Original Article

Energy Based Pharmacophore Modelling and Docking Studies for Determining Potent Inhibitor Against M2 Proton Channel of Influenza Virus

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ABSTRACT

M2 protein, a crucial glycoprotein present on the viral envelope of Influenza A virus plays an important role in the replication and budding of influenza A virus in the host organism. Due to its primal role in the life cycle of influenza A virus, it is targeted by many potent drugs like amantadine and rimantadine. The emergence of M2 protein mutants in the recent years has rendered these drugs ineffective. Keeping this in our minds, an investigation was performed to determine potent inhibitors of M2 protein using computational strategies like e-pharmacophore based virtual screening and molecular docking. A three dimensional pharmacophore model was generated based on the chemical features using PHASE module of Schrödinger Suite. Subsequently, molecules with same pharmacophoric features were screened from a total of 8621 molecules available in the DrugBank database using the generated pharmacophore hypothesis. This was followed by molecular docking of the screened molecules using three methodologies namely HTVS, SP and XP. Ligand filtration was also done to obtain an efficient collection of hit molecules by analysing pharmacokinetic properties by using Qikprop module. Consequently, our study ascertained nine molecules namely, DB00374, DB00770, DB05015, DB08868, DB00631, DB00983, DB01262, DB00837 and DB01048 which were found to possess anti-viral properties. It is also worth mentioning that antiviral activity of these compounds has been previously reported in recent literature.

Key words: Influenza A virus, M2 protein, E-Pharmacophore model, Virtual screening, DrugBank database.

INTRODUCTION

Influenza A is a genus of the Orthomyxoviridae family of viruses which is characterized by segmented, negative-strand RNA genomes with 11 genes. It has a roughly spherical shape and an external structure with approximately 500 spike-like projections.[1] These studded glycoproteins – Haemagglutinin (HA) and Neuraminidase (NA)- are present in a ratio of approximately four to one.[2] The trimer, HA is not only crucial for the formation of replication-competent influenza viruses but also binds to sialic acid residues on cell surfaces facilitating virus entry and cell infection.[3] NA on the other hand has major enzymatic activity in cleaving sialic acid residues and causing the release of newly formed virions from the surface of infected cells.[3] Another crucial protein on the viral envelope is the M2 transmembrane homotetramer protein, which forms the pH-gated proton channel.[4] This channel gets activated by low pH resulting in weakening of the interactions of M1 matrix protein which connects RNA-nucleoprotein core to the membrane envelope. Ultimately, this leads to the release of viral transcriptase into the cytosol of the host.[5] During the viral replication and budding stage, the trans- golgi network carries viral glycoproteins from the endoplasmic reticulum to the host cell surface.[6] Low pH might also cause premature fusion activity leading to conformational

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changes in the HA which renders it inactive. M2 protein plays a major role in neutralizing this acidic pH.[7] All these activity in the influenza viruses causes the infection in the host organism. Subsequently, influenza viruses cause a contagious respiratory illness that is accompanied by fever, headaches, fatigue, cough and generalized weakness.[8] Sometimes its complications increase when it includes secondary bacterial pneumonia, post-influenza encephalitis, changes in cardiac electrocardiogram and secondary bacterial infections.[9] These viruses not only affects humans but also other species like birds, horses and pigs.[9,10]

Emergence of distinct variants of Influenza A virus carrying different combinations of NA and HA glycoproteins have led to three major pandemics of influenza in the 20th century.[11] The Spanish flu of 1918, the Asian flu of 1957 and the Hong Kong flu in 1968 were all caused by variants of Influenza A carrying the H1N1, H2N2 and H3N2 combinations.[12] It has been estimated that this virus causes approximately half a million deaths worldwide every year.[9,10] The severity of each successive pandemic increases due to two reasons. Firstly, Influenza A virus is susceptible to consistent and unpredictable antigenic variations.[8] This was seen in all the three outbreaks of 1918, 1957 and 1968, in which each of them was caused by different antigenic subtypes of influenza.[12] This kind of antigenic instability renders the treatment and prevention of influenza infection relatively or totally ineffective even in case of vaccinated ones.[8,13] Secondly, the high incidence of mutations in influenza A virus as a result of antigenic drift and shift led to change in the influenza viruses every 20-30 years which ended up in new, more toxic and drug resistance virus.[9] Moreover, these shifts also made the job of developing anti-influenza virus drugs very difficult due to rapid mutations in the virus.[14] At the current time, only 2 classes of influenza antiviral agents-M2 ion channel inhibitors and NA inhibitors are being used.[14] It has been reported in recent literatures that the resistance to the antiviral drugs, amantadine and rimantadine, against M2 proton channels has reached more than 90% in humans, birds and pigs.[14] Moreover, rimantadine which has been shown effective in treatment and prevention of influenza has adverse effects involving the gastrointestinal and central nervous systems.[15] These adverse events at the recommended dose in controlled clinical trials include insomnia, dizziness, headache, nervousness, fatigue, nausea, vomiting, anorexia, dry mouth and abdominal pain.[16] All these factors point strongly towards the emerging need for development of novel drugs against influenza A virus which are not susceptible to the mutations in the target protein.[13] In such case the dire need of finding novel drugs for the treatment of influenza virus infection comes to surface. The discovery and development of a new drug is a costly and tedious process involving many trials and errors, hence computer aided molecular design method have been identified as the most promising candidate to concentrate on experimental methods of drug discovery.[17] The most commonly used computational strategies in number of drug discovery projects are docking and molecular simulation approaches. Docking programs are employed to evaluate the binding affinity of ligands with the crystal structure of proteins available in physical or virtual database.[18,19] On the other hand, molecular dynamics simulation is performed to understand the complete and accurate protein-ligand binding mechanism by providing a realistic degree of flexibility in the system under study.[19-22] In the recent era of drug designing, pharmacophore modelling method is broadly used to generate a 3D pharmacophore model which represents the binding mode of ligands with a specific target in study and is represented as 3D arrangement of various chemical features present in the ligands.[23] In contrast, the e-Pharmacophore method generates energetically optimized, structure-based pharmacophore that can be used to rapidly screen millions of compounds while utilizing the Glide XP scoring function. This scoring function aids to accurately characterize protein-ligand interactions, resulting in improved database screening enrichments.[24] Subsequently, a virtual screening process is used in accordance with the molecular docking which has acquired the position of a dynamic and cost-effective technology in finding out novel drug like compounds or so called "hits' in the pharmaceutical industry.[25] Moreover, the technological advances in this screening process allows chemists to promptly screen large databases for effective therapeutics. Of note our study also aims at determining effective drug candidate against M2 proton channel by repositioning of existing drugs using a virtual screening protocol. This would potentially reduce the expense and time associated with early-stage testing of hit compounds.

MATERIALS AND METHODS

Dataset Preparation

A dataset comprising of six potent drugs against influenza A virus infection were taken. All these six drugs, namely rimantadine, amantadine, oseltamivir, zanamivir, peramivir and laninamivir are set of FDA approved drugs which were retrieved from Pubchem database. The drugs, rimantadine and amantadine are the well known inhibitors of M2 proton channel while the other

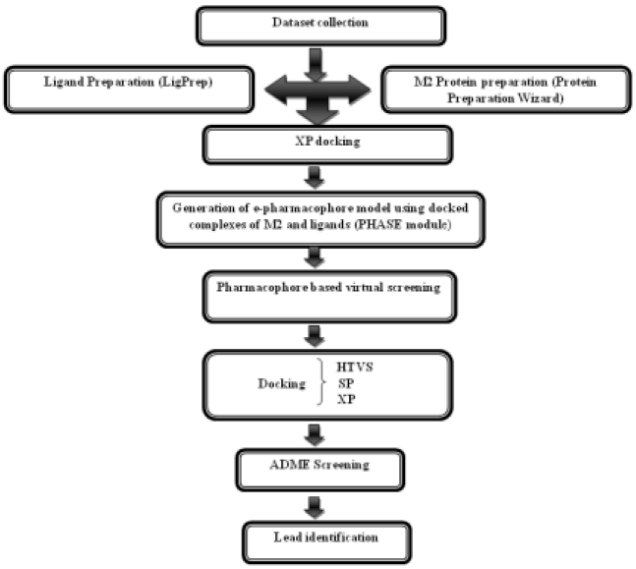

Figure 1: Virtual screening workflow of the lead identification based on e- pharmacophore based drug design.

four are anti-viral drugs against the NA protein of influenza A virus. On the contrary the M2 protein with the resolution of 3.5 Å was retrieved from PDB. The PDB ID of the protein used is 3C9J. Lastly, the molecules used for virtual screening process were taken from DrugBank database. A systematic representation of the activities performed is illustrated in Figure 1.

Ligand Preparation

The drugs retrieved from Pubchem database were prepared using the LigPrep software of Schrödinger Suite. The process of ligand preparation involves 2D to 3D structure conversion, ascertaining ligand's ionisable state at pH 7 and stereoisomers generation, which results in the energy minimization of ligands.[26] Eventually, all the six ligands were prepared under the force field of OPLS-2005. The options of desalt and generate tautomers were deselected in order to keep the ligands in its original state.

Protein Preparation

The 3D structure of M2 protein with PDB ID 3C9J was treated by protein preparation wizard of Schrödinger Suite. This process rectifies any structural faults which may be antecedent in the molecules. Consequently, the protein structure cognitive contents were analyzed. The protein was preprocessed and the water molecules were eliminated up to a distance of 5.0 Å. In addition, heteroatoms which do not have any influence on the protein structure, function or conformation were removed from the protein. The structure was then optimized and minimized using OPLS-2005 force field. Lastly, geometry of the protein was refined with restrained minimization, in which missing residues and loops were evaluated. This process was carried out in such a way that the junction of atom had a default value of RMSD of 0.3 Å.[27]

Molecular Docking and E-Pharmacophore Model Generation

The prepared ligands were XP (Extra Precision) docked with the prepared M2 protein using GLIDE module of the Schrödinger Suite. XP docking is an incisive method which has the ability to bring out physical insight into the binding affinity of a ligand for the binding site of the protein.[28] In addition the XP descriptor generated provides atomic level information such as hydrophobic, hydrogen bond, electrostatic, pi-pi stacking and pi cation interaction.[29] A grid for docking was generated using the GLIDE grid generation option available in the Schrödinger Suite. The broadly used antiviral, rimantadine, against M2 protein is used as the reference ligand to generate grid. Consequently, a rectangular box was generated which was centred on the binding site of M2 protein. Ultimately, the prepared ligands were docked against the grid obtained for M2 protein structure.

Furthermore, these docked complexes were employed to develop an e-pharmacophore model. The technique of e-pharmacophore modelling includes usage of structure and energy based scoring function in GLIDE.[28] The models were generated with six inbuilt chemical features in PHASE 3.0 module of Schrondinger Suite. These features consists of hydrogen bond acceptor (A), positive ionisable group (P), hydrophobic group (H), negative ionisable group (N), hydrogen bond donor (D) and aromatic ring (R). Eventually, a best pharmacophore hypothesis was selected which was then used to screen the database.

Virtual Screening

The DrugBank database was screened against the pharmacophore model with an aim to identify structurally diverse compounds as "hits" that are suitable for further optimization towards the development of a novel drug. The method of molecular docking was used to generate a score putative protein-ligand complexes based on their determined binding affinities. This technique has been effectively used in devising potential ligands with good binding efficiency for the residues of active site.[29] In the present analysis, the e-pharmacophore based virtual screening deferred a set of molecules with similar pharmacophoric features as present in the model. Additionally, the molecular docking was performed using GLIDE module to narrow down the hit molecules with higher binding efficiency towards the M2 protein. The grid generated for the M2 protein is used again for the

docking process. Consequently, the ligands were docked against the generated grid in a gradual process, using GLIDE high throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP) mode. The best molecules with good glide scores were further analysed.

RESULTS

Energy - based pharmacophore model

Pharmacophore modelling is a technique which substantially identifies novel moieties with biological activity.[30] The strategy of e-pharmacophore combines the structure and energy based values in scoring function using GLIDE module. The PHASE module Schrödinger Suite is used for generating the model.[31] Consequently, the docked complexes of M2 protein and the ligands were employed to create a pharmacophore model which is energetically efficient. The six intrinsic pharmacophoric features existing in the PHASE module are taken into account while generating the model. A two featured pharmacophore hypothesis (DD) (Figure 2) consisting of two hydrogen bond donor group was generated. This hypothesis was observed to possess higher statistical values which comprises of higher correlation and lower RMSD. It was also observed that the energy score of both the features namely D13 and D19 was less than 1.0 kcal/mol with a value of -1.04 kcal/mol and -2.03 kcal/mol respectively. The pharmacophore model was further employed for screening DrugBank database for lead molecules.

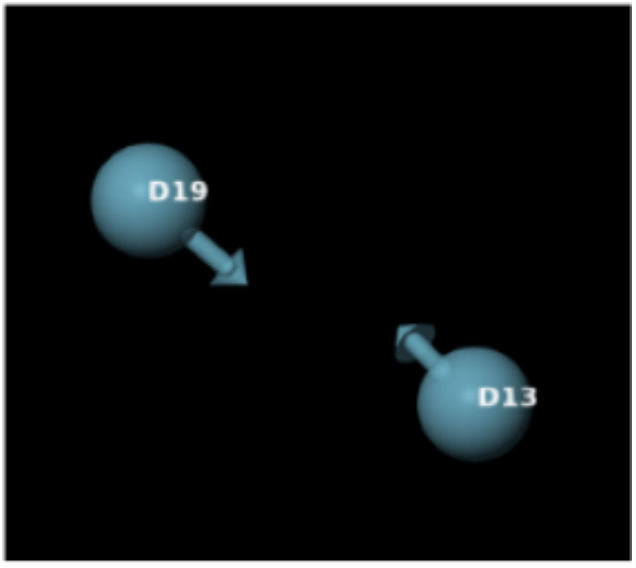

Figure 2: Best hypothesis with two pharmacophoric features namely two hydrogen bond donors.

E- Pharmacophore Based Virtual Screening

The screening process was performed against the DrugBank database with the help of DD hypothesis as search query. The DrugBank database is a comprehensive, freely accessible, online database containing information on wide range of drugs and drug targets.[32] Firstly, PHASE database was created using Lipinski's filter which estimates passive oral absorption based on log P, H-bond donors, H-bond acceptors and molecular weight. A set of 3000 molecules were yielded from a total of 8621 molecules. Eventually, these compounds were docked against the generated energy optimized pharmacophore model which served as a search platform for determining ligands with same pharmacophoric features. The molecules with a fitness score more than 1.4 were further screened using docking based screening process to determine potent NA inhibitor.[33]

Docking based Virtual Screening

The GLIDE module of Schrödinger Suite was employed to screen the molecules based on docking approach. HTVS and SP docking use the same scoring function, but HTVS reduces the number of intermediate conformations throughout the docking funnel, and also reduces the thoroughness of the final torsional refinement and sampling. The docking algorithm itself is essentially the same. XP does more extensive sampling than SP. It also employs a more sophisticated scoring function with greater requirements for ligand-receptor shape complementarity. These removes out the false positives from the hit molecules obtained. The PHASE search resulted in 2227 molecules as matches for the queried hypothesis. In a view of ranking the screened ligands a systematic procedure consisting of three steps of increasing complexity was performed. High throughput virtual screening reduced the molecules down from 2227 molecules to 1229 which was then followed by SP and XP virtual screening leading to the final number of 581 "hit" molecules. It is noteworthy to mention that 29 out of 581 molecules were found to be approved drugs. Hence these molecules were carried further for ADME analysis.

ADME properties

Along with the Lipinski rule of five, we utilized another crucial method of screening namely the ADME properties. This is performed to avoid failure of candidate molecules due to poor pharmacophore profile in the later stages of development. In the present study QikProp module was used for ADME properties analysis.[34] The 29 molecules were analyzed for #stars and CNS values. The descriptor #stars indicates the number of properties

that fall outside the recommended range. Therefore, a lower number of #stars denotes the better drug-like molecule. On the other hand, the CNS descriptor value predicts the central nervous system activity of each compound. For instance, the CNS value lies between -2 (inactive) and +2 (active). Rimantadine has in some cases shown adverse effects on the central nervous system of the patients like insomnia, dizziness, headache, nervousness, fatigue and nausea.[16] Hence in our study CNS activity was taken as -2 which signify it inactivity on the CNS. Interestingly, 9 hits namely, DB00374, DB00770, DB05015, DB08868, DB00631, DB00983, DB01262, DB00837 and DB01048, out of 29 molecules were found to possess 0 as #stars value and CNS value of -2. The glide scores and values of descriptors for the 9 screened molecules are shown in Table 1 and Table 2 respectively.

DISCUSSION

In this study, we have made an attempt at finding novel drugs that would inhibit the action of M2 proton channel by using already existing potent anti-viral drugs. Computational approaches such as virtual screening and molecular docking process have been used, which efficiently aids in instantaneous discovery of possible lead compound for protein inhibition. An amalgamation of method of e-pharmacophore based virtual screening and molecular docking approach was employed to identify potent M2 inhibitor. A total of 8621 compounds from the DrugBank database were screened by applying Lipinski's filter. The common e- pharmacophore DD was generated using complexes of known antiviral drugs and the M2 protein. Subsequently, common pharmacophore based screening was successfully applied to screen the compounds. The screening led to the curtailing of vast number of molecules to 2227 molecules. These 2227 molecules not only fit the hypothesis but also ratified other important criteria like the lipinski's rule of five and ADME properties. Further, the identified hits were screened based on HTVS, SP and XP docking approaches which yielded 29 hit compounds.

In addition it was observed that 9 "hits" out of 29 lead compounds found in our investigation have certain characteristic that calls for special attention here. Firstly, all of them are approved drugs and is inactive on the central nervous system with -2 as CNS value. Moreover, the number of properties or descriptor values that fall outside the 95% range of similar values for known drugs that is the star activity is 0. All of them were also found to interact with the amino acid residues present in the active side of the M2 channel. The interaction pattern of these 9 molecules is given in Table 3. It is clear from the table that these molecules were interacting with the amino acid residues namely Asp44, His37 and Leu43 which are known for their important role in the activation of M2 proton channel and the proliferation of the virus.[35] The interaction profile of the hit molecules with M2 protein is shown in Figure 3. In addition, superimposed images of lead molecules with the pharmacophore model and binding site of M2 protein are shown in Figure 4 and Figure 5 respectively.

In our study two out of 9 hit molecules namely, Abacavir (DB01048) and Treprostinil (DB00374) were found to have cyclopentane in their structure which contributes to the molecule's anti-viral properties. Cyclopentane (C_5H_{10}) is an alicyclic hydrocarbon consists of a ring of five carbon atoms each bonded with two hydrogen atoms above and below the plane is found out to have some significant antiviral properties. Moreover, it was also found that cyclopentane not only has anti-viral properties but instead was found specific against influenza virus too, which strengthens our research authenticity.[36] In an experiment conducted at Institute for Antiviral Research, Utah State University, Logan, Utah, a novel series of cyclopentane derivatives was found to exhibit potent and selective inhibitory effects on three different strains of influenza A virus-(H1N1, H3N2, and H5N1) by inhibiting viral cytopathic effect and NA glycoprotein activity. They were even found to have slightly more potency than zanamivir and oseltamivir carboxylate.[36] Three out of nine of the "hit" molecules found in our investigation were purine or pyrimidine analogs namely- Abacavir (DB01048), Clofarabine (DB00631), and Decitabine (DB01262). Fascinatingly, majority of the anti-viral drugs permitted worldwide for clinical use are analogs of either purine or pyrimidine such as idoxuridine, ganciclovir, zalcitabine, didananosine and others.[37,38,39,40,41,42] Many purine nucleoside and their derivatives have shown to have prominent anti-viral activity against some of the most deadly viruses found like human immune-deficiency virus (HIV), herpes simplex virus (HSV), vaccinia virus, respiratory syncytial virus, hepatitis B virus (HBV), human cytomegalovirus (HCMV) and varicella zoster virus (VZV).[43-48] Abacavir (DB01048), a guanosine analogue is known for its activity against retrovirus (HIV, Moloney sarcoma virus) and herpes simplex virus (HSV) activity in cell culture.[38] In a study conducted in University of Alabama at Birmingham, USA, the structural similarities between M1 matrix protein of Influenza A virus (having RNA nucleocapsid-binding activities) and HIV matrix and capsid proteins was ascertained, establishing an evolutionary link between retroviruses and negative strand

Table 1: Glide Score and Energy involvement of M2 protein and screened hit molecules.				
Sl. No.	**Entry ID**	**Glide Energy (kcal/mol)**	**Glide Score (kcal/mol)**	**XP Score (kcal/mol)**
1.	Rimantadine	-23.15	-1.905	-1.905
2.	4676	-28.29	-5.096	-5.096
3.	4680	-27.425	-4.526	-4.526
4.	4893	-24.567	-2.7	-2.7
5.	4896	-19.941	-2.717	-2.717
6.	4898	-22.162	-2.673	-2.673
7.	4903	-23.278	-2.676	-2.676
8.	4959	-23.787	-2.931	-2.931
9.	5122	-25.982	-2.954	-2.954
10.	5194	-22.814	-2.046	-2.046

Table 2: Absorption, distribution, metabolism, and excretion (ADME) properties of screened hit molecules.														
DrugbankID	**M.W[a]**	**SASA[b]**	**FOSA[c]**	**Donor HB[d]**	**Acceptor HB[e]**	**HOA[f]**	**QplogKP[g]**	**QplogBB[h]**	**QplogS[i]**	**QplogPo/w[j]**	**QplogPw[k]**	**QplogPoct[l]**	**stars[m]**	**CNS[n]**
Rimantadine	179.307	677.53	267.221	1	1	2	-3.876	0.9798	-4.881	3.241	10.212	17.009	1	-2
4676	390.519	753.91	469.14	3	6.15	2	-3.244	-2.103	-5.538	4.173	11.275	20.832	0	-2
4680	354.486	765.495	528.413	3	7.4	2	-4.224	-2.958	-3.713	3.029	11.548	21.187	0	-2
4893	318.347	574.81	22.564	3	8.7	3	-3.927	-2.086	-2.835	0.476	16.271	19.949	0	-2
4896	307.475	702.933	451.864	4	4.4	3	-3.945	-1.279	-3.217	3.178	10.002	18.611	0	-2
4898	303.68	493.876	104.119	4	9.1	2	-4.949	-1.376	-2.693	-0.328	16.823	19.919	0	-2
4903	344.41	662.627	247.884	4	7.2	2	-5.212	-1.536	-2.722	1.592	14.879	21.575	0	-2
4959	228.207	418.066	124.172	4	9.1	2	-5.897	-1.821	-1.469	-1.868	16.664	17.452	0	-2
5122	334.777	594.782	84.238	3	4.25	3	-2.779	-1.119	-3.261	2.671	13.466	18.344	0	-2
5194	286.336	537.427	254.337	4	6.2	3	-3.309	-1.095	-3.041	1.271	13.801	18.479	0	-2

[a] Molecular weight in g/mol
[b] Solvent-accessible surface area
[c] Hydrophobic component of the SASA
[d] Donor hydrogen bond
[e] Acceptor hydrogen bond
[f] Human oral absorption
[g] Predicted skin permeability
[h] Predicted brain/blood partition coefficient.
[i] Predicted aqueous solubility
[j] Predicted octanol/water partition coefficient
[k] Predicted water/gas partition coefficient.
[l] Predicted octanol/gas partition coefficient
[m] Number of property or descriptor values that fall outside the 95% range of similar values for known drugs
[n] Predicted central nervous system activity on a –2 (inactive) to +2 (active) scale

Table 3: Analysis of intermolecular interaction of screened hit molecules with M2 protein.

Sl. No.	Drug Bank ID	Type of Interaction	Interacting Atoms of Protein-Ligand Complex	Distance (Å)
1.	DB00374	Hydrogen Bond	Asp44…Lig(OH) Arg53…Lig(O^-)	2.1213 2.006
2.	DB00770	Hydrogen Bond	Arg53…Lig(=O) Arg53…Lig(=O) Asp44…Lig(OH) Asp44…Lig(OH)	1.73728 1.83319 1.85406 2.20399
3.	DB05015	Pi –Pi Bond Hydrogen Bond	Phe47…Lig Phe47…Lig Leu46…Lig(OH) Asp44…Lig(NH)	5.496 4.14489 2.38624 1.83008
4.	DB08868	Hydrogen Bond	Asp44…Lig(NH_3^+) Asp44…Lig(OH)	1.72663 1.80215
5.	DB00631	Hydrogen Bond Pi – Pi Bond	Asp44…Lig(OH) Asp44…Lig(OH) His37…Lig	1.79588 2.02396 3.98925
6.	DB00983	Hydrogen Bond Pi – Pi Bond	Asp44…Lig(NH_2^+) Leu43…Lig(NH) Phe47…Lig	1.64987 2.14668 5.08969
7.	DB01262	Hydrogen Bond	Asp44…Lig(=NH) Asp44…Lig(-NH)	1.78752 2.01874
8.	DB00837	Hydrogen Bond	Asp44…Lig(NH^+) Asp44…Lig(NH_2)	1.69174 1.64347
9.	DB01048	Hydrogen Bond Pi – Pi Bond	Asp44…Lig(NH) Phe47…Lig Phe48…Lig	1.75519 5.2178 4.15663

RNA viruses.[39] An intriguing feature of Abacavir is also that it has cyclopentane in its structure making it a cyclopentyl nucleoside. All these evidence suggests that Abacavir could have potential activity against influenza virus too. Clofarabine (DB00631), another purine nucleoside, has a substitute of chlorine in its purine structure and fluorine in its ribose. Like Abacavir, it too has been observed to have potent anti- HIV 2 activity. [40,41]

Pyrimidine nucleosides also have shown prominent anti-viral activity against diseases caused by viruses like HIV, herpes simplex virus type 1 (HSV-1) and type 2(HSV-2), varicella-zoster virus (VZV), cytomegalovirus (CMV) and adenovirus (types 2, 3 and 4).[49,50] Decitabine (DB01262), an analogue of cytidine with an extra hydroxyl and nitrogen group is one such example of a pyrimidine nucleoside found in our study. This molecule has shown inhibition action against HIV along with other drugs like Resveratrol and Gemcitabine and therefore is being used to pioneer novel HIV – drug combination therapies.[51,52,53] Cytidine itself has anti-viral properties against Hepatitis B and HIV virus.[54,55] Another "hit" molecule that needs to be mentioned is Formoterol (DB00983). In a study conducted in Utrecht University, Netherlands this drug was effective against all enterovirus and rhinoviruses tested.[56] It is a known fact that enteroviruses are positive-sense single-stranded RNA viruses like the retroviruses. As previously mentioned the evolutionary link between positive and negative strand RNA viruses suggests the use of these "hit" molecules for inhibition of influenza virus.

Alprostadil (DB00770) and Treprostinil (DB003740) are two of our hit molecules that are prostaglandins and its analogues. A significant disclosure made during our investigation was that prostaglandins possesed anti-viral activity against many DNA and RNA viruses according to the many studies conducted.[57] Prostaglandins are lipid autacoids which sustain homeostatic functions and mediate pathogenic mechanisms, including the inflammatory response.[58] In our study Δ^{12}-Prostaglandin J2 exceptionally stands out notably for two reasons. Firstly that it has known to show therapeutic effectiveness against influenza A virus (H1N1) both *in vitro* and *in vivo* as it has an interesting ability to interfere with virus replication at multiple levels and secondly that it is a natural cyclopentenone metabolite of prostaglandin D_2.[59] In another study conducted on vero cells the inhibitory effect of cyclopentenone prostaglandin A1 (PGA1) was seen on Mayaro virus replication.[57] The relevance of cyclopentane in our study as an anti-influenza drug has already been established. There are other varieties of prostaglandins especially of the A series that have also shown anti –viral activities against deadly viruses like

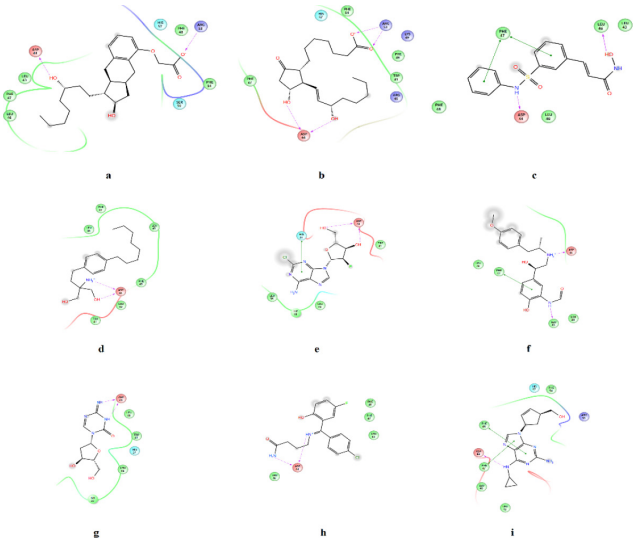

Figure 3: Two dimensional interaction map of selective hits to the active site of M2 protein. a DB00374, b DB00770, c DB05015, d DB08868, e DB00631, f DB00983, g DB01262, h DB00837, i DB01048.

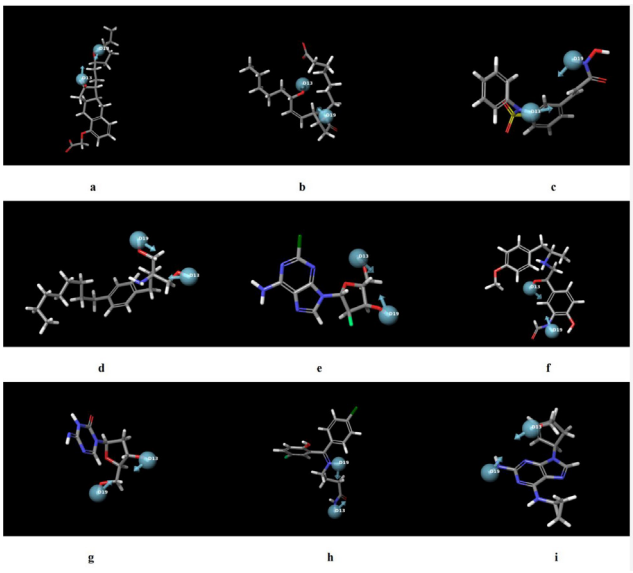

Figure 4: Superimposibility of Screened hit molecules with the generated e-pharmacophore hypothesis. a DB00374, b DB00770, c DB05015, d DB08868, e DB00631, f DB00983, g DB01262, h DB00837 and i DB01048.

herpes simplex virus type 1,vaccinia virus, sendai and vesicular somatostatis virus and encephalomayocaditis virus.[60,61,62] In a study conducted at University of Science and technology, China, Alprostadil (DB00770) was shown to be effective against severe hepatitis viral infection.[63] All of these evidences indicate prostaglandins to be a deserving anti- influenza drug candidate in our study. The basis of the above argument is that alprostadil is a

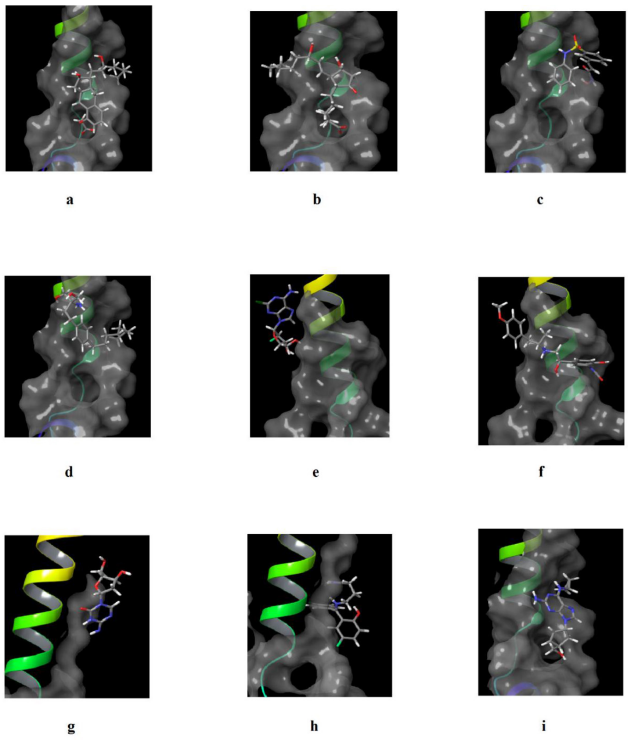

Figure 5: Three dimensional conformations of "Hit" compounds along with the corresponding hit molecules a DB00374, b DB00770, c DB05015, d DB08868, e DB00631, f DB00983, g DB01262, h DB00837 and i DB01048.

prostaglandin itself-PGE1 whereas Treprostinil is a synthetic analogue of prostacyclin –PGI2.

In course of our analysis of the various "hit" molecules, certain constituent structures and its analogues stood out distinctly for its anti-viral activity. One such structure was of Phenoxy acetic acid, a constituent of Treprostinil (DB00374). A riveting discovery was made in a study conducted at Shandong Academy of Agricultural Sciences, China, while searching for natural products having anti-influenza virus, they found out that compounds having structural fragment of phenoxy acetic acid had improved activity against NA glycoprotein of Influenza A virus.[64] In another two studies conducted individually it was observed that pyrazoline derivatives derived from phenoxy acetic acid had higher anti-viral activity against a broad panel of viruses in different cell cultures.[65,66,67] Another such constituent structure having anti-viral activity was diphenylmethane of Progabide (DB00837). Intriguingly, diphenylmethane and its derivatives are active against a host of viral infections like Bovine-viral diarrhoea virus, A-PR8 virus, poliomyelitis virus, adeno-virus, vaccinia virus and MHV3 virus.[56,68] Overall, the results from our analysis along with available experimental evidences comprehend that these 9 lead molecules could be used as promising candidate for M2 protein inhibition.

CONCLUSION

The current study has been carried out with an aim to find potent M2 proton channel inhibitor using computational techniques such as e-pharmacophore modelling and molecular docking approach. The common pharmacophore hypothesis (DD) was generated using complexes of known antiviral drugs and the M2 protein which consisted of two pharmacophoric features; two hydrogen bond donors (D). This was followed by e-pharmacophore based and docking based virtual screening. These two processes were successfully applied to screen compounds from a total of 8621 molecules of DrugBank database. This led to the curbing of molecules to 29 molecules which was further screened using ADME analysis. Subsequently, the ADME analysis yielded a total of 9 hit molecules whose pharmacokinetic properties lay in the acceptable range. Additionally, their binding patterns with M2 protein were also studied. It is also noteworthy to mention that different scaffolds of these 9 lead molecules have been reported for its prominent antiviral properties in recent literatures. Hence, these hit compounds could be used as potential drugs for the inhibition of M2 proton channel. Thus, this study has explicitly demonstrated the importance of e-pharmacophore modelling in drug discovery. We believe that the results from our analysis are of immense importance to researchers seeking to develop a novel M2 proton inhibitor and the molecules could act as prototype candidates for further research on influenza treatment.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

HTVS: High throughput virtual screening; **SP:** Standard precision; **XP:** Extra precision; **HA:** Haemagglutinin; **NA:** Neuraminidase; **ADME:** Absorption, Distribution, Metabolism and Excretions.

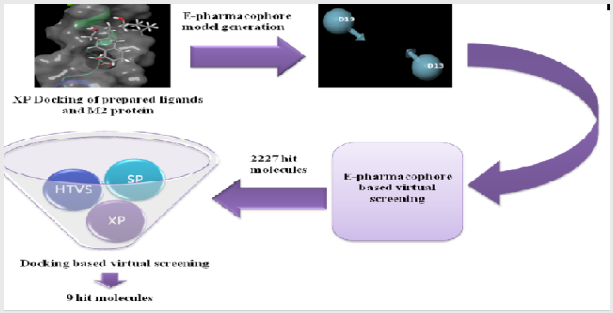
REFERENCES

1. Li XB, Wang SQ, Xu WR, Wang RL, Chou KC. Novel inhibitor design for hemagglutinin against H1N1 influenza virus by core hopping method. PloS One. 2011;6(1):e28111.
2. Hinshaw VS, Webster RG, Bean WJ, Sriram G. The ecology of influenza viruses in ducks and analysis of influenza viruses with monoclonal antibodies. Comparative Immunology, Microbiology and Infectious Diseases. 1980;3(1-2): 155-64.
3. Matrosovich MN, Matrosovich TY, Gray T, Roberts NA, Klenk H. Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. Journal of Virology. 2004;78(22):12665-7.
4. Wang C, Lamb RA, Pinto LH. Activation of the M2 ion channel of influenza virus: a role for the transmembrane domain histidine residue. Biophysical journal. 1995;69(4):1363-71.
5. Ciampor F, Cmarko D, Cmarková J, Závodská E. Influenza virus M2 protein and haemagglutinin conformation changes during intracellular transport. Acta Virol. 1995;39(3):171-81.
6. Chizhmakov IV, Geraghty FM, Ogden DC, Hayhurst A, Antoniou M, Hay AJ. Selective proton permeability and pH regulation of the influenza virus M2 channel expressed in mouse erythroleukaemia cells. Journal of Physiology. 1996;494(2):329-36.
7. Shimbo K, Brassard DL, Lamb RA, Pinto LH. Ion selectivity and activation of the M2 ion channel of influenza virus. Biophysical journal. 1996;70(3):1335-46.
8. Gong J, Xu W, Zhang J. Structure and Functions of Influenza Virus Neuraminidase. Current Medicinal Chemistry. 2007;14(1):113-22.
9. Jeffery KT, David MM. 1918 Influenza: The mother of all pandemics. Emerging Infectious Diseases. 2006;12:15-22.
10. Wright PF, Webster RG. Orthomyxoviruses. Fields Virology publication. 2001;1533-79.
11. Patterson KD. Pandemic and epidemic influenza 1830–1848. Social Science and Medicine. 1985; 21(5):571-80.
12. Kilbourne ED. Influenza pandemics of the 20th century. Emerging infectious diseases. 2006;12(1):9.
13. Durbin JE, Fernandez-Sesma A, Lee CK, Rao TD, Frey AB, Moran TM, *et al*. Type I IFN Modulates Innate and Specific Antiviral Immunity. Journal of Immunology. 2000;164(8):4220-8.
14. Huang RB, Du QS, Wang CH, Chou KC. An in-depth analysis of the biological functional studies based on the NMR M2 channel structure of influenza a virus. Biochemical and Biophysical Research Communications. 2008;377(4):1243-47.
15. Hayden FG, Belshe RB, Clover RD, Hay AJ, Oakes MG, Soo W. Emergence and Apparent Transmission of Rimantadine-Resistant Influenza A Virus in Families. New England Journal of Medicine. 1989;321(25):1696-702.
16. Hall CB, Dolin R, Gala CL, Markovitz DM, Zhang YQ, Madore PH, *et al*. Children with influenza an infection: treatment with rimantadine. Pediatrics. 1987;80(2):275-82.
17. Kaur P, Sharma V, Kumar V. Pharmacophore Modelling and 3D-QSAR Studies on N □ -Phenylpyrazinones as Corticotropin-Releasing Factor 1 Receptor Antagonists. International Journal of Medicinal Chemistry. 2012; 2012.
18. Rajendran V, Purohit R, Sethumadhavan R. *in silico* investigation of molecular mechanism of laminopathy caused by a point mutation (R482W) in lamin A/C protein. Amino acids. 2012;43(2):603-15.
19. Purohit R. Role of ELA region in auto-activation of mutant KIT receptor: a molecular dynamics simulation insight. Journal of Biomolecular Structure and Dynamics. 2014;32(7):1033-46.
20. Rajendran V, Sethumadhavan R. Drug resistance mechanism of PncA in Mycobacterium tuberculosis. Journal of Biomolecular Structure and Dynamics. 2014;32(2):209-21.
21. Kamaraj B, Rajendran V, Sethumadhavan R, Kumar CV, Purohit R. Mutational analysis of FUS gene and its structural and functional role in amyotrophic lateral sclerosis 6. Journal of Biomolecular Structure and Dynamics. 2015;33(4):834-44.
22. Kamaraj B, Purohit R. Mutational Analysis on Membrane Associated Transporter Protein (MATP) and Their Structural Consequences in Oculocutaeous Albinism Type 4 (OCA4)-A Molecular Dynamics Approach. Journal of cellular biochemistry. 2016;117(11):2608-19.
23. Kaserer T, Beck KR, Akram M, Odermatt A, Schuster D. Pharmacophore Models and Pharmacophore-Based Virtual Screening: Concepts and Applications Exemplified on Hydroxysteroid Dehydrogenases. Molecules. 2015;20(12):22799-832.
24. Loving K, Salam NK, Sherman W. Energetic analysis of fragment docking and application to structure-based pharmacophore hypothesis generation. Journal of Computer-Aided Molecular Design. 2009;23(8):541-54.

25. Muralidharan AR, Selvaraj C, Singh SK, Nelson JCA, Geraldine P, Thomas PA. Virtual screening based on pharmacophoric features of known calpain inhibitors to identify potent inhibitors of calpain. Medicinal Chemistry Research. 2014;23(5):2445-55.
26. Aparna V, Dineshkumar K, Mohanalakshmi N, Velmurugan D, Hopper W. Identification of natural compound inhibitors for multidrug efflux pumps of Escherichia coli and Pseudomonas aeruginosa using *in silico* high-throughput virtual screening and *in vitro* validation. PLoS One. 2014;9(7):e101840.
27. Vass M, Schmidt É, Horti F, Keