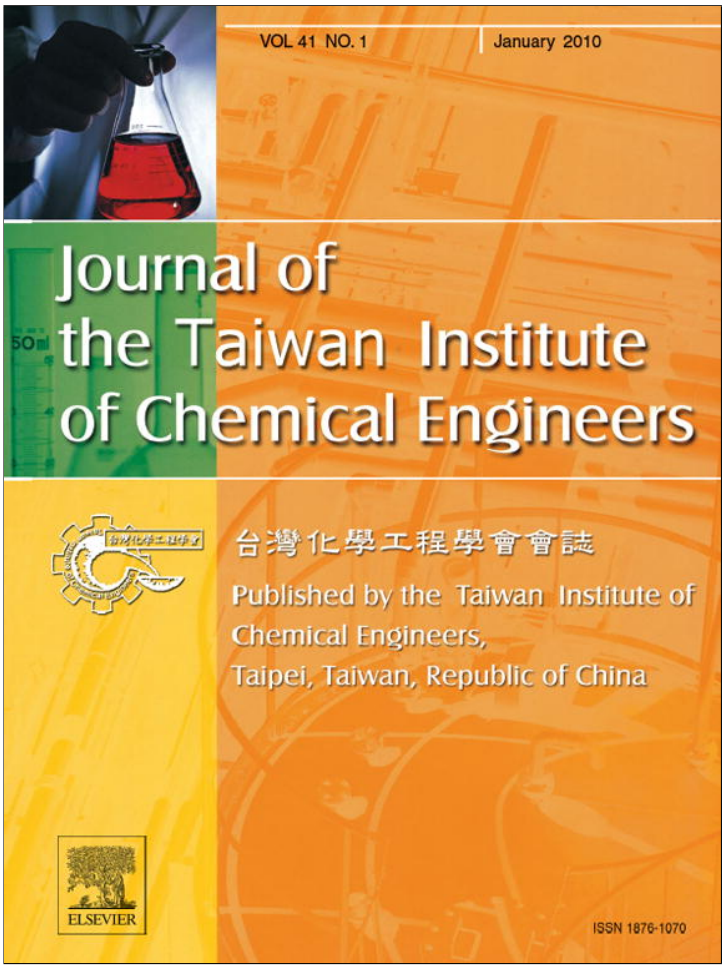

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Drug design for Influenza A virus subtype H1N1

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ABSTRACT

An outbreak of influenza A virus subtype H1N1, also known as swine flu, in Mexico was occurred in April 2009. To design drugs for treating this epidemic is urgency. In this study, we employed the new sequences (2009) to build the N1 simulation structure by homology modeling, which has been checked for high reliability by Verify Score and Ramachandran plot. The latest H1 homology model was employed from Chen's report. 365,602 compounds from NCI database have been screened by docking study of H1 and N1, respectively. And then, nine candidates were screened and suggested as potent dual target candidates from the docking studies. In our investigation, drug resistance was found by our molecular simulation in the new N1 modeling structure to oseltamivir. However, the mechanism is still not clear; further clinical investigations are urgently required.

1. Introduction

Since March 2009, an outbreak of H1N1 influenza in Mexico has led to hundreds of confirmed cases and a number of deaths. On April 28, the new strain was suspected to infect more than 2500 individuals worldwide and 152 attributed deaths. The U.S. Centers for Disease Control and Prevention warned that the outbreak could be pandemic. On April 27, 2009, the World Health Organization raised their alertness level from 3 to 4 worldwide in response to sustain human-to-human transfer of the virus, and the situation was raised to level 5 on April 29. Moreover, on June 11, 2009, the WHO declared an H1N1 pandemic, moving the alert level to phase 6, marking the first global pandemic since 1968. Hence, there is an urgent need to find the resolution for this international problem. Unfortunately, H1N1 virus was reported that it has gained drug resistant for oseltamivir (Collins *et al.*, 2008; Hauge *et al.*, 2009; Moscona, 2009). Hence, a new drug is required against this epidemic.

The membranes of influenza virus contain haemagglutinin (HA) and neuraminidase (NA), they both are glycoproteins. Haemagglutinin has 16 subtypes (H1, H2, H3, . . ., H16) and neuraminidase (N1, N2, N3, . . ., N9) has 9 subtypes. They assort the type of influenza A viruses (Mukhtar *et al.*, 2007; Shirvan *et al.*, 2007). Cell-surface sialic acid receptor to bind to initiate virus infection was mediated by HA, and sialic acid was removed from virus by NA. By the above two steps, cellular glycoproteins improve virus releasing and the spread of infection to new cells, respectively (Raymond and Leach, 2007; Takabatake *et al.*, 2007). By blocking haemagglutinin or neuraminidase could prevent virus from invading into host cells (Russell *et al.*, 2006; Shimbo *et al.*, 2007). Both zanamivir (Relenza) and oseltamivir (Tamiflu) are neuraminidase inhibitors (Collins *et al.*, 2008; Ho *et al.*, 2007). Influenza A virus subtype H1N1 is the most common cause of influenza in humans (Palese, 2004). Some strains of H1N1 are human endemic; such as the pandemic flu in 1918, 50–100 million people were killed worldwide (Kash *et al.*, 2006; Kobasa *et al.*, 2007). Less virulent H1N1 strains which roughly caused half of flu infections in 2006 has still existed (Cheung *et al.*, 2002; Kash *et al.*, 2006; Kobasa *et al.*, 2007; Palese, 2004); other strains of H1N1 in swine and fowls are endemic. In the past few years, many reports indicated that virtual screening techniques were feasible (Chen and Chen, 2007; Chen, 2008a,b,c; Chen, 2009a,b,c; Chen *et al.*, 2008, 2009a, b). The experimental procedure flow chart was revealed in Fig. 1. In this

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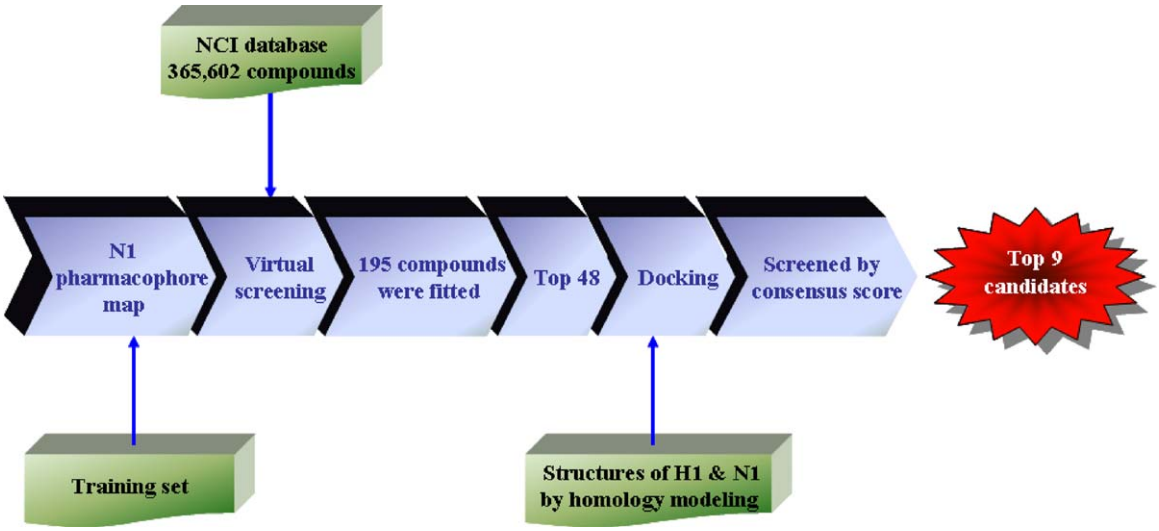

Fig. 1. The flow chart of overall experimental procedures in this study.

study, we have built the latest N1 structure model by homology modeling. In the other hand, the latest H1 homology model was employed from Chen's report (Chen *et al.*, 2009a, b). 365,602 compounds from NCI database have been screened by docking study of H1 and N1, respectively. We aimed at figuring out potent candidates for N1 and H1 for the 2009 outbreak of influenza A H1N1.

2. Materials and methods

2.1. Sequence alignment and homology modeling

All programs in this study were performed by Discovery Studio 2.0 (Accelrys, San Diego, CA, USA). The new sequences (2009) of H1 and N1 were downloaded from NCBI influenza virus sequence database. The templates of H1 and N1 were downloaded from protein data bank (PDB). Their structures had been released in 2004 and 2006, respectively (PDB ID: 1RD8 and 2HU0). The multiple sequence alignment method was based on the CLUSTAL W program and progressive pairwise alignment algorithm (Thompson *et al.*, 1994). The alignment scoring matrix was set in BLOSM by default. 1RD8 and 2HU0 were applied to build the latest structure of the H1 and N1 sequence, respectively.

2.2. NCI database screening

NCI database, which contented 365,602 compounds, was provided by National Center for High-performance Computing. The catalyst compare/fit algorithm was employed to screen the compounds from NCI database, and then, the docking protocol of LigandFit was used to rank the compounds by scoring functions.

2.3. Docking study

All of the compounds were built and energy minimized under MM2 force field by ChemOffice 2005. The LigandFit program performed the docking simulation at the binding site by Discovery Studio 2.0. During the docking procedure, ligands were flexible whereas the receptor was fixed. The ligand flexibility was carried out by In Situ Ligand Minimization based on CHARMm force field. Docking score (DS) was employed to score the docking results. Candidate ligand poses are evaluated and prioritized according to the DockScore function. There are two types of DockScore. One is based on a force field approximation, the other on the Piecewise Linear Potential function (PLP)

$$\text{DockScore (force field)} = -\left(\frac{\text{ligand}}{\text{receptor interaction energy}}\right) - \text{ligand internal energy} \quad (1)$$

$$\text{DockScore (PLP)} = -(\text{PLP potential}) \quad (2)$$

As shown in Eq. (1), there are two energy terms in the force field version of DockScore, internal energy of the ligand and the interaction energy of the ligand with the receptor. The interaction energy is taken as the sum of the van der Waals energy and electrostatic energy. The computation of the interaction energy can be quite time consuming. To reduce the time needed for this calculation, a grid-based estimation of the ligand/receptor interaction energy is employed. Piecewise Linear Potential is a fast, simple, docking function that has been shown to correlate well with protein–ligand binding affinities. PLP scores are measured in arbitrary units, with negative PLP scores reported in order to make them suitable for subsequent use in consensus score calculations. Higher PLP scores indicate stronger receptor–ligand binding (larger pK_i values). Additionally, PMF was computed by summing pairwise interaction terms

