PHARMACOPHORE MODELLING, DOCKING AND BIOLOGICAL EVALUATION OF VANILLIN DERIVATIVES AS NEURAMINIDASE INHIBITORS

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ABSTRACT

A series of vanillin derivatives (**1**-**3**) were synthesized and their proposed structure was established by elemental analyses, FTIR and ^{1}H-NMR spectral. In this paper we employed A 3D pharmacophore model based on selected ten established NA inhibitors as training set with diverse molecular structures and performed docking simulation using PDB-2HU0 to observe the ligand-protein complex interaction. The best hypothesis of the pharmacophore model consist one hydrogen bond acceptor (HBA), one hydrogen bond donor (HBD) and one negative ionizable (NI) features used to predict the neuraminidase (NA) inhibition activities of the synthesized vanillin derivatives (**1**-**3**). All synthesized vanillin derivatives were tested against NA of *clostridium perfringens* on MUNANA assay for validation. The 4-[(4-Hydroxy-3-methoxybenzylidene)amino]-1,5-dimethyl-2-phenylpyrazolidin-3-one (**1**), 2-methoxyphenol-2,3,4-trimethyl-5-phenyl-1,3-oxazolidine (**2**) and 2-Methoxy-4-(phenylminomethyl)phenol (**3**) was found to inhibit the NA enzymes. These compounds exerted significant NA inhibition with IC_{50} **0.73** mg/mL, **0.09** mg/mL and **0.26** mg/mL, respectively which is compared to the NA inhibitor, N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (DANA) of IC_{50} **0.2** mg/mL.

Keywords: vanillin, neuraminidase, pharmacophore, inhibitor

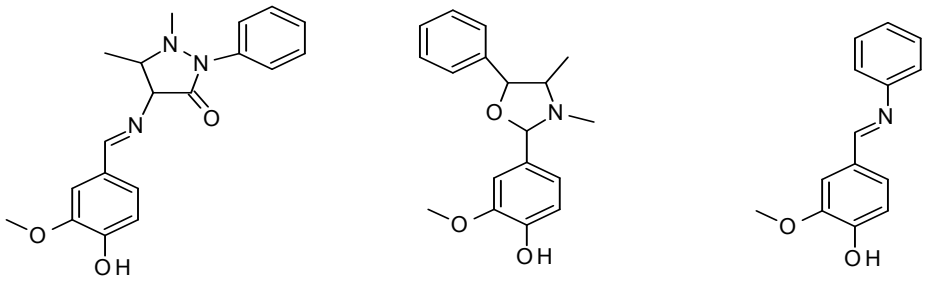
INTRODUCTION

Influenza virus causes global health problems in humans as a consequence of antigenic drift and shift which promotes unpredictable mutation and reassortment of viral strains.[1-2] Some of the highly pathogenic avian Influenza Virus (HPAIV) strains such as H5N1, have occasionally infected humans and pose a severe threat due to their high pathogenicity with high mortality rates.[3] The most impact epidemiologic evidence for increased influenza-related fatality comes from the 1918, 1957 and 2009 pandemics.[4] The flu pandemic of 2009 caused by a new A/H1N1 strain which was characterized by a high transmission rate but low virulence was less critical but still urgently need for searching novel antiviral agents.[5-7]

The available antiviral drugs such as amantadine, rimantadine, zanamivir and oseltamivir are selective for influenza A viruses but the usage are limited due to their side effects and resistant to the new viral strains. The influenza A/H1N1 2009 isolates are also resistant to amantadine and rimantadine and to both, oseltamivir and peramivir as well.[8-9] Vaccine development in the control of influenza epidemics are not fully succeeded due to the highly variable mutation of influenza virus. Effective and safe anti-influenza therapeutics are lacking. In the market, recent pandemics that involved human cases of the influenza have put an awareness to control the spread of the disease.[10]

