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Docking, synthesis and bioassay studies of imine derivatives as potential inhibitors for dengue NS2B/NS3 serine protease

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ABSTRACT

Objective: To search imine derivatives as new active agents against dengue type 2 NS2B/NS3 using molecular docking, since there is no effective vaccine against flaviviral infections.
Methods: In this research, molecular docking was performed for a series of imine derivatives and the information obtained from the docking studies was used to explore the binding modes of these imine derivatives with dengue type 2 NS2B/NS3 serine protease. A set of imine were synthesized and bioassay study of the inhibitory activities of these compounds was then performed.
Results: The results indicated that MY8 and MY4 have the ability to inhibit DEN2 NS2B/NS3 proteolytic activity.
Conclusions: These two compounds were chosen as the reference for the next stage in drug design as new inhibitor agents against NS2B/NS3.

1. Introduction

Dengue is a serious re-emerging vector borne viral infection disease with the number of dengue epidemic increasing substantially in the past 10 years[1]. Dengue infection is caused by dengue virus which is a member of the Flaviviridae family. There are four serotypes of dengue viruses (DEN1, DEN2, DEN3, DEN4). Dengue virus type 2 (DEN2) is the most prevalent in all the serotypes of dengue virus[2].

Dengue virus is transmitted through the bite of a domestic mosquito, *Aedes aegypti*, and in some occasion, *Aedes albopictus*. A mosquito feeding on a person during the first to fifth days of illness can transmit the virus to another person. Following the virus incubation period of 8–10 days in the vector, the virus can be transmitted by an infected mosquito to susceptible individuals through blood feeding[3].

Currently, in the clinical research there are some of anti-dengue therapies but no approved vaccines to avoid the dengue infection and in addition there are also no antiviral drugs to treat this infection[4-6]. The presence of four dengue virus serotypes and the prior lack of a suitable animal model for dengue made the growth of dengue vaccines become more complicated. There have been several reports on the search for possible antiviral agents for dengue. Kiat *et al.*[7] reported two compounds isolated from finger root (*Boesenbergia rotunda*) *i.e.* 4-hydroxypanduratin A and panduratin A, which showed good inhibitory activities against DEN2 serine protease at K_i values of 21 and 25 μmol/L, respectively.

In another work, Velmurugan *et al.*[8] reported a series of biguanidine compounds to have potential as inhibitors for DEN2 NS2B/NS3 serine protease. They observed improved potency of these compounds with possible specificity towards dengue and West

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Nile virus compounds with biguanidine arm at various positions in the ring.

There are many reports on the bioactivity of imine derivatives. For example, imine had been reported to be an agent against migration inhibitory factor[9]. In another example, Yang and his co-workers[10] reported imine to be a novel class of potential inducible nitric oxide synthase inhibitors. Imine derivatives have also been shown to be potential antihypertensive agents[11]. However, to the best of our knowledge, there have been no reports on imine derivatives with antiviral activities, in particular towards dengue virus. In this study, we aim to investigate the activity of imine derivatives in inhibiting the serine protease activities of DEN2.

Using the DEN2 homology model reported by Wichapong and co-workers[12] as the target, the imine derivatives were used as ligands (Figure 1) then it docked into the active site of NS2B/NS3 serine protease. This target model was chosen since Wichapong and co-workers reported that DEN2 homology model was built based solely on the X-ray structure of the homolog West Nile virus NS2B/NS3 protease-inhibitor complex and was in good agreement with the experimental data.

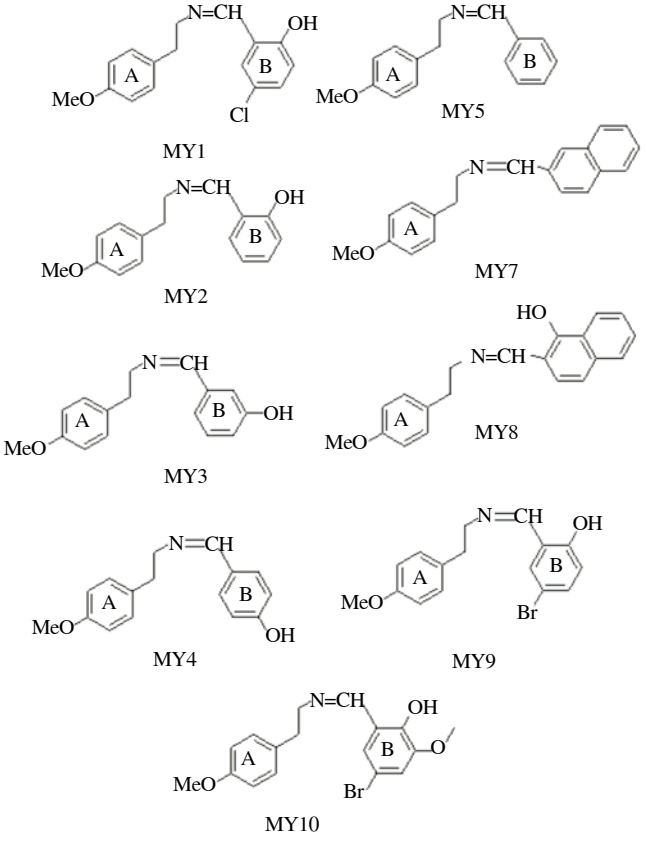


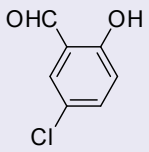
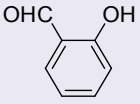
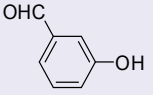
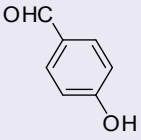
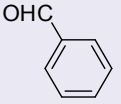
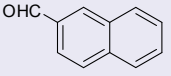
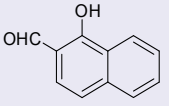
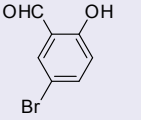
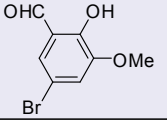
Figure 1. Molecular structures of imine derivatives.

2. Materials and methods

2.1. Synthesis and bioassay of imine derivatives

In this study, the synthesis of various imine derivatives (Schiff bases) was carried out by reacting 2 mmol of various aldehydes listed in Table 1, with equimolar amount of 2-(4-methoxyphenyl) ethylamine in 20 mL ethanol under reflux condition. After the completion of the reactions (3–5 h), the reaction mixtures were concentrated and the Schiff base products were isolated using recrystallization technique[13].

Table 1
List of aldehydes used in the reactions to produce imine derivatives.

Entry	Aldehyde	Product	Yield
1		MY1	92%
2		MY2	87%
3		MY3	85%
4		MY4	94%
5		MY5	78%
6		MY7	72%
7		MY8	75%
8		MY9	78%
9		MY10	92%

The bioassay protocol used was modified from the method published by Kiat and co-workers[7]. Reaction mixtures, with total volume of 200 µL, were prepared. The reaction mixtures comprised 100 µmol/L fluorogenic peptide substrate (Boc-Gly-Arg-Arg-MCA), 2 µmol/L DEN2 NS2B/NS3 protease complex, with or without MY1, MY2, MY3, MY4, MY5, MY7, MY8, MY9 and MY10 of varying concentrations, buffered at pH 8.5 by 200 mmol/L Tris-HCl. MY1-10 were initially prepared in dimethylsulfoxide and assayed at five different concentrations, *i.e.* 25–400 mg/L. The reaction mixtures were incubated at 37 ℃ for 30 min before addition of the final fluorogenic peptide substrate. The mixture was then further incubated at 37 ℃ for 30 min. All the measurements were done in triplicates and the readings were taken using Tecan Infinite M200 PRO fluorescence spectrophotometer. Substrate cleavage was observed by optimizing and monitoring the emission at 440 nm upon excitation at 350 nm. The readings were then used for calculating IC_{50} values (in µmol/L) of MY1-10 presented in Table 2 using non-

linear regression curves fitting in GraphPad Prism 5.0 software[14].

Table 2

IC_{50} values of MY1-10 calculated from protease inhibition bioassay, using non-linear regression model in GraphPad Prism 5.0 software.

No.	Compound	IC_{50} (μmol/L)
1	MY1	282.2
2	MY2	286.9
3	MY3	2721.0
4	MY4	193.2
5	MY5	414.2
6	MY7	1313.0
7	MY8	174.6
8	MY9	270.8
9	MY10	244.9

2.2. Molecular docking

The docking of these nine imine derivatives onto the active site of DEN2 serine protease was done using MOE software packages (Chemical Computing Group Inc). The docking operation began with the protein preparation and then following with ligand preparation. The hydrogen atoms were added for serine protease, following with the minimizing of its backbone. All ligands should be minimized before the docking process. Monte Carlo method with CHARMM force field integrated with the MOE software packages (Chemical Computing Group Inc) with a grid box measuring 10.68Å × 6.34Å × 10.41Å and dimension along the x, y, z axes, respectively, and without any constrains. When the docking process is complete, the best conformation will select with the lowest complexed energy. The geometry of the complexes (enzyme-ligand) by using the same force field (*i.e.* CHARMM) was then minimized until the gradient was 0.01 kcal/mol/Å. The minimization processes were performed by relaxing the structure step by step where minimization was done chronologically on the heavy atoms, followed by the backbone atoms then the alpha carbon atoms, and lastly, all of the atoms[15].

2.3. Calculation of complexation energy

The interaction energies (*i.e.* van der Waals and electrostatic energies) and binding energies (*i.e.* for binding energy calculation, it was carried out by using Poisson–Boltzmann with nonpolar surface area as one of the implicit solvent model) were calculated for these complexes using Discovery Studio 2.1 software package (Accelrys). Complexation energy can be defined as sum or total of interaction energy and binding energy[16,17].

3. Results

3.1. Bioassay of imine derivatives

Inhibition assay against NS2B/NS3 proteolytic activity involved the usage of Boc-Gly-Arg-Arg-MCA as substrate. At 400 mg/L inhibitor concentration, MY1, MY2, MY4, MY8, MY9 and MY10 exhibited more than 80% inhibitory activities as shown in Figure 2. However, only MY4, MY8 and MY9 were able to achieve 90% inhibition. At 200 mg/L, except MY3 and MY7, all inhibitors showed more than 60% inhibition. MY3 and MY7 were shown to inhibit the proteolytic activity in less than 30% and 50%, respectively, even with the highest concentration (400 mg/L) used in this study.

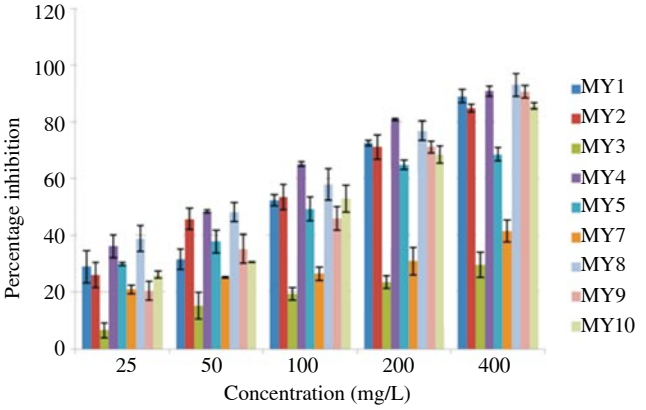


Figure 2. Plot of experimental value *vs.* predicted value.

From Table 2, the bioassay results showed MY8 to be the best inhibitor amongst the nine ligands tested with IC_{50} of 174.6 μmol/L, followed by MY4 with IC_{50} of 193.2 μmol/L, MY10 (IC_{50} of 244.9 μmol/L) and MY9 (IC_{50} of 270.8 μmol/L). Meanwhile, MY3 was shown to have the lowest inhibition activity with IC_{50} of 2721.0 μmol/L.

3.2. Docking of imine derivatives to DEN2 NS2B/NS3

Docking of nine imine derivatives onto the active site of DEN2 serine protease was performed using CHARMM force field with a grid box and without any constraint. From the docking results, the lowest binding energy value was selected as the best docked poses. Superimposition was done for the selected of the best docked poses of these ligands as depicted in Figure 3. The best docking poses with the spatial arrangement of compound MY8 is presented in Figure 4. The spatial arrangements of compound MY4, MY7 and MY3 are depicted in Figures 5–7, respectively. The docking results of these imine derivatives are shown in Table 3.

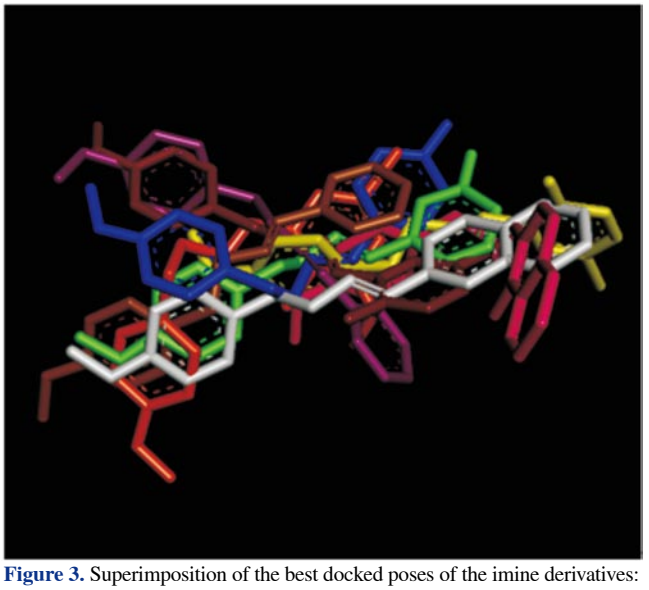


Figure 3. Superimposition of the best docked poses of the imine derivatives: MY1 (maroon), MY2 (purple), MY3 (green), MY4 (blue), MY5 (brown), MY7 (white), MY8 (pink), MY9 (orange) and MY10 (yellow).

Table 3
Docking results.

No	Molecule	S	Econf	Eplace	Escore1	Erefine	Escore2	IE	BE	CE
1	MY1	–2.9	2.2	–8.8	–0.0	–19.2	–2.9	–18.3	–39.0	–57.3
2	MY2	–2.9	2.6	–10.1	–1.7	–8.7	–2.9	–18.9	–48.6	–67.6
3	MY3	–2.9	4.0	–10.1	–1.4	–15.7	–2.9	–13.4	–22.9	–36.3
4	MY4	–2.9	3.2	–9.3	1.1	–13.4	–2.9	–19.3	–49.9	–69.2
5	MY5	–2.9	2.0	–10.1	–0.5	–19.0	–2.9	–14.9	–42.9	–57.9
6	MY7	–2.1	2.0	–10.6	4.5	–15.0	–2.1	–11.4	–18.5	–30.0
7	MY8	–2.4	4.0	–11.5	0.5	–13.2	–2.4	–29.8	–59.2	–89.0
8	MY9	–2.5	2.3	–10.4	–0.0	–19.4	–2.5	–14.3	–42.5	–56.8
9	MY10	–2.2	3.6	–9.0	0.8	–12.2	–2.2	–19.7	–27.5	–47.2

S, Econf, Eplace, Escore, Erefine, IE, BE and CE are in kcal/mol and defined as the final energy score (which is the score of the last stage), the energy of conformer, score from the placement stage, score from rescoring stage, score from refinement stage, interaction energy, binding energy and complexation energy, respectively.

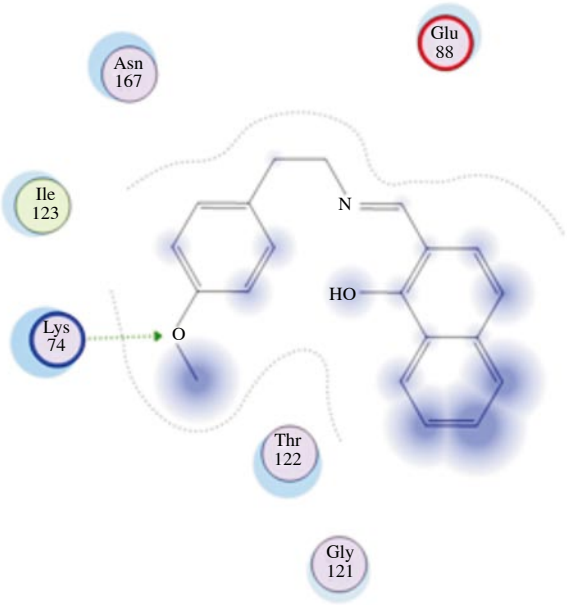


Figure 4. Spatial arrangement of the binding site residues of DEN2 protease surrounding MY8.
MY8 is shown as line; binding residues are shown as balls.

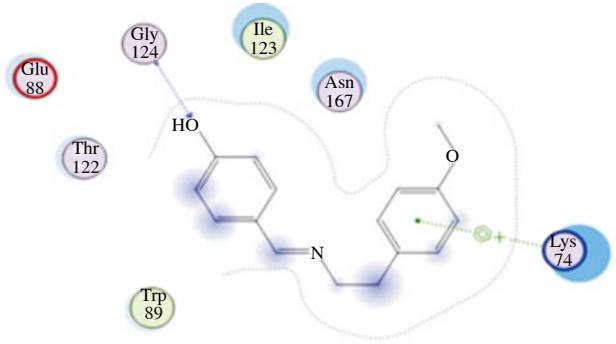


Figure 5. Spatial arrangement of the binding site residues of DEN2 protease surrounding MY4.
MY4 is shown as line; binding site residues are shown as balls.

