

Design, Synthesis and Biological Evaluation of some Chalcone Derivatives as Potential Pancreatic Lipase Inhibitors

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ABSTRACT

Obesity is a growing global health problem, but few drugs are available for the treatment of obesity. Several classes of compounds have been studied and demonstrated for the human lipase inhibition activity - a target in obesity prevention. This study was about design, synthesis and biological evaluation of some synthetic chalcones as pancreatic lipase inhibitors.

FlexX software integrated in LeadIT was used for molecular docking studies of 66 chalcone derivatives. 6 derivatives with low docking scores (good binding affinity) were selected for synthesis using both classical and microwave-assisted Claisen-Schmidt condensation reactions.

Biological evaluation on pancreatic lipase indicated that some chalcones showed good lipase inhibition activities and that there were correlations between *in silico* model and biological activities. These presented the possibility to apply virtual screening tools in finding potential agents with high obesity-prevention capacity.

Keywords: lipase inhibitory activity, chalcone,

INTRODUCTION

Obesity has been cited as a major social issue, both in developed and developing countries. According to statistics by WHO, the number of overweight adults reached 1.4 billion in 2008. Of these 500 million people was identified as clinical obese and the rate has nearly doubled since 1980. Obesity would be no cause for patients' concern if it was not the reason behind the surging risks of dyslipidemia, diabetes mellitus type 2, coronary artery disease, stroke, as well as musculoskeletal and airway disorders, prostate cancer, etc [5]. There is a huge potential market for anti-obesity drugs [4], which contributed to roughly 2-6% healthcare expenditure in many developed nations and it was estimated to reach \$3,7 billion in 2008. There is, however, quite a few active substances approved by FDA for long-term obesity treatment like orlistat (pancreatic lipase inhibitor), lorcaserin (serotonin 2C receptor activator) alongside with short-term drugs like phentermine, zonisamide [5], [9].

Drugs are used for patients with BMI over 30 or over 27 in case they suffer from some accompanying diseases such as hypertension, insulin resistance [20]. Many indications was used to be adopted for many years. Nevertheless, almost the anti-obesity drugs allowed on the market has been withdrawn for their adverse effects. Numerous drugs today (with new indications or combination) are under study, of which lorcaserin (Belviq[®]) is the first substance within 13 years to be approved by FDA on June 27 this year [18]; and phentermine/topiramate combination (Qsymia[®]) was adopted by FDA for the long-term obesity treatment on July, 17, 2012 [19].

Orlistat (prescription branded-name Xenical[®], Roche and OTC low-dose Alli[®], GSK) is the long-term anti-obesity drug approved by FDA in the US (1999). Orlistat is not misused because of its inability to absorption (<1%) and it is also safe for central nervous system. As a

pancreatic lipase inhibitor, orsilat is responsible for lowering fat absorption by more than 30% and is not associated with trypsin, chymotrypsin and phospholipase. Orsilat also inhibits gastric lipase, carboxyesterase and phospholipase A2 [1].

Pancreatic lipase is a common target for anti-obesity drug research. Pancreatic lipase (triacylglycerol acyl hydrolase) plays an essential role in the digestion of triacylglycerols. Excreted in the duodenum, pancreatic lipase hydrolyzes 50-70% fat (at a maximum rate of 140 grams per minute) in the presence of colipase and Ca^{2+} . Several classes of substances exhibit pancreatic lipase inhibitory activity, including flavonoids, saponins [4],[17], and substances of microbial sources such as lipstatin, panclicins, etc [2].

The chalcone derivatives (of flavonoid group) are both naturally occurred and synthesized in a straightforward fashion in laboratory. As human safety is revealed [3], chalcone analogs are given priority for the investigation into pancreatic lipase inhibitory activity. This opens up new directions in bioactivity studies at Department of Pharmaceutical Chemistry, University of Medicine and Pharmacy in HCMC, besides prostaglandin E2 inhibitory [15], antioxidant and antifungal activity [6], antimicrobial activity of chalcones analogs and heterocyclic chalcones [14].

MATERIALS AND METHODS

Docking

In terms of properties of protein structure collected from database banks and the aim of evaluating the possibility of human lipase inhibitor, protein of PDB code 1LPB fixes requirements for a human lipase model, having targeted ligand is the inhibitor in the active site of enzyme, with high resolution of 2.46 Å.

Protein 1LPB was protonated by MOE 2008.10. Then, 1LPB's pocket was created by LeadIT 2.1.1. Water molecules and ions were removed. Binding site chosen was of 6.5 Å surrounding co-crystallized ligand-methoxyundecylphosphinic acid (MUP). Binding site includes paramount amino acids in triple catalysis Ser152, Asp176. Chalcone formulas were docked to the target protein by LeadIT 2.1.1. Co-crystallized ligand also went through *redock* (the technique of docking co-crystallized ligand in the active site of enzyme) with similar parameters; based on the values of root-mean-square deviation (RMSD) it is likely to evaluate the precision of ligand preparation procedure and docking method.

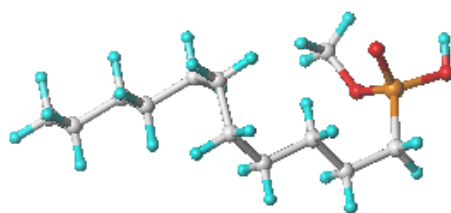


Figure 1. 3D structure of methoxyundecylphosphinic acid (MUP)

Synthesis - microwave-assisted method

To a 100 mL Erlenmeyer flask containing an acetophenone derivative (5 mmol) and a benzaldehyde derivative (5.5 mmol) dissolved in 96% ethanol (6 mL), 60 % KOH solution (2 mL) was added dropwise. The reaction mixture was immersed in an ice bath, stirred frequently and placed in 300 W microwave oven within 6-10 minutes. Time for every reaction was set in 30 seconds, the flask was then allowed to cool before the next turn, in case ethanol was overheated, evaporated vigorously and the mixture was extruded from the flask or caught fire. The process was monitored by thin layer chromatography. After completion, the mixture

was allowed to stand to room temperature and laid overnight. The mixture was acidified by 10% HCl solution, cooled and stirred frequently to pH 4. The obtained precipitation was filtered, washed to neutralized pH. The product was recrystallized by ethanol or ethanol-water mixture [9].

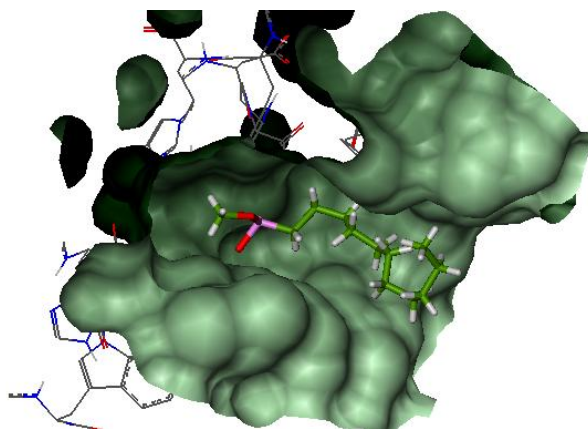


Figure 2. Co-crystallized ligand in the active site of enzyme

Porcine Pancreatic Lipase Inhibitor Assay

Porcine pancreatic lipase is often the prevalent choice for pancreatic lipase inhibitory activity, since there is roughly a 85 percentage of homologous genes between human and porcine [11]. The assay also fits the cost and the goal of this study. Based on the absorbance variation of the reaction mixture, emulsified triolein was used as a substrate for the lipase inhibition assay [7]. The substrate, the inhibitor and lipase were mixed and absorbance at a wavelength of 450 nm was monitored, the time T_0 was recorded. The reaction was kept in a suitable condition ($37\text{ }^\circ\text{C}$) and absorption was recorded at T_{30} .

Variation of the absorbance is designated as $[A_{450}(T_0) - A_{450}(T_{30})]$, for both the test and the blank sample. % Inhibition was calculated as following (13):

$$\% \text{ Inhibition} = \frac{\Delta A_{450 \text{ blank}} - \Delta A_{450 \text{ test}}}{\Delta A_{450 \text{ blank}}} \times 100\%$$

RESULTS AND DISCUSSIONS

Docking

The result of docking with 66 chalcones and four other agents with IC_{50} values on protein 1LPB by LeadIT 2.1.1 after formulas were optimized and prepared by Sybyl-X 1.1. 10 (Formulas with the highest docking scores were illustrated in table 1)

Table 1. Doking results of 10 formulas with the highest docking score

No	Structures	Scores	Hydrogen Bonds	π - π Bonds
1	S16	-26,0210	Gly76, Asp79, Ser152, His263	Phe215
2	S51	-25,1390	Ser152, His263, Gly76, Asp79	
3	S27	-24,9490	Ser152, His151, Gly76	
4	S28	-24,8970	Tyr114	
5	S35	-24,8510	Ser152, Gly76	
6	S15	-24,8140	Ser152, Phe215, Gly76, Asp79	Tyr114
7	S3	-24,436	Ser152, His263, Gly76, Asp79	Tyr114
8	S56	-23,9090	His151	Tyr114
9	S43	-23,7900	Ser152, Phe77	Tyr114
10	S19	-23.466	Ser152, His263, Gly76, Asp79	Phe215

Interactions between some ligands and receptors were illustrated in **Figure 1** and **Figure 2**.

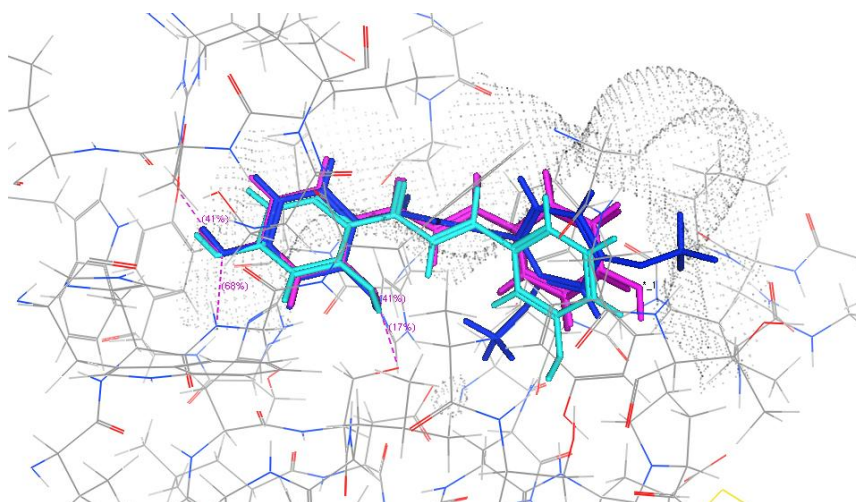


Figure 3. 3D interactions between four ligands (S51, S19, S15, S3) and their receptor.

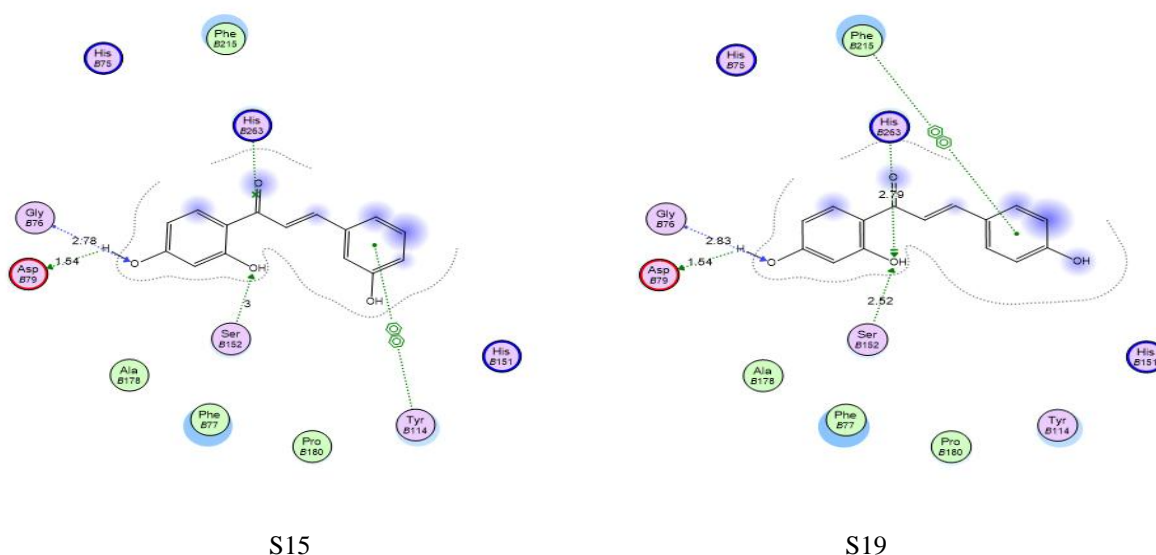


Figure 4. 2D interaction between ligands (S15, S19) and their receptor.

Pancreatic Lipapase Inhibitor

Result of identifying effective lipase inhibition of 10 formulas is illustrated in table 2.

Table 2. Result of identifying effective lipase inhibition of chalcone derivatives

	Structures	IC ₅₀ (μM)	Docking scores (kJ/mol)
1	S15 - 3,2',4'-trihydroxychalcon	<10	-24,8140
2	S3 - 2',4'-dihydroxychalcon	26,16 ± 0,16	-24,436
3	S43 - 2',4'-dihydroxy-3,4-dimethoxychalcon	<10	-23,79
4	S19 - 4,2',4'-trihydroxychalcon	<10	-23,466
5	S65 - 2'-hydroxy-4-(N,N-dimethyl)aminochalcon	104,47 ± 3,01	-20,843
6	S42 - 2'-hydroxy-3,4-dimethoxychalcon	58,29 ± 6,90	-19,944
7	S66 - 2'-hydroxy-4-clorochalcon	167,15 ± 4,03	-19,2751
8	S6 - 2'-hydroxy-2-clorochalcon	61,09 ± 2,88	-19,1640
9	S38 - 2'-hydroxy-4-methoxychalcon	83,12 ± 2,99	-18,9240
10	S25 - 3-nitrochalcon	---	-21,014
11	Orlistat	1,87 ± 0,54	---

Correlations between docking scores and IC₅₀ of biologically evaluated derivatives are presented in **Figure 5**, in which values of IC₅₀ are presented in forms of pIC₅₀.

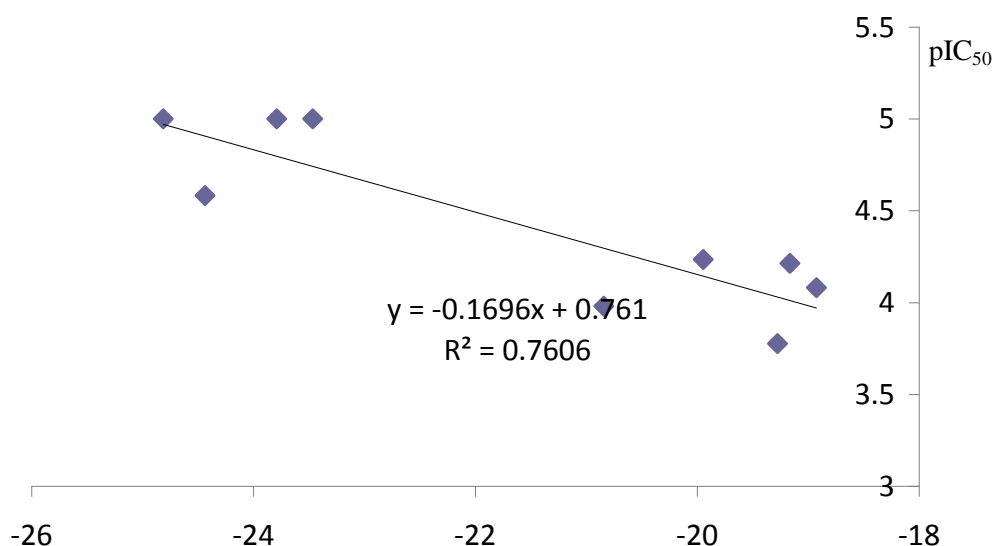


Figure 5. Correlation between IC₅₀ and docking scores

Based on ligand-receptor interactions and substances with IC₅₀ < 10 μM, some structure activity relationship of chalcones were proposed. Carbonyl group in chalcone structure is important for its bonding to amino acids of threesome catalysis. Having hydroxyl substituents at 2', 4' positions showed better potential lipase inhibitor than having only one hydroxyl substituent at 2' position. Amino group at 4' position shows good effect on inhibiting enzyme when having additional hydroxyl group or nitro group at position 3 on ring B. In addition, positions of substituents on ring B such as hydroxyl at position 3 (S15) have better potential comparing with hydroxyl group at position 4 (S19).

For the purpose of selecting some of chalcone derivatives by using docking model running on computer, this research is hoped to find out potential chalcone compounds which are able to resist pancreatic lipase activities. Based on docking outcomes, it is found that chalcones with many hydroxy or amino substituents not only lead to good cohesion results but also be capable of creating bonds with some critical amino acids. Among the derivatives used in the docking model, some of them were studied for many other effects such as anti-inflammatory, anti-bacterial, anti-tumor abilities (S19, S14, S18...) [12],[15]. A new point of this research is that in the list of used compounds, there are some derivatives containing two hydroxy substituents at meta-position (resorcinol derivatives). These derivatives bring about favorable docking results. However, in terms of synthesis, the two -OH substituents at meta-position are oxidized easily in alkaline environment at appropriate temperature. Comparing the docking results to IC₅₀ of published compounds with R²=0.986 proves that there is correlation between docking results and testing results. This is to say, the compounds with lower IC₅₀ lead to lower negative values in docking result.

When it comes to the synthesis process, in the trend of “green chemistry”, the research is chose to be ran in a safe method reducing poisonous solvents (in order to protect the environment), using microwave in a short period of time (for purpose of saving electricity and water). Nevertheless, using microwave-assisted Claisen-Schmidt synthesis reaction requires intense caution in working with equipment than heating under reflux on magnetic stirrers. The solvent for microwave reaction is ethanol-water, hence when microwave energy is transmitted to the reaction's mixture; ethanol is boiled easily and creates a high vapor pressure, resulting in overflow the reaction's mixture. Therefore, the reaction needs to be carried out in short periods of time (from 15 to 30 seconds) and alternately be cooled with or without inert gas environment (N₂, argon as the condition of our laboratory). Among a number of compounds

which is chosen to synthesize, isoliquiritigenin (S19; 2',4,4'-trihydroxychalcone) - a natural compound extracted from liquorice - was studied for many different effects and anti-lipase ability ($IC_{50} = 7, 3 \mu M$ [3]). Because it is a chalcone derivative containing three hydroxy substituents, to synthesize S19 all of factors such as temperature, alkaline environment and time of reaction need to be controlled in the case of using Claisen-Schmidt reaction with microwave or hydroxyl substituent in the chain of acetophenone must be protected against oxidation reactions and coupling reactions of 2'-hydroxy.

Results of testing anti-lipase activity show that many compounds are potential on this target. Based on **Figure 5**, with $R^2=0.7606$ there is a correlation between docking model outcomes and results of active tests. The active test carrying out with ten compounds brings about a result that 9 of them have anti-lipase ability. Specially, S25's ability cannot be determined because of its insolubility at necessary concentration. Meanwhile, other multiple hydroxy substituent compounds such as S15, S43, and S19 have IC_{50} value under $10 \mu M$. IC_{50} value of published compounds, S19 ($IC_{50} 7, 3 \mu M$ [3], experimental value $< 10 \mu M$) and orlistat ($IC_{50} 0, 5 \mu M$ [10], experimental value $= 1, 87 \mu M$) demonstrate that chalcone synthetic derivatives are possible to inhibit lipase activity. Thanks to the research, chalcone derivatives are proven to have the anti-pancreatic lipase, applying into obesity treatment.

CONCLUSIONS

In this study, we screened 66 chalcone derivatives, synthesized 6 biologically potential derivatives and evaluated 9 derivatives for their lipase inhibitory activities. The methods were mainly composed of three stages. The results indicated that there were correlations between *in silico* models and biological activities, hence also presented the possibility to apply virtual screening tools in finding potential agents with high obesity-prevention capacity.

Acknowledgments

This work was supported by the Vietnam's National Foundation for Science and Technology Development - NAFOSTED (Grant # 104.01.2012.78 to Thanh-Dao Tran).

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