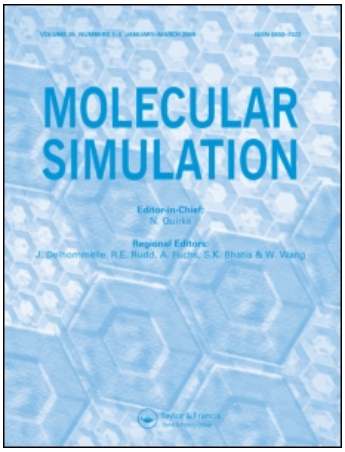

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Novel hemagglutinin inhibitors for H1N1 influenza virus screening from TCM database

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The emergence of Tamiflu (oseltamivir)-resistant viral strains in pandemic of H1N1/09 influenza virus has raised global awareness of anti-viral drug resistant issue. There is an urgent demand for developing new anti-influenza compound. The purpose of this research is to design novel haemagglutinin (HA) inhibitor for inhibiting viral entry into the host cell. We performed structure-based drug design to analyse interactions between the potent inhibitor and HA. A traditional Chinese medicine (TCM) database was used for *in silico* screening process. The docked TCM constituents were input into *de novo* evolution to generate derivatives. Selected derivatives were then docked back to HA binding site. We identify four key features from top 10 docked derivatives' binding conformations and structure scaffolds. The addition of 2-aminopyridinium group has the greatest influence in the binding ability of TCM derivatives and is, therefore, suggested to be the key point in designing HA inhibitors.

Keywords: H1N1; Tamiflu; docking; structure-based drug design; traditional Chinese medicine

1. Introduction

With the advancement in computational technology, molecular modelling is becoming an integral part of material science, new polymer design and even pharmaceutics development [1–6]. In particular, *in silico* screening, or structure-based drug design, that utilises computational chemistry and molecular mechanics, permits detailed investigation of potential drug–receptor interactions, while also offering speed enhancement in drug discovery.

The need for rapid assessment of compound libraries to search for potential medicinal compound is most recently reflected in the pandemic of H1N1 influenza virus, in which the viral strain resistant to the front-line antiviral drug Tamiflu was identified [7–9]. Tamiflu, a neuraminidase (NA) inhibitor, inhibits the spread of new influenza viral progeny by binding to viral surface glycoprotein, NA [10–12]. However, the segmented RNA genome of influenza viruses is prone to antigenic drift (sequence base mutations) and antigenic shift (genetic recombination), both of which are known causes for NA inhibitor resistance [13–16].

In addition to NA, influenza surface membrane also contains another glycoprotein, haemagglutinin (HA). HA facilitates viral entry through binding to host surface sialic acid residues [17]. Hence, blocking of HA sialic acid binding site could inhibit viral entry into the host cell and then, indirectly, could prevent viral replication. When compared to NA inhibitors, HA inhibitor, theoretically, should be a more effective anti-influenza strategy, since NA inhibitor, in case of Tamiflu, are ineffective in the late stage of viral infection [18,19]. This lack of efficacy is most attributed to the build-up of virus number in the host body.

Presently, there are no clinically available HA inhibitor despite numerous efforts. In the hope of finding new anti-viral compounds, we conducted structure-based drug design to analyse interactions between potential ligands and HA sialic acid binding site. There were many successful cases of applying structure-based drug design to investigate ligand–protein or protein–protein interactions [20,21], and in the past, our group has applied the computer-aided drug design technology to develop new therapeutic compounds and has also developed new scoring function for assessing binding affinity [22–38]. Of all the different HA subtypes (H1–H16), we concentrated our efforts on H1, the HA subtype found on the recent pandemic H1N1/09 virus. We performed

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in silico screening of a traditional Chinese medicine (TCM) database (Available at http://tcm.cmu.edu.tw/) to find constituents most fitted to the binding pocket of H1. TCM constituents have been studied in the past to investigate anti-cancer [39] and anti-inflammatory [40,41]; however, their anti-influenza potencies have not been closely examined. Natural compounds, such as those from TCM, cover diverse range of chemical structures and, therefore, could be extremely novel compounds for protein targets.

2. Methods

2.1 *Software*

Structure modelling, validation of homology model, virtual screening and generations of derivatives were performed by Discovery Studio Client v2.5.0.9164 (Dock Score 2.5). The 2D and 3D structures of TCM constituents within the TCM database were built by using ChemBioOffice 2008 (CambridgeSoft, Inc., Cambridge, MA, USA). The TCM database can be accessed at http://tcm.cmu.edu.tw/

2.2 *Sequence comparison and homology modelling of H1N1/09 HA*

As shown in Figure 1, sequence alignment was performed prior to homology modelling to ensure the sequence identity and similarity shared between the template sequence and the target sequence. The gene sequence of HA, subtype H1, was obtained from GenBank: ADI99650. The H1 template was downloaded from the Protein Data Bank, PDB ID: 1RDB [42]. After homology modelling, Ramachandran plot and Profiles-3D were used to validate the reliability and the compatibility of the modelled H1 structure.

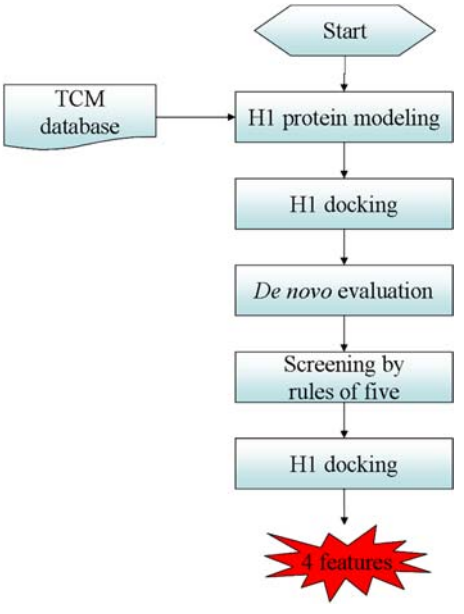


Figure 1. Flowchart of the entire experiment.

2.3 In silico *screening (docking)*

LigandFit module of DS 2.5 was used to dock TCM constituents into H1. All protein and small molecules were pre-applied with a forcefield of Chemistry at Harvard Macromolecular Mechanics. Ligand binding site of H1 was set to the *N*-Acetyl-D-Glucosamine (NAG) binding site found in the HA crystal. NAG molecule was also used as a control to filter TCM constituents.

2.4 *Derivatives generation (*de novo *evolution)*

Candidate compounds obtained from previous docking screening were input into *de novo* evolution, in which the TCM compound scaffolds were used as the starting basis for further modification. Fragments that complement the H1 binding sites were fused to the TCM scaffolds.

After *de novo* evolution, Lipinski's Rule of Five [43] was used to screen out derivatives that are orally unstable or inapplicable compounds. Derivatives past the screen were then docked back to H1 ligand binding site to assess binding affinity and receptor–ligand interactions.

3. Results and discussion

3.1 *Homology modelling*

The sequence alignment of H1 protein and target sequence gives a sequence identity of 83.2% and a similarity of 92.1% (Figure 2). This high sequence identity and similarity between the target and the template sequence provide supports for using the template structure (H1: 1RD8) as modelling basis.

To validate the modelled H1 structure, Ramachandran plot was used to examine the compatibility of dihedral angles of protein backbone atoms. As the plot illustrates in Figure 3, 94.4% of residues are found in the acceptable regions, while only 2.5% of residues are in disallowed regions (Figure 3). As for Profiles-3D validation of protein structure, all the binding site residues (sequence 12–65) are found to have compatibility scores greater than zero (Figure 4). Based on the above results, we have confidence in the reliability of our H1 model (Figure 5).

3.2 *Docking results*

From docking, we selected the top 20 TCM constituents (Table 1) for further structural modification. The *de novo* evolution products of these TCM were screened by Lipinski's Rule of Five and were docked back to the H1

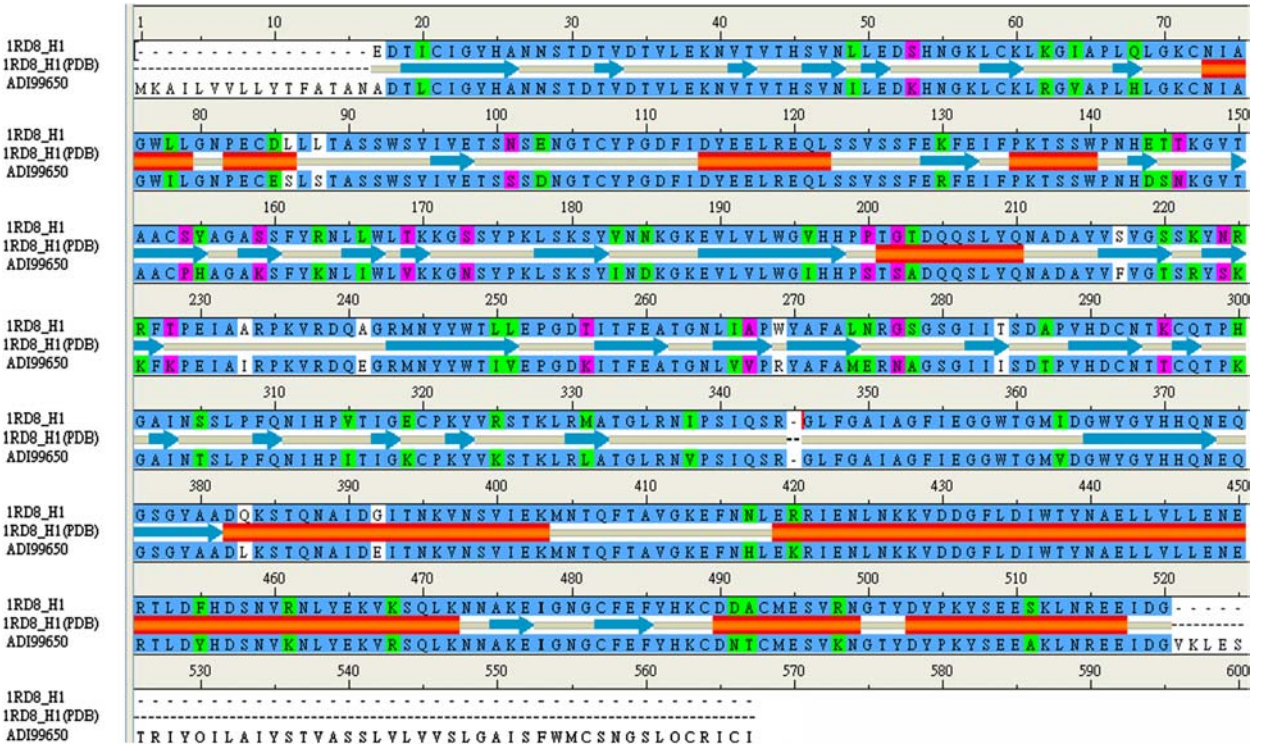


Figure 2. Sequence alignment of H1 (sequence identity: 83.2% and similarity: 92.1%).

protein. We used DS as the primary scoring function for ranking derivatives. The DS of the control, NAG, was used as the filtering basis. Derivatives with DS higher than control were further judged based on piecewise linear potential (PLP), PLP2, and -potential of mean force. The top 10 derivatives are shown in Table 2. The top 10 *de novo* products are mostly derived from xylopine and rosmaricine, and they all share the addition of benzene ring and positive charged 2-aminopyridinium (Table 3).

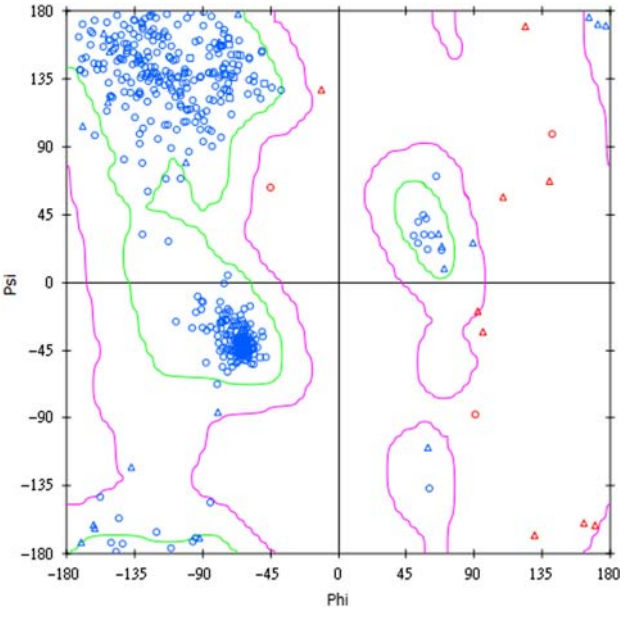


Figure 3. Ramachandran plot of H1. Residues of about 94.4% are in the allowed region, while only 5.6% of residues are in the semi-allowed region or disallowed region.