Considering, the threat of influenza endemic and pandemic, it was of importance to synthesize new lead of NA inhibitors. Influenza NA is a major drug target for the influenza infections therapy.[11] Therefore it is an attractive target for designing inhibitors against influenza viruses despite of the conserved catalytic site in all influenza A and B virus.[12] In this study, 4-hydroxy-3-methoxybenzaldehyde or vanillin used as the starting material for the syntheses of proposed NA inhibitors. Vanillin is widely used as an intermediate in the chemical and pharmaceuticals industries.[13-15] Vanillin has been used as a starting material to synthesize drugs such as papaverine, *l*-dopa, *l*-methyldopa and trimethoprim as antimicrobial agent.[16] Vanillin derivatives are of considerable interest in this work was due to its potential biological activities, such as antiviral, antibacterial, antimalarial and antitumor activities.[17-18]

In order to synthesize the NA inhibitors, pharmacophore model approach was applied to explore common chemical characteristics of chemical structures with great diversity. A reliable pharmacophore model was used as a query for searching chemical databases to find new lead compounds. In this paper, we now report the synthesis and characterization of vanillin derivatives namely, 4-[(4-Hydroxy-3-methoxybenzylidene)amino]-1,5-dimethyl-2phenylpyrazolidin-3-one (**1**), 2-methoxyphenol-2,3,4-trimethyl-5-phenyl-1,3-oxazolidine (**2**) and 2-Methoxy-4-(phenylminomethyl)phenol (**3**) (Figure 1) used as a pharmacophore model to evaluate and validate NA inhibition activity.



1 **2** **3**

Figure 1. Proposed chemical structure of vanillin derivatives (**1** - **3**).

MATERIALS AND METHODS

Pharmacophore studies

CATALYST, Discovery Studio (DS) 2.5, Accelrys Inc. USA (www.accelrys.com) software packages and CS ChemDraw Ultra 7.01, Cambridge Soft Corp. USA (http://www.cambridgesoft.com) and LigandScout 3.03 Inte:Ligand GmbH were utilized in this research work.

Hypotheses generation

The pharmacophore model was generated using a series of training set consists of ten established NA inhibitors. Genetic algorithm and multiple linear regression analysis were employed to select optimal combinations of pharmacophoric models and 2D descriptors against compounds of training set.[19] Training set was shown in Figure 2 and the generated pharmacophore model was shown in Figure 3.