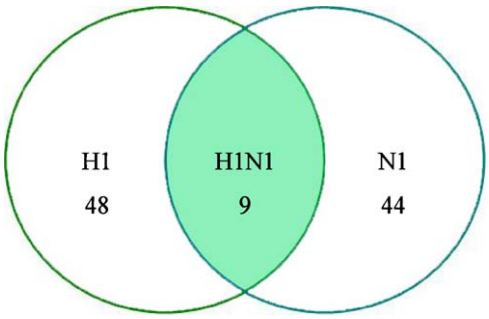

Fig. 2. The screening results of H1 and N1 by docking study. There are 48 and 44 compounds listed in H1 and N1 docking results, respectively. There are 9 compounds overlapped in the set-theoretic intersection.

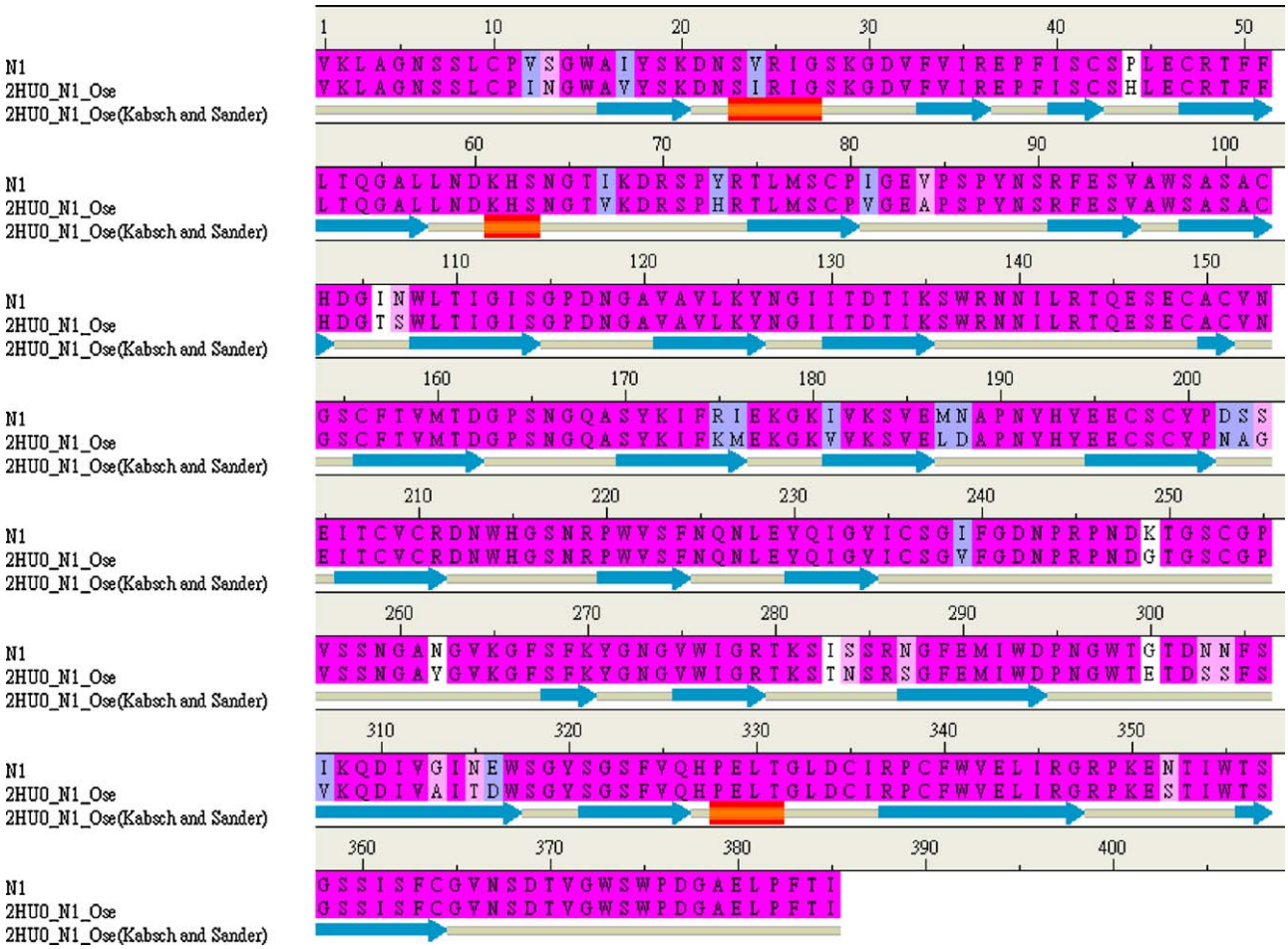

Fig. 3. The results of sequence alignment analysis. The sequence identity and similarity between the latest N1 sequence and 2HU0 are 91.4% and 95.6%, respectively.