3.3 *Characteristics of* de novo *product binding conformations*

Our docking results show that all the top 10 derivative compounds have binding interaction with residues, Arg238 and Asp103, that were deemed important in the previous literature [42] (Figure 6). Further analyses of derivative binding conformations suggest that salt bridge

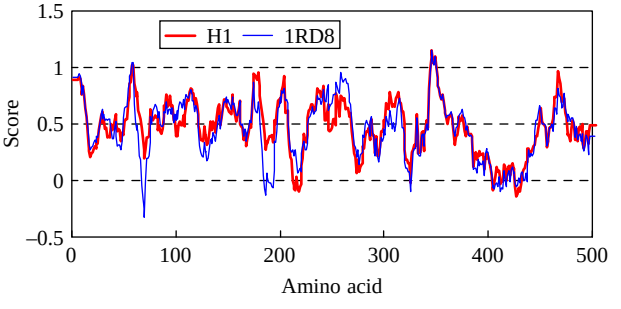


Figure 4. Result of 3D-profile for H1 model structure. A score greater than zero indicates high reliability in the modelling structure.

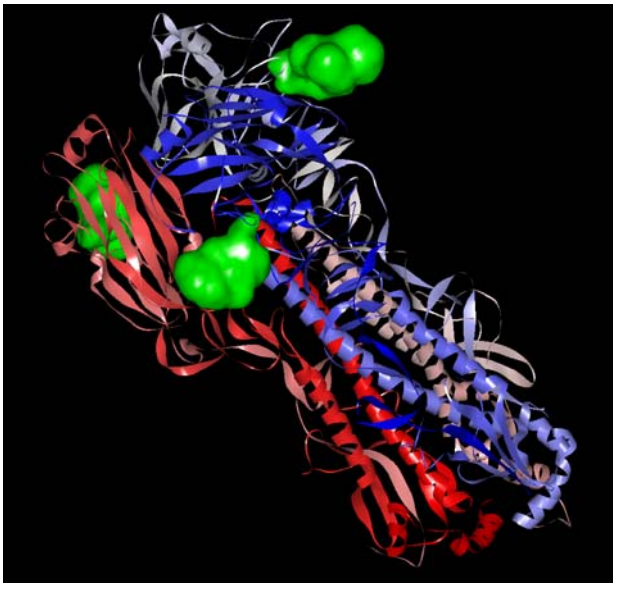


Figure 5. Modelling structures of H1. The binding sites are indicated in green (colour online).

interaction, hydrophobic interaction, hydrogen-bond interaction and π-stacking interaction are the most important non-bonded interactions. Based on these non-bonded interactions and derivative binding conformations, we summarised four key features (Figure 7) that are important for ligand–H1 binding.

Table 1. The docking results of nature compounds. Only top 20 compounds of the database are shown here.

Name	DS	PLP1	PLP2	PMF
Glycyrrhizic acid	94.86	55.27	57.91	107.59
Cynarin	93.52	54.20	62.49	68.70
Rosmarinic acid	92.10	71.65	76.59	42.55
Ergotamine	91.27	52.73	52.63	76.26
Tangshenoside I	87.77	64.95	78.17	64.28
Amentoflavone	86.28	56.85	61.44	95.28
Digallic acid	85.08	60.46	58.72	52.13
Glutinic acid	84.01	38.45	41.30	67.78
Eugeniin	83.74	62.25	70.18	68.99
Xylopine	79.59	30.64	32.89	59.54
Picrocrocinic acid	79.16	37.20	36.88	62.62
Rosmaricine	79.09	38.12	36.51	71.28
3-*O*-feruloylquinic acid	79.07	73.57	59.51	55.08
Procyanidin C1	79.06	46.63	51.98	74.49
Songbeisine	78.65	30.74	32.56	60.77
Galloyl-oxypaeoniflorin	78.32	81.94	88.67	82.60
Heliotrine	78.17	28.52	26.83	50.39
Schaftoside	77.57	62.71	63.10	77.72
Cholic acid	76.65	67.88	67.88	54.08
Rutin	74.47	80.21	87.42	76.89
NAG*	54.82	62.68	65.35	73.39

Notes: DS, dock score; PLP, piecewise linear potential; PMF, potential of mean force and NAG*, control set.

Table 2. The docking results of top 10 derivatives.

Name	DS	PLP1	PLP2	PMF
Xylopine_2	144.18	37.33	40.03	45.62
Rosmaricine_14	138.92	31.03	31.41	82.25
Rosmaricine_15	135.67	37.86	37.50	99.24
Rosmaricine_5	97.92	79.98	84.81	93.56
Rosmaricine_16	96.53	75.60	78.85	88.38
Rosmaricine_23	95.85	69.71	67.90	91.22
Rosmaricine_12	95.52	38.66	37.16	81.00
Rosmaricine_6	95.43	41.85	39.70	81.50
Rosmaricine_21	95.23	42.88	41.21	79.77
Rosmaricine_11	94.73	74.06	76.23	91.65
NAG*	54.82	62.68	65.35	73.39

Notes: DS, dock score; PLP, piecewise linear potential; PMF, potential of mean force and NAG*, control set.

3.3.1 NH_2^+ and NH_3^+ groups

Xylopine, rosmaricine and their derivatives all contain protonated amine group that can form interaction with hydrogen bond or salt bridge interaction with binding site residues. As shown in Figure 6, the protonated amine group could form salt bridge interaction with Glu83 or with Asp103.

3.3.2 2-Aminopyridinium and benzene groups

2-Aminopyridinium group is found on the top three derivatives, Xylopine_2, Rosmaricine_14 and Rosmaricine_15, while the benzene ring is present in the rest. Addition of 2-aminopyridinium group gives significant increase in binding affinity and also influences the binding conformations. Xylopine_2, Rosmaricine_14 and Rosmaricine_15 all have salt bridge interactions with Asp103 (Figure 6(a) and (b)). In contrast, the other derivatives lack the positive charge characteristic of 2-aminopyridinium and, therefore, can only form hydrophobic interactions with hydrophobic residues. These results demonstrate the importance of the 2-aminopyridinium in the H1 inhibitor design.

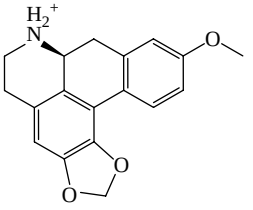
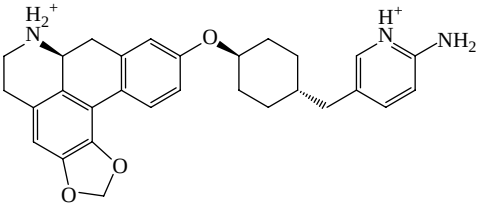
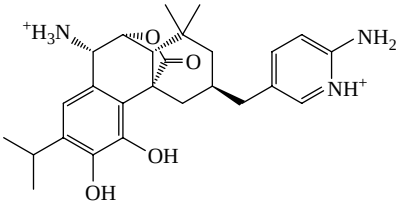
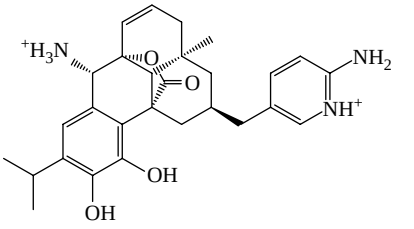
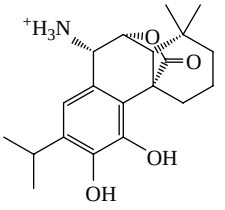
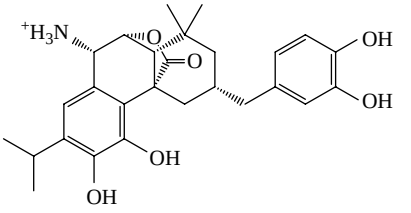
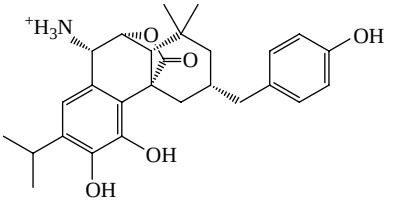
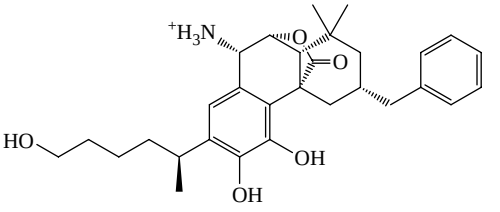
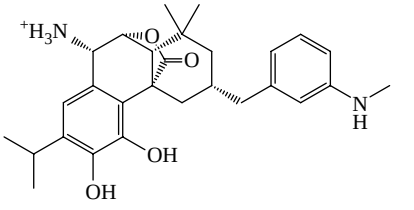
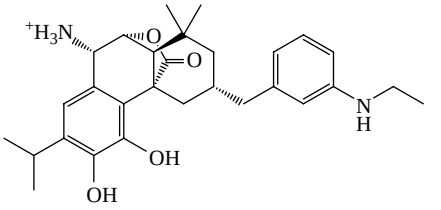
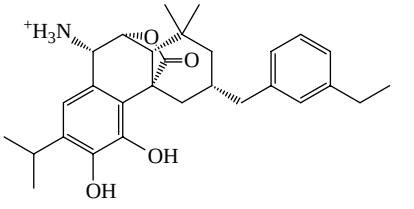
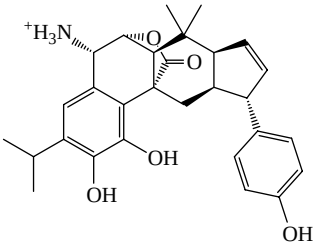
3.3.3 Isopropyl group

Presence of isopropyl group is important for 2-aminopyridinium-substituted rosmaricine derivatives to have hydrophobic interaction with hydrophobic residues of the H1 binding site (Figure 6(a) and (b)). This interaction is suggested to stabilise the previously discussed salt bridge interaction with Asp103 and to prevent flipping in the binding conformation.

3.3.4 Carbonyl group

Carbonyl group is a common characteristic found in rosmaricine derivatives. The electronegative oxygen of

Table 3. The scaffold of original compounds and top 10 derivatives.

Name	Derivatives
Xylopine	Xylopine_2
	
	Rosmaricine_14
	
	Rosmaricine_15
	
Rosmaricine	Rosmaricine_5
	
	Rosmaricine_16
	
	Rosmaricine_23
	
	Rosmaricine_12
	
	Rosmaricine_6
	
	Rosmaricine_21
	
	Rosmaricine_11
	

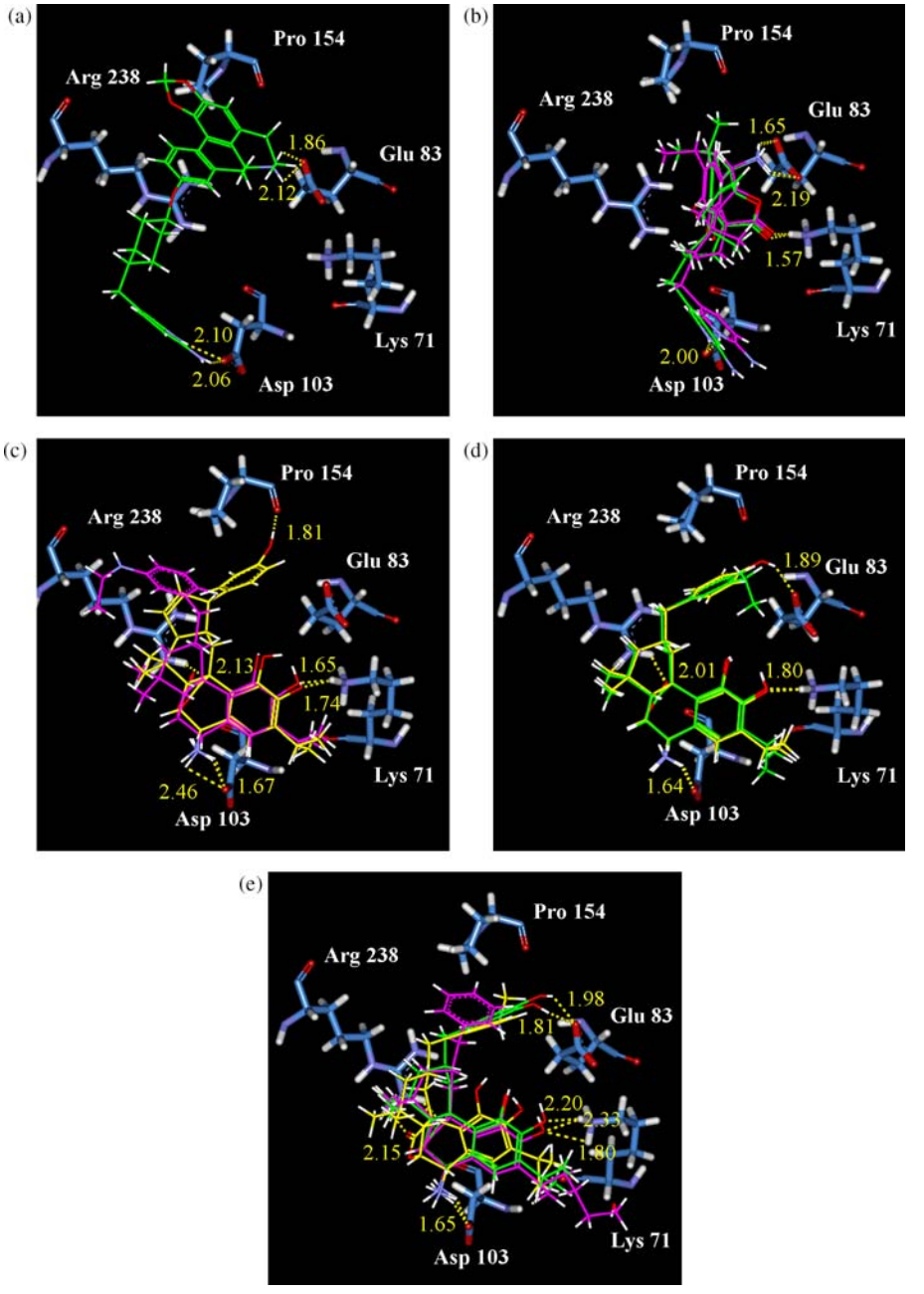


Figure 6. Docking pose of top 10 derivatives in H1 – (a) Xylopine_2, (b) Rosmaricine_14 (purple) and Rosmaricine_15 (green), (c) Rosmaricine_6 (purple) and Rosmaricine_11 (yellow), (d) Rosmaricine_16 (yellow) and Rosmaricine_21 (green), (e) Rosmaricine_5 (green), Rosmaricine_12 (yellow) and Rosmaricine_23 (purple) (colour online).

carbonyl could act as a hydrogen bond acceptor in forming hydrogen bond interaction. The presence of carbonyl group in Rosmaricine_14 and Rosmaricine_15 allows with hydrogen bond interaction the Lys71 of H1.

4. Conclusion

Our docking and *de novo* design give 10 candidate compounds that bind to key residues, as found in the previous literature and as well as non-mutated residue in H1 binding site. Of the four key features obtained from structural analysis, the addition of 2-aminopyridinium has positive contribution in the binding affinity of xylopine and rosmaricine to H1. We hope that these results can have positive contribution to H1 inhibitor design and that the compounds suggested in this study can be further developed into anti-influenza compounds.

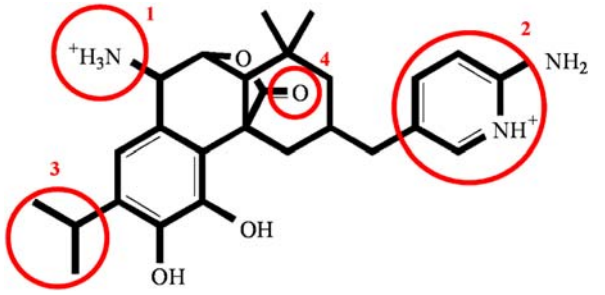


Figure 7. The four points in Xylopine_2 and Rosmaricine_14 for designing inhibitors of H1.

Acknowledgements

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