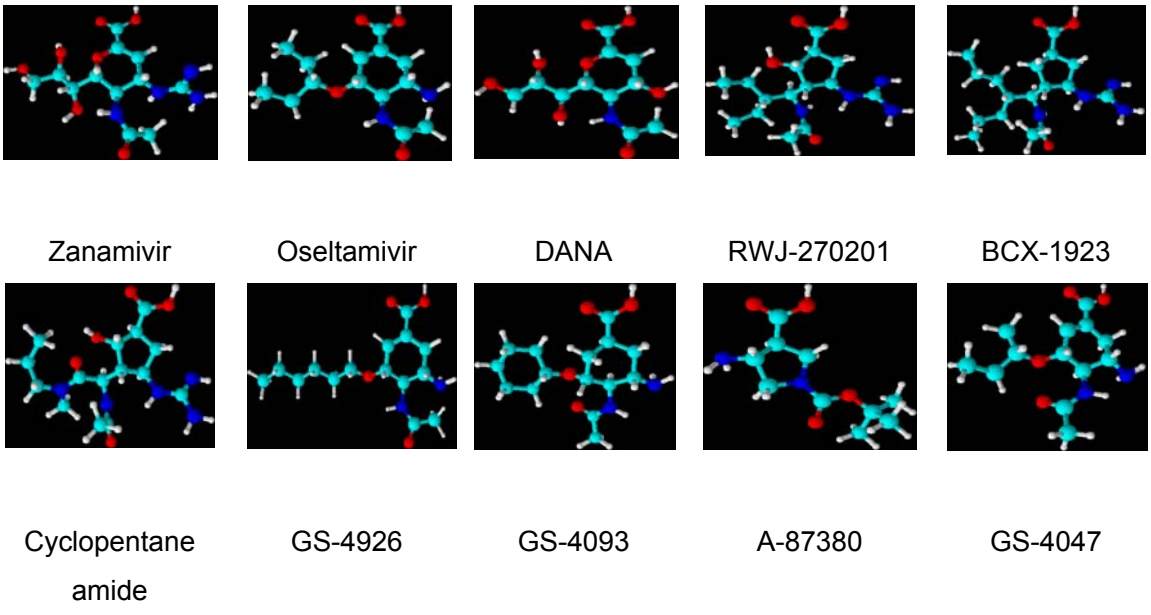


Figure 2. Structure of compounds used in training set 1.

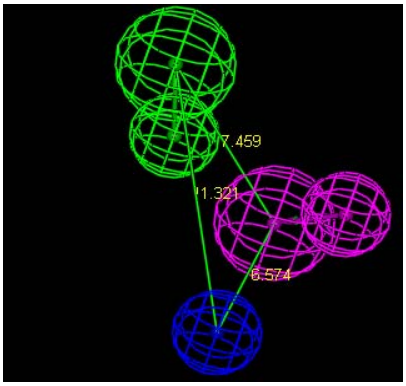


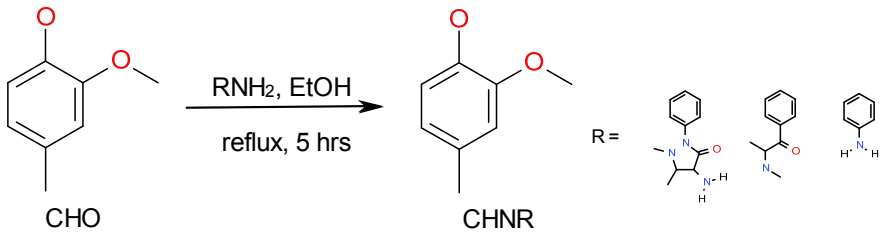
Figure 3. Pharmacophore model of hypothesis 1 of the top rank score generated from the training set

Synthesis

General methods: Chemicals were purchased from Aldrich, Fluka, Acros and J. T. Baker. Vanillin derivatives were synthesized by condensation reaction using Schlenk apparatus under dry nitrogen. The condensation was monitored by silica gel TLC. Detection was done with UV (254 nm). Infrared spectra were recorded on a Perkin Elmer Spectrum GX Fourier Transform Spectrphotometer using KBr disc from 4000-375 cm^{-1}. Electronic spectra were recorded on a Perkin Elmer Lambda25 from 260-550 nm in DMF. ^{1}H-NMR was recorded on a JEOL 500 MHz, tetramethyl silane (TMS) was used as an internal standard spectrometer. The elemental analysis was performed using Flesh EA 1112.

Preparation of vanillin derivatives 1 - 3

The synthetic route to prepare vanillin derivatives is described in Scheme 1.



Scheme 1. The general synthesis route of Schiff base reaction

Synthesis of (4R,5R)-4-{[(E)-(4-hydroxy-3-methoxyphenyl)methylidene]amino}-1,5-dimethyl-2-phenylpyrazolidin-3-one (**1**)

Compound **1** was prepared by Schiff base condensation reaction An ethanolic solution (20 mL) of compound **1** (0.01mol) was added to an ethanolic solution (20 mL) of vanillin (4-hydroxy-3-methoxybenzaldehyde) (0.01mol) and the solution was refluxed for about 5 hours with vigorous stirring and allowed to cool. Then, it was cool down in crushed ice for the compounds to be formed. The compounds were filtered off and recrystallized from ethanol. The reaction was monitored by TLC.

Yield: 80%, IR (KBr) v 3444 (O-H), 1630 (C=N), 1037 (C-O), 1732 (C=O) cm^{-1}; ^{1}HNMR (400 MHz, MeOD) δ 2.44 (s, 3H, -CCH_3), 3.14 (s, 3H, -NCH_3), 3.84 (s, 3H, -OCH_3), 6.84 (d, 1H, *J*=8 Hz), 7.18 (dd, 1H, *J*=2,2,8 Hz), 7.34 (s, 1H), 7.36-7.39 (m, 2H), 7.43 (d, 1H, *J*=2 Hz), 7.51-7.55 (m, 2H), 9.47 (s, 1H, -N=CH) ppm; Anal. Calc. For $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38%. Found: C,67.20; H,6.19; N, 12.32%.

Synthesis of 4-(3,4-dimethyl-5-phenyl-1,3-oxazolidin-2-yl)-2-methoxyphenol (**2**)

Compound **2** was synthesized using the similar method (**1**) and the reaction gave 70% yield. IR (KBr) v 3403 (O-H), 1614 (C=N), 1037 (C-O), 1614 (C-N) cm^{-1}; ^{1}HNMR(400 MHz, MeOD) 1.21 (d, 3H, *J*=7 Hz, -NCH_3), 2.19 (s, 3H, -CCH_3), 2.50-2.57 (m, 1H), 3.91 (s, 3H, -OCH_3), 4.71 (d, 1H, *J*=8 Hz), 4.90 (s, 1H), 6.82 (d, 1H, *J*=8 Hz), 6.98 (dd, 1H, *J*=2, 2, 8 Hz), 7.18 (d, 1 H, *J*=2 Hz), 7.31-7.34 (m, 1H), 7.37-7.41 (t, 2H, *J*= 8,8 Hz), 7.44 (s, 1H), 7.45 (d, 1H, *J*=2 Hz) ppm; Anal. Calc. for $C_{15}H_{21}NO_3$: C, 86.01; H, 8.42; N, 5.60%. Found: C, 85.97; H, 8.37; N, 5.54%.

Synthesis of 2-methoxy-4-[(E)-(phenylimino)methyl]phenol (**3**)

Compound **3** was synthesized using the similar method (**1**) and the reaction gave 70% yield. IR (KBr) v 3089 (O-H), 1622 (C=N), 1156 (C-O) cm^{-1}; ^{1}HNMR(400 MHz, MeOD) 3.94 (s, 3H, -OCH_3), 6.90 (d, 1H, *J*=8 Hz), 7.22 (m, 3H), 7.31 (dd, 1H, *J*=2,2,8 Hz), 7.38-7.45 (m, 2H), 7.62 (d, 1H, *J*=2 Hz), 8.41 (s, 1H, -N=CH_3) ppm. Anal. Calc. for $C_{14}H_{13}NO_2$: C, 86.12; H, 6.71; N, 7.17%. Found: C, 86.06; H, 6.66; N, 7.13%.

Neuraminidase (NA) inhibition assay

Reagents and apparatus: 2-(4-methylumbelliferyl)-α-D-acetylneuraminic acid (MUNANA), *N*-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (DANA), 2-N-Morpholino-ethanesulfonic acid (MES), Neuraminidase from c*lostridium perfringens* (*C. welchii*) were purchased from Sigma. $CaCl_2$, NaOH and ethanol were purchased from R&M Chemicals. Microplate 96 F was purchased from BMG Co., Germany.

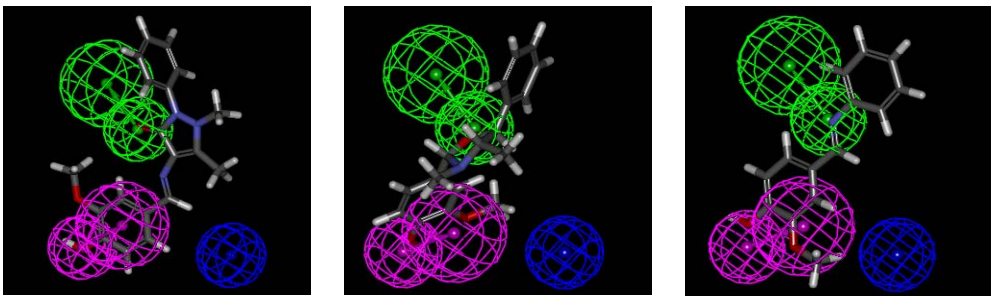
MUNANA assay

MUNANA is the substrate of NA. Cleavage of this substrate by NA produces a fluorescent product, which can emit an emission wavelength of 460 nm with an excitation wavelength of 355 nm. NA enzyme activity and drug inhibition assays were based on the method of Potier *et al*., and Blick *et al*.[20-21] The intensity of fluorescence reflects the activity of NA sensitively. Each assay was done in triplicate. The fluorescence involving 4-MUNANA at excitation 365 nm and emission at 450 nm was measured using MODULUS Multi-well Plate Reader machine.

RESULTS AND DISCUSSIONS

Pharmacophore model established for influenza virus NA inhibitors was applied to detect inhibitory activities of the synthesized vanillin derivatives using Schiff based reaction and has high solubility in water and organic solvent such as MeOH. The conformations of compounds **1**, **2** and **3** fits Hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) features which is in good accordance based on the training set, essential to possess NA inhibition activity. Figure 4 illustrates the mapping of the synthesized compounds against the pharmacophore model.

From the preliminary studies, we found that all three vanillin derivatives showed good alignment but not fully fit all features especially negative ionisable (NI) due to lack of -COOH group. Compounds **1**, **2** and **3** fitted well to both HBA and HBD of the vanillin scaffold. Methyl (-CH_3) group of pyrazolidine of **1** and oxazolidine of **2** and their benzene ring also plays an important role as hydrophobic interactions which are important for the interaction between these ligands and NA enzymes.



1 **2** **3**

Figure 4. Mapping of **1**, **2** and **3** onto pharmacophore model. Green: Hydrogen bond acceptor (HBA), Magenta: hydrogen bond donor (HBD) and blue: negative ionisable (NI)

The inhibitory effects of the vanillin derivatives (**1**-**3**) against NA were first reported. Their structural scaffolds are different from those of known NA inhibitors approved for influenza treatment such as oseltamivir and zanamivir. All synthesized compounds exhibit predicted inhibitory activity through the pharmacophore mapping. Fluorometric determinations were quantified with MODULUS Multi-well Plate Reader and the biological evaluation was plotted in Figure 5 data.

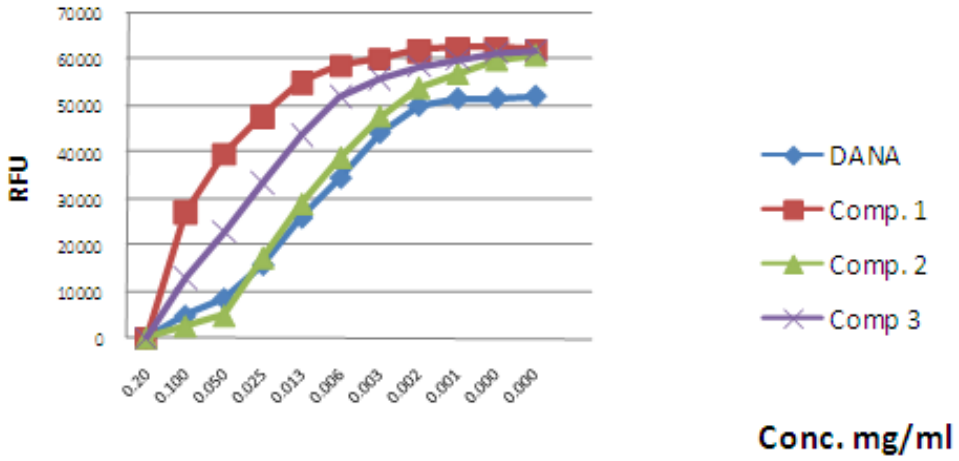
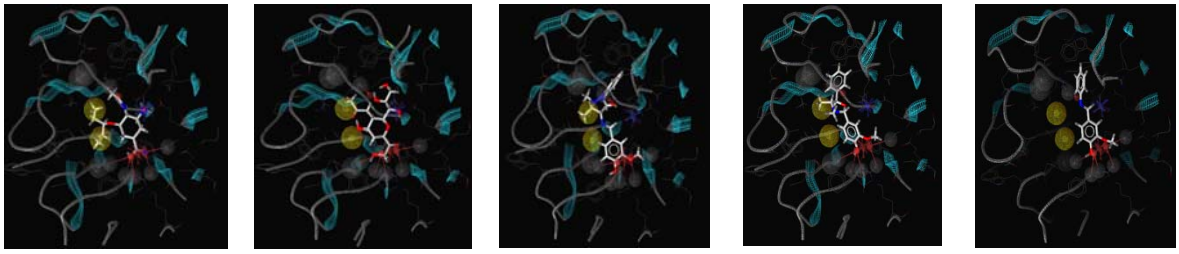


Figure 5. A plot of NA activity percentage *vs* concentration of compound **1, 2, 3** compare to DANA

It was found that compounds **1**-**3** showed significant NA inhibition, with IC_{50} of 0.73 mg/mL, 0.09 mg/mL and 0.26 mg/mL, respectively compare to DANA (control) which possess IC_{50} of 0.2 mg/mL. Compound **2** exert the strongest NA inhibition activity compare to **1**, **3** and DANA. The docking studies verified the interactions within the NA active site as illustrated in Figure 6 using protein data bank (PDB), code 2HU0 using LigandScout 3.03 software.



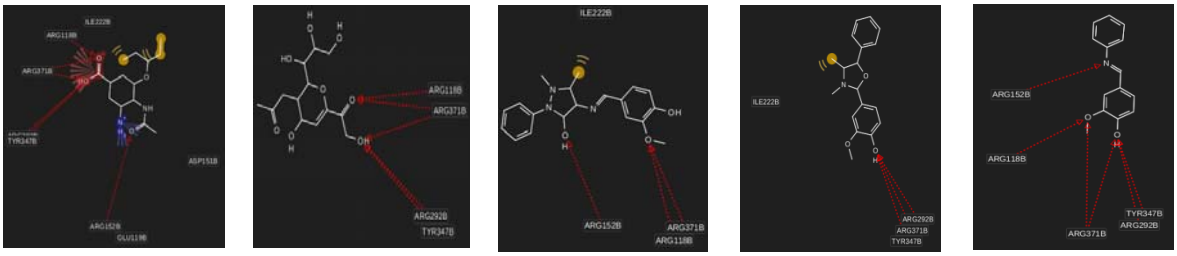


Figure 6. Interaction of oseltamivir, DANA and compounds **1** – **3** in NA (PDB:2HU0) active site.

From the docking simulation, it was shown that compound **2** binds to NA active site *via* Arg371, Arg118 and TYR347 including hydrophobic interaction in the Ile222 region *via* methyl group of the oxazoline ring which act as an anchor in the active site compare to DANA (control). Whereas compound **1** showed two interactions *via* ARG371 and Arg118 at the methoxy group which shows a weaker interaction compare to compound **2** which binds three residues at hydroxyl (OH) group. Both, **1** and **3** showed inhibition activity lower than DANA.

CONCLUSION

It was found that compounds **1**-**3** possess weaker NA inhibition activity compare to oseltamivir carboxylate which is a current marketed drug available for the treatment of influenza disease but the these compounds could be further investigated for the development of novel anti-influenza agents in near future.

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