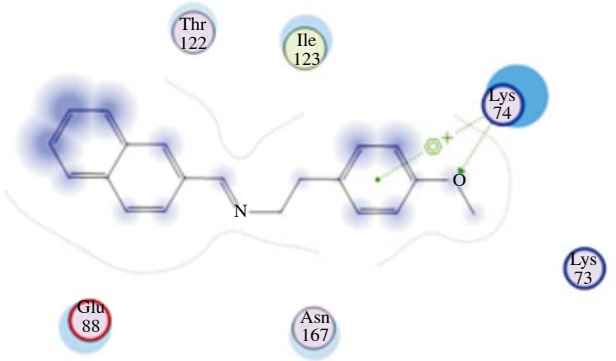


Figure 6. Spatial arrangement of the binding site residues of DEN2 protease surrounding MY7.
MY7 is shown as line; binding site residues are shown as balls.

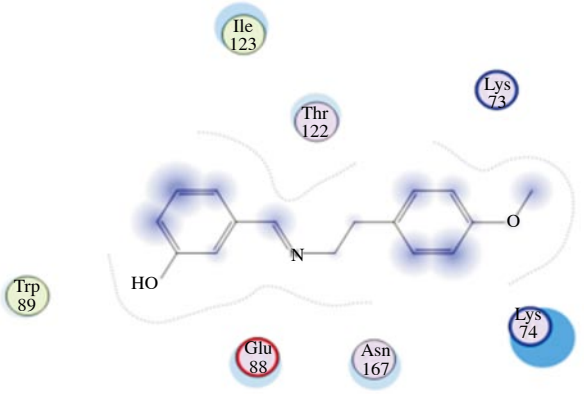


Figure 7. Spatial arrangement of the binding site residues of DEN2 protease surrounding MY3.
MY3 is shown as line; binding site residues are shown as balls.

4. Discussion

Base on the docking results (*i.e.* complexation energy), it seemed that MY8 (–89.09 kcal/mol) and MY4 (–69.21 kcal/mol) are the most active inhibitor against DEN2 NS2B/NS3 while MY3 (–36.38 kcal/mol) and MY7 (–30.01 kcal/mol) are the non active inhibitor. The spatial arrangements of the ligands in the enzyme active site were then studied. Several residues played important roles in determining the binding interactions with the ligands. The binding of MY8 to the active site of the protease showed that a hydrogen bond was formed between the methoxyl O atom of the ligand's ring A and H atom of Lys74. In addition, through the van der Waals specificity pocket the hydrophobic residues such as Gly121 and Asn167 were interacted with the ligand. These interactions indicate

MY8 to be an active inhibitor against DEN2 NS2B/NS3. Moreover, the experimental value for IC_{50} of MY8 was seen consistent with the lowest complexation energy, which indicated that MY8 can be used as good inhibitor for dengue infection[17].

For MY4, docking results showed hydrogen bond interaction between the hydroxyl O atom of the ligand's ring B and the H atom of Gly124. Hydrophobic binding pocket is observed with the interaction of the ligand and residue Asn167. Based on the docking results, it could be assumed that MY4 has the ability to inhibit the DEN2 serine protease. However, its activity was presumably not as good as MY8 as its complexation energy was smaller than that of MY8.

Compound MY7 ligand's hydrogen bonding interaction is observed between the ligand's ring A with Lys74. The complexation energy value for MY7 obtained at –30.01 kcal/mol is lower than those for MY8 and MY4. This seems to indicate MY7 to be less active than MY8 and MY4 in inhibiting DEN2 protease activity[16].

For MY3, no hydrogen bonding interactions were observed at the binding site. From the docking results, the complexation energy value also indicated that MY3 showed the lowest inhibitory activity, obtained at –36.38 kcal/mol. This seemed to indicate MY3 to be a less active ligand in inhibiting DEN2 protease activity.

A series of imine derivatives were synthesized and assayed for inhibition activities against DEN2 NS2B/NS3 protease. They were then docked onto the active site of the serine protease. Docking results corroborated well with the experimental results where MY8 was observed to be the most active while MY3 was the least active inhibitor. Thus, MY8 could be developed further as a potential lead inhibitor of DEN2 NS2B/NS3 serine protease toward the design of anti-dengue therapeutics.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References

[1] National Institute of Allergy and Infectious Diseases. Bethesda: National Institute of Allergy and Infectious Diseases; 2017. [Online] Available from: https://www.nih.gov/about-nih/what-we-do/nih-almanac/national-institute-allergy-infectious-diseases-niaid [Accessed on 30th August, 2017]

[2] Salazar MI, Pérez-García M, Terreros-Tinoco M, Castro-Mussot ME, Diegopérez-Ramírez J, Ramírez-Reyes AG, et al. Dengue virus type 2: protein binding and active replication in human central nervous system cells. *ScientificWorldJournal* 2013; **2013**: 904067.

[3] Centers for Disease Control and Prevention. Dengue and dengue hemorrhagic fever. San Juan: Centers for Disease Control and Prevention; 2008. [Online] Available from: https://www.cdc.gov/dengue/resources/Dengue&DHF%20Information%20for%20Health%20Care%20Practitioners_2009.pdf [Accessed on 30th August, 2017]

[4] Nguyen TTH, Lee S, Wang HK, Chen HY, Wu YT, Lin SC, et al. *In vitro* evaluation of novel inhibitors against the NS2B-NS3 protease of dengue fever virus type 4. *Molecules* 2013; **18**: 15600-12.

[5] Sajin AK, Rathnan RK, Mechoor A. Molecular docking studies on phytocompounds from the methanol leaf extract of *Carica papaya* against envelope protein of dengue virus (type-2). *J Comput Methods Mol Des* 2015; **5**(2): 1-7.

[6] Thomas SJ. Developing a dengue vaccine: progress and future challenges. *Ann N Y Acad Sci* 2014; **1323**: 140-59.

[7] Kiat TS, Pippen R, Yusof R, Ibrahim H, Khalid N, Rahman NA. Inhibitory activity of cylohexenyl chalcone derivatives and favanoids of fingerroot, *Boesenbergia rotunda* (L.), towards dengue-2 virus NS3 protease. *Bioorg Med Chem Lett* 2006; **16**: 3337-40.

[8] Velmurugan D, Mythily U, Rao K. Design and docking studies of peptide inhibitors as potential antiviral drugs for dengue virus ns2b/ns3 protease. *Protein Pept Lett* 2014; **21**(8): 815-27.

[9] Dios A, Mitchell RA, Aljabari B, Lubetsky J, O'Connor K, Liao H, et al. Inhibition of MIF bioactivity by rational design of pharmacological inhibitors of MIF tautomerase activity. *J Med Chem* 2002; **45**: 2410-6.

[10] Yang W, Hu Y, Yang YS, Zhang F, Zhang YB, Wang XL, et al. Design, modification and 3D QSAR studies of novel naphthalin-containing pyrazolines derivatives with/without thiourea skeleton as anticancer agents. *Bioorg Med Chem* 2013; **21**: 1050-63.

[11] Ismail MA, Nabil Aboul-Enein M, Abouzid KA, Abou El Ella DA, Ismail NS. ACE inhibitors hypothesis generation for selective design, synthesis and biological evaluation of 3-mercapto-2-methyl-propanoyl-pyrrolidine-3-imine derivatives as antihypertensive agents. *Bioorg Med Chem* 2009; **17**: 3739-46.

[12] Wichapong K, Pianwanita S, Sippl W, Kokpol S. Homology modeling and molecular dynamics simulations of Dengue virus NS2B/NS3 protease: insight into molecular interaction. *J Mol Recognit* 2010; **23**: 283-300.

[13] Yaeghoobi M, Rahman NA, Ng SW. 4-Chloro-2-[(E)-2-(4-methoxyphenyl)-ethyliminomethyl]phenol. *Acta Cryst* 2009; **65**: O1070..

[14] Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. *FASEB J* 1987; **1**: 365-74.

[15] Yaeghoobi M, Frimayanti N, Chee CF, Ikram KK, Najjar BO, Zain SM, et al. QSAR, *in silico* docking and *in vitro* evaluation of chalcone derivatoves as potential inbhitors for H1N1 virus neuraminidase. *Med Chem Res* 2016; **25**: 2133-42.

[16] Frimayanti N, Chee CF, Zain SM, Rahman NA. Design of new competitive dengue NS2B/NS3 protease inhibitors-a computational approach. *Int J Mol Sci* 2011; **12**: 1089-100.

[17] Zamri A, Frimayanti N, Teruna HY. Docking and molecular dynamic simulations: study of 1,3, 4-oxadiazole-chalcone hybrid derivatives to search new active anticancer agents. *Thai J Pharm Sci* 2016; **40**(4): 179-84.