ar-CH=CH-CH), 7.927 (d, thiazole C-H), 7.705 (d, thiazole C-H), 7.705-7.262 (m, Ar-C-H), 5.412 (s, N-H of thiazole), 4.082 (s, N-H of benzimidazole). ¹³C NMR (100 MHz, CDCl₃, δ (ppm)): 163.930 (thiazole N=C-S), 159.201 (benzimidazole N-C=N), 140.019 (C=C-C=N), 138.621 (thiazole S-C=C-N), 135.528 (Ar-C=C-C), 130.172, 128.003, 125.990, 118.693, 116.089, 114.007, 104.97, 64.481 (Benzene ring carbon atoms), 119.309 (Ar-C=C-C), 108.109 (thiazole S-C=C-N), 41.998. LCMS calculated for [M⁺]: 345.42, found: 345.23.

In vitro PL inhibition assay

The assay was performed by MUPs Institute of Pharmacy, Degaonrisod, India. The lipase was prepared by dissolving the crude PPL in 50 mM phosphate buffer (1 mg/ml) and centrifuging for 5 min at 12 000 × g. An enzyme stock concentration of about 0.1 mg/ml of enzyme stock was obtained after dissolving 1 mg of solid PPL powder in 1 ml of buffer. The inhibitory capacity of the compounds was evaluated using a modified version of the Lewis method. Lipase activity was quantified by measuring the hydrolysis of *p*NPB to *p*-nitrophenol at 405 nm using *p*NPB as the substrate. To perform lipase tests, the test compounds at concentrations 1, 5, 10, 25, 50 and 100 µg/mL were incubated in reaction buffer for 10 min with PPL and *p*NPB (50mM potassium phosphate buffer at pH 7.2, 0.5 percent Triton X-100). *p*NPB was solubilised in 1% DMSO prior to being diluted with the reaction buffer in order to achieve a final concentration of 2.5 mM.

Experiments were carried out at 37°C, with results provided as the average of three replicates. Orlistat acted as a positive control in this study, while DMSO was employed as a negative control. At 37°C, one unit of activity was defined as the rate at which 1 µmol of *p*-nitrophenol is produced per minute of reaction time.

When PPL was incubated with the test chemicals, the amount of lipase activity decreased by a percentage, which was represented as a percentage drop in activity. The percentage of lipase inhibition was estimated using the following formula:

$$\text{Lipase inhibition (I\%)} = 100 - \left[\frac{B - b}{A - a} \right] \times 100$$

Where, “A” represents the activity without an inhibitor, “a” represents the negative control without an inhibitor, “B” represents the activity with an inhibitor, and “b” represents the negative control with an inhibitor.

CONCLUSION

The present study aimed to utilize the power of *in silico* and *in vitro* techniques in order to finally discover a novel potent inhibitor of PL in the most elegant and cost-effective manner. A number of novel thiazole-benzimidazole conjugates were designed initially and were screened *in silico* for their ADMET and drug-likeness properties, in addition to their binding affinities to PL. Results of the *in silico* assays showed that compounds 3, 6 and 8 were the most promising PL inhibitors and so these derivatives were further investigated for their *in vitro* PL inhibitory activity. The *in vitro* enzymatic assay validated the *in silico* assays' results and successfully identified compound 8 as a potent PL inhibitor that possessed comparable activity to the approved drug orlistat. The uniqueness of this study lies in its use of *in silico* studies to determine the compounds with the highest probability of success and thus reduce cost, time and effort. Moreover, our study showed that results of the *in vitro* assay was in line with that of the *in silico* assays which further indicates the validity of our approach and the importance of *in silico* techniques in reducing time and effort. Therefore, this study managed to discover a novel potent PL inhibitor, compound 8, and showed the importance of utilizing both *in silico* and *in vitro* techniques in drug discovery to save time, effort and resources.

ACKNOWLEDGEMENT

This research has been funded by Scientific Research Deanship at University of Hail, Saudi Arabia through Project number RG-21 173.

Financial and Funding source

Scientific Research Deanship at University of Hail, Saudi Arabia through Project number RG-21 173.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ADMET: Chemical absorption, distribution, metabolism, excretion, and toxicity; **FT-IR:** Fourier transform infrared; **H-NMR:** Proton Nuclear Magnetic Resonance; **¹³C-NMR:** Carbon Nuclear Magnetic Resonance; **LCMS:** Liquid Chromatography Mass Spectrometry; **PL:** Pancreatic Lipase; **HBD:** Hydrogen Bond Donors; **HBA:** Hydrogen Bond Acceptors; **RTB:** Rotatable bonds; **TPSA:** Total Polar Surface Area; **BBB:** Blood Brain Barrier; **TLC:** Thin-Layer Chromatography; **DMF:** Dimethyl Formamide.

SUMMARY

In this study, 10 novel thiazole-benzimidazole conjugates were designed and screened *via in silico* experiments, such as ADMET analysis and molecular docking, to identify the most promising PL inhibitor properties. It was conducted that compounds 3, 6 and 8 had the most optimum drug-likeness properties, and the highest binding affinity to PL, therefore, these promising derivatives were then synthesized and subjected to an *in vitro* PL inhibition assay to validate the results of the *in silico* experiments. The synthetic compounds were fully characterized *via* FTIR, ¹H-NMR, ¹³C-NMR and LCMS. Results of the enzymatic assay revealed that 3, 6 and 8 inhibited PL potently with high inhibition rates of greater than 80% at the highest tested dose. Compound 8 was the most active derivative and displayed comparable activity to Orlistat.

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Cite this article: Break MKB, Younes KM, Huwaimel B, Abouzied AS. Discovery of A Novel Synthetic Thiazole-benzimidazole Conjugate that Acts as A Potent Pancreatic Lipase Inhibitor using *in silico* and *in vitro* Approaches. *Ind. J. Pharm. Edu. Res.* 2023;57(1):218-27.