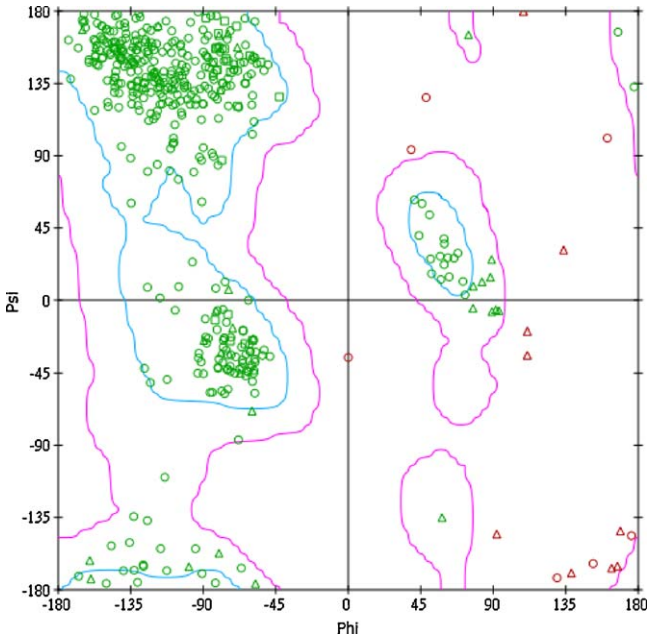

Fig. 4. Ramachandran plot of modeling structure of N1. Glycine is labeled by triangles. It shows only 3.4% out of the region of possible angle formations.

over all interatomic pairs of the receptor–ligand complex. Otherwise, the consensus score (CS) protocol calculates the consensus scores of a series of docked ligands for which other scores have been previously computed. For each selected scoring function, the ligands are listed by score in descending order. The consensus scores for each molecule were employed as a judgment viewpoint for ranking the investigated compounds. For each selected scoring function, the ligands are selected by score in descending order. The consensus scores were employed

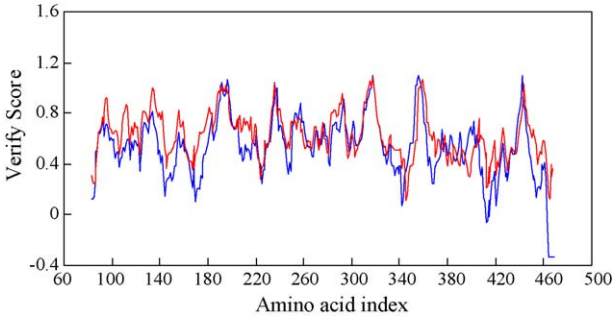

Fig. 5. The Verify Score diagram validates the N1 homology model. The amino acid from 119 to 293 is the putative major binding site. The blue line and the red line are the latest N1 sequence and the template (PDB ID: 2HU0), respectively.

as a judgment for ranking the potent candidates. Comparing the docking results of H1 and N1, the overlapped compounds in the set-theoretic intersection were suggested as the candidates for dual target inhibitors (Fig. 2).

3. Results and discussion

3.1. The results of homology modeling

The homology model of H1 had been published in our previous paper (Chen *et al.*, 2009a, b). The sequence identity and similarity were 70.8% and 78.9%, respectively. The results of alignment of N1 sequences were shown in Fig. 3. The sequence identity and similarity of N1 sequences were 91.4% and 95.6%, respectively. Accordingly, the alignment results were employed to build new homology model. Reliability of new homology model for N1 was identified by Ramachandran plot and Verify Score (Figs. 4 and 5). The Ramachandran plot indicated the region of possible angle formations by φ (phi) and ψ (psi) angles. The conventional terms represented the torsion angles on either side of alpha carbon in peptides. The results of Ramachandran plot showed only 3.4% were out of the region in our homology modeling. In Fig. 5, the putative region of binding site on N1 sequence was amino acid index 119–293. The results showed that some amino acids had lower scores than zero, however, they did not locate on the region of binding site. Thus, it is reasonable that all the findings are reliable and these factors could not affect the overall judgments.

3.2. The results of docking study

All the mentioned compounds provided by the NCI database were docked into H1 and N1 structures, respectively. The

Table 1
The docking results of the fifty compounds with H1.

Name	LigS1	LigS2	−PLP1	−PLP2	Jain	-PMF	-PMF04	DS	CS
Destomycin-A	4.72	4.35	46.64	46.71	0.35	67.82	20.32	47.29	8
NCI0624650	5.14	4.52	33.96	44.38	1.92	67.09	29.22	44.93	7
NCI0607158	4.32	4.03	39.37	33.30	−1.46	59.57	18.16	46.00	6
NCI0605741	4.56	4.33	46.85	46.78	2.36	41.36	7.12	43.15	6
NCI0608647	4.94	4.20	31.56	31.19	−0.52	76.04	36.82	42.07	6
BB-K-89	3.96	4.24	45.99	45.78	−0.80	57.22	12.29	41.90	6
Protoverine	4.24	4.42	43.88	52.14	−0.29	34.51	−1.08	41.52	6
IPX-750	4.80	4.54	49.87	57.00	1.56	34.77	3.13	40.23	6
NCI0353858	4.21	3.49	21.40	26.58	0.35	69.00	39.62	64.60	5
NCI0605737	4.75	3.91	27.83	30.26	-0.41	74.57	39.51	44.73	5
Gentamycin-A	4.15	4.36	40.58	39.27	-0.23	29.49	4.30	38.40	5
Kanamycin-C	4.19	4.60	52.82	57.44	0.26	34.32	12.73	38.02	5
NCI0685277	4.43	4.29	41.48	44.62	1.00	28.83	−1.88	37.69	5
NCI0685281	4.43	4.29	41.48	44.62	1.00	28.83	−1.88	37.69	5
Apramycin	5.01	4.64	66.28	67.52	1.05	30.64	1.82	31.62	5
NCI0608643	4.07	4.41	36.85	35.90	−1.96	27.31	1.35	41.15	4
NCI0606258	3.39	4.19	35.53	33.49	-1.69	61.99	18.05	41.09	4
NCI0608650	4.12	3.62	24.44	24.57	−1.43	65.67	34.59	39.85	4
GP-1–515	4.26	4.00	30.92	29.08	−0.26	30.53	7.32	38.81	4
GP-515	4.26	4.00	30.92	29.08	−0.26	30.53	7.32	38.81	4
NCI0671266	4.13	3.36	23.89	27.73	−0.66	85.59	44.36	38.81	4
4″-Deoxytobramycin	4.63	4.42	33.02	33.74	−1.79	79.24	38.29	33.01	4
Sphingosine-phosphate-1	4.06	4.27	44.73	44.77	−1.52	51.41	11.03	25.87	4
Dihydroacarbose	5.24	4.55	48.60	52.66	−2.66	22.15	−1.27	6.86	4
Zanamivir	4.34	3.80	29.32	31.90	−1.08	85.92	37.05	45.00	4
NCI0611895	3.59	3.99	21.88	18.33	−2.57	59.60	28.34	42.46	3
NCI0607157	4.05	3.79	39.61	37.31	−1.63	31.77	5.06	41.12	3
Actinospectinoic acid	2.91	3.15	10.84	15.82	−2.45	49.60	19.44	38.84	3
Phosphatidylserine	3.48	3.51	16.75	13.26	−0.37	50.90	23.99	32.84	3
NCI0275619	2.88	3.50	23.40	26.12	0.60	53.55	26.13	32.71	3
Etimicin	1.10	3.86	38.96	37.94	−1.32	47.11	11.55	31.51	3
Gentamicin	1.10	3.86	38.96	37.94	−1.32	47.11	11.55	31.51	3
Gentamycin-C1A	1.10	3.86	38.96	37.94	−1.32	47.11	11.55	31.51	3
Benanomicin-B	2.94	4.03	48.00	41.39	−3.23	33.47	2.83	30.71	3
NCI0608654	2.56	3.24	26.48	27.34	−1.30	42.56	21.02	41.98	2
Methyl-oligobiosaminide	3.12	3.33	27.05	34.86	−1.21	44.88	25.47	38.43	2
Dibekacin	2.55	3.20	21.82	20.73	−3.97	52.53	15.62	35.29	2
Hydroxyvalidamine	3.18	3.12	13.78	16.33	−1.14	52.67	26.77	33.87	2
Oseltamivir	3.05	3.99	50.47	46.67	−0.88	−6.35	−30.19	23.90	2
NCI0158489	4.06	3.90	29.95	34.48	−1.29	37.31	13.05	39.63	1
NCI0345087	3.10	3.41	26.09	29.65	−1.64	37.33	13.89	24.72	1
NCI0521703	2.01	3.79	25.97	23.76	−0.76	26.03	−12.23	24.67	1
NCI0521704	2.01	3.79	25.97	23.76	−0.76	26.03	−12.23	24.67	1
SCH-21561	1.11	2.86	13.83	12.37	−4.14	40.90	17.74	24.23	1
Pradimicin-FL	3.55	3.98	37.29	33.07	−5.95	40.70	8.46	14.06	1
Desmosine	1.75	3.38	35.05	27.79	−4.73	17.63	−1.30	34.04	0
NCI0187635	−6.82	−11.83	−23.22	−12.94	−2.91	38.35	6.38	26.00	0
NCI0187646	−6.82	−11.83	−23.22	−12.94	−2.91	38.35	6.38	26.00	0
LU-15-089	1.12	3.35	35.11	33.51	−2.38	−9.65	−9.14	25.60	0
Streptoimidazolidine	1.46	2.87	15.22	13.37	−6.45	39.55	11.67	24.47	0

DS: docking score; CS: consensus score.

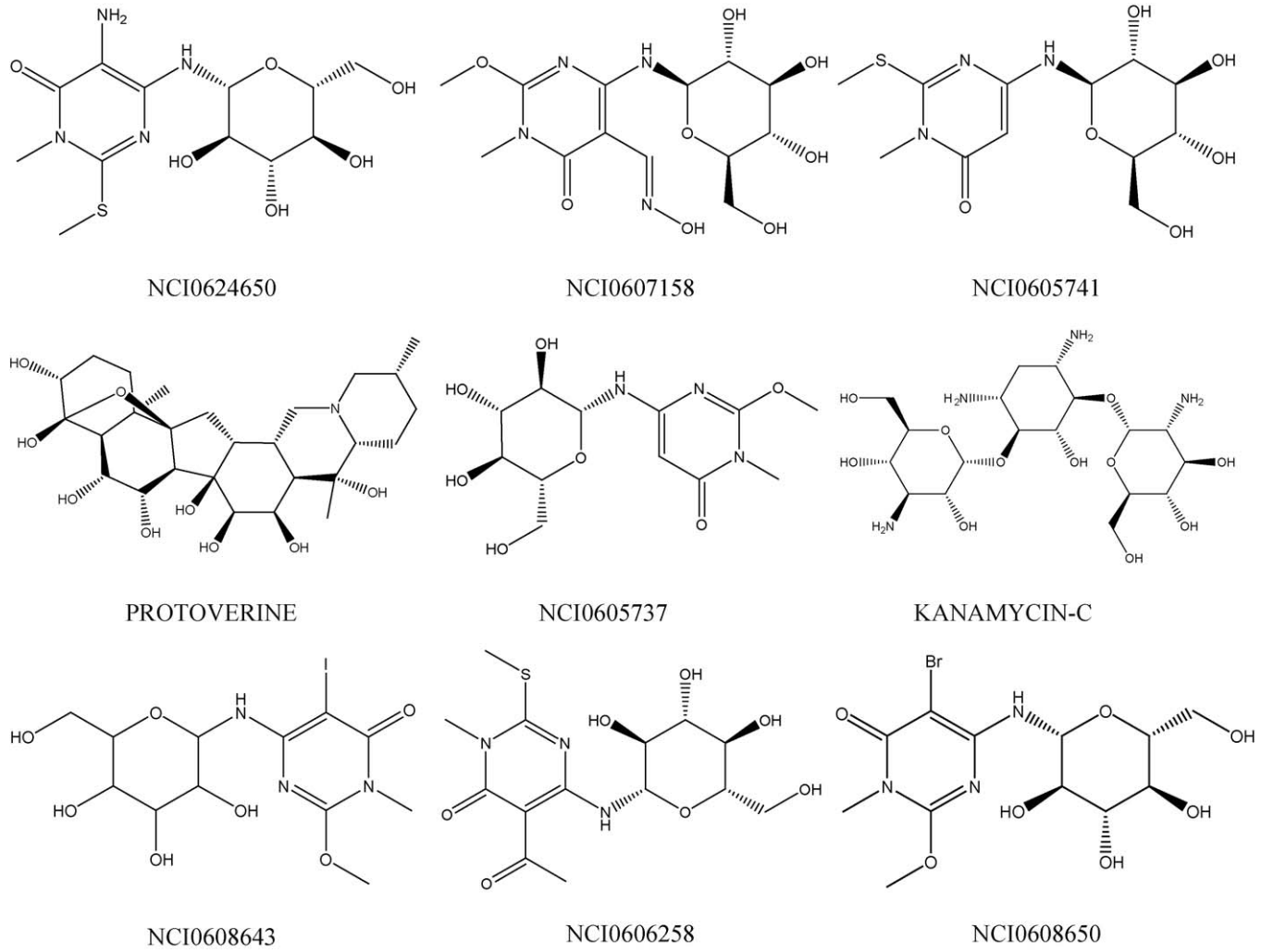

Fig. 6. The chemical structures of the 9 candidates.

docking results of the fifty compounds with H1 were shown in Table 1. NCI0353858 had the highest docking score, even higher than the standard controls, zanamivir and oseltamivir. In fact, zanamivir and oseltamivir were commonly used as inhibitors for NA, drugs for previous H1N1. As expected, zanamivir had a high affinity for H1 in the identification and judgment of this study. Interestingly, a few compounds, such as NCI0353858, DESTOMYCIN-A, and NCI0607158, were more suitable than zanamivir.

In Table 2, zanamivir had 78.41 as its docking score, which means that zanamivir may still had a high efficacy for the latest N1 in 2009. However, the other commonly used previous drug oseltamivir had only 44.91 as its docking score. In our previous study, oseltamivir had 53.3 in docking score for N1 in 2004 (data not shown). Thus, the latest N1 might have developed its drug resistance to oseltamivir, from the evidence above. The results were consistent with a few recent literatures (Collins *et al.*, 2008; Hauge *et al.*, 2009; Moscona, 2009). In Table 2, protoverine and NCI0607158 had even higher docking score than zanamivir, and they were suggested as potent dual target compounds.

3.3. The results of virtual screening by scoring functions

There were top 9 dual-target inhibitor candidates selected from docking results by scoring functions: NCI0624650, NCI0607158, NCI0605741, protoverine, NCI0605737, Kanamycin-C, NCI0608643, NCI0606258, and NCI0608650 (Fig. 6). Compared with zanamivir, NCI0607158 has not only higher docking score but also consensus score. Possibly, NCI0607158 might have high activity in *in vitro* and/or *in vivo* study. The docking poses in H1 and N1 of these nine candidates were shown in Fig. 7. In H1, the residuals of the binding site were like fingers to clutch the ligands by several hydrogen bonds. The half-opened access shape of H1 binding site may increase the difficulty for forming the ligand–protein complex. Among them, NCI0353858 has been reported that it might be the potent H1 inhibitor by Chen *et al.* (2009a, b). Considered from the viewpoint of increasing the binding affinity for N1, the PLP score might play an important role in this study. PLP scores showed a consistent trend with that of docking scores (Table 2). In our study, the major explanation for its lower binding affinity of oseltamivir in N1 was that it had lower PLP score in its docking analysis outcome with the latest N1 structure. Extending

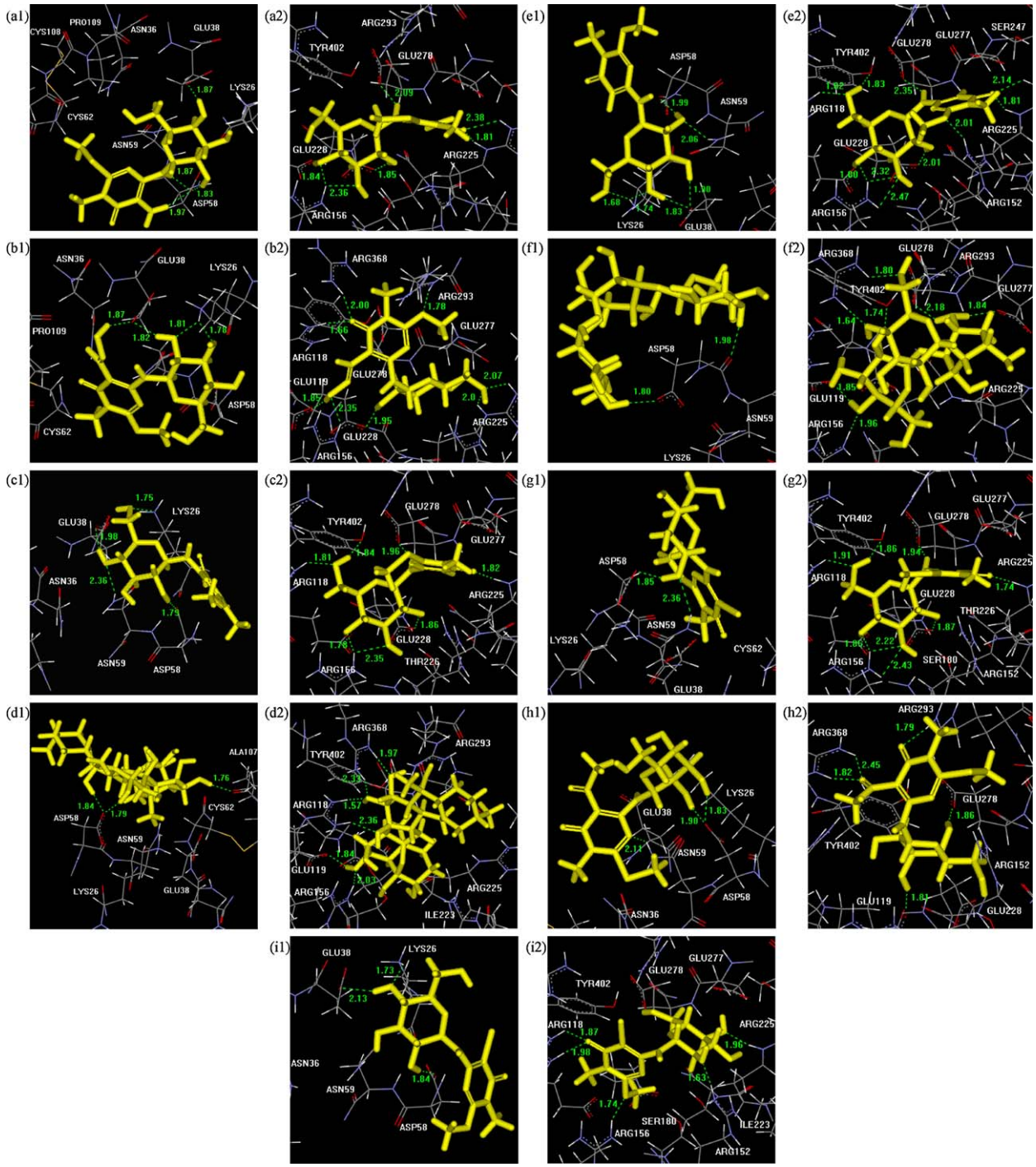

Fig. 7. The docking poses of the 9 candidates in H1 (a1–i1) and N1 (a2–i2), respectively. (a) NCI0624650, (b) NCI0607158, (c) NCI0605741, (d) protoverine, (e) NCI0605737, (f) KANAMYCIN-C, (g) NCI0608643, (h) NCI0606258, and (i) NCI0608650.

Table 2
The docking results of the 46 compounds with N1.

Name	LigS1	LigS2	−PLP1	−PLP2	Jain	−PMF	−PMF04	DS	CS
Kanamycin-C	6.94	6.92	93.83	105.07	6.43	230.59	165.70	72.23	8
Protoverine	6.94	6.14	71.04	84.73	4.19	244.60	130.43	81.01	8
Zanamivir	5.68	5.73	72.21	77.73	1.49	200.16	123.85	78.41	8
NCI0624650	6.28	5.96	73.34	70.98	4.13	195.08	124.74	70.28	8
NCI0611895	6.86	6.97	69.55	75.67	5.62	195.20	136.36	76.18	8
Apramycin	6.67	6.07	97.07	92.38	4.31	210.78	152.16	68.39	8
NCI0608654	6.81	6.41	74.08	79.23	5.06	191.55	125.29	68.81	8
NCI0608643	6.80	6.26	72.65	75.62	5.25	194.81	125.48	70.23	8

Table 2 (*Continued*)

Name	LigS1	LigS2	−PLP1	−PLP2	Jain	−PMF	−PMF04	DS	CS
NCI0608650	6.61	5.96	66.67	73.31	4.26	185.81	121.62	74.41	8
NCI0605741	6.71	6.35	69.09	73.33	4.77	190.73	121.03	73.67	8
NCI0605737	6.60	6.05	67.56	75.32	5.15	188.56	118.85	71.18	8
NCI0607157	6.68	6.04	69.36	77.12	5.74	178.45	128.09	77.22	8
NCI0607158	6.78	6.23	73.52	80.63	5.21	204.98	135.52	83.52	8
NCI0606258	6.52	5.93	68.04	73.57	5.37	201.98	128.54	77.03	8
Oseltamivir	4.93	4.31	33.85	38.08	2.84	180.44	98.36	44.91	7
Methyl-oligobiosaminide	6.30	5.92	68.37	79.88	2.42	190.64	131.79	71.41	7
Dibekacin	6.55	6.30	70.76	74.23	4.92	220.30	153.95	64.58	7
Benanomicin-B	7.54	5.88	104.70	101.44	4.78	216.61	139.35	19.84	7
NCI0671266	6.71	6.30	72.77	77.41	2.10	206.59	149.51	82.10	7
BB-K-89	7.15	6.46	97.05	76.41	5.38	179.67	127.28	59.88	7
NCI0158489	6.62	6.14	66.14	66.96	3.49	175.24	117.37	72.43	7
Destomycin-A	6.34	5.10	76.54	71.82	3.19	208.11	155.67	80.09	6
SCH-21561	5.53	4.92	72.56	78.35	6.08	204.71	135.57	35.61	5
LU-15-089	6.53	6.52	87.94	91.05	4.52	172.35	92.19	62.60	5
4″-Deoxytobramycin	6.09	6.00	69.01	65.97	2.51	185.19	131.35	56.64	5
NCI0685277	6.25	5.55	68.65	80.38	3.35	180.02	108.89	61.29	5
NCI0685281	6.25	5.55	68.65	80.38	3.35	180.02	108.89	61.29	5
NCI0608647	5.92	6.51	71.24	67.83	4.95	110.64	75.49	73.38	5
IPX-750	6.31	5.88	59.10	72.04	3.16	128.60	90.63	71.99	4
Etimicin	5.98	5.64	74.61	64.91	5.47	204.90	129.22	52.50	4
Gentamicin	5.98	5.64	74.61	64.91	5.47	204.90	129.22	52.50	4
Gentamycin-C1A	5.98	5.64	74.61	64.91	5.47	204.90	129.22	52.50	4
Desmosine	6.93	5.94	78.44	83.02	1.73	171.65	115.90	45.11	4
Gentamycin-A	6.79	5.26	71.71	64.17	2.03	218.87	159.36	60.45	4
Actinospectinoic acid	6.49	5.96	64.50	65.78	2.46	217.50	139.58	63.91	4
NCI0345087	6.37	5.83	68.01	65.70	3.72	165.36	111.23	58.00	3
Sphingosine-phosphate-1	5.91	4.95	63.62	73.98	−1.28	180.82	83.79	53.95	2
Phosphatidylserine	5.57	5.53	47.51	49.41	4.05	119.48	82.96	68.30	2
NCI0521703	5.37	6.01	46.60	46.86	4.40	116.76	95.14	57.74	2
NCI0521704	5.37	6.01	46.60	46.86	4.40	116.76	95.14	57.74	2
Hydroxyvalidamine	5.41	5.77	58.93	60.98	4.48	104.51	67.53	58.55	1
NCI0275619	4.41	4.68	40.72	46.14	3.24	105.74	71.61	56.66	1
GP-1-515	5.27	4.82	34.81	28.74	−0.14	118.00	94.03	59.51	0
GP-515	5.27	4.82	34.81	28.74	−0.14	118.00	94.03	59.51	0
NCI0187635	3.96	4.82	49.58	37.41	1.12	124.82	79.27	53.53	0
NCI0187646	3.96	4.82	49.58	37.41	1.12	124.82	79.27	53.53	0

DS: docking score; CS: consensus score.

the side chain for increasing positive charge may have the effect of improving the binding affinity. The two compounds, protoverine and NCI0607158, had longer molecular length than the other 7 candidates (Fig. 6).

4. Conclusion

In this study, we have built the latest N1 structure model by homology modeling, which has high reliability by Verify Score plot and Ramachandran plot. In addition, the latest H1 homology model was also employed into consideration from our previous paper. Up to 365,602 compounds from NCI database have been screened by docking study of H1 and N1, respectively. After proceeding concisely presented in Fig. 1, nine compounds, NCI0624650, NCI0607158, NCI0605741, protoverine, NCI0605737, Kanamycin-C, NCI0608643, NCI0606258, and NCI0608650 were selected as potent dual-target candidate drugs for H1N1 (Fig. 6). On the other hand, the latest N1 structure might have drug resistance to oseltamivir, suggesting that oseltamivir may not work so well for treatment of this wave of H1N1 influenza. Hopefully, we have proposed some useful candidates for H1N1, and put forward a constructive concept of designing H1N1 inhibitors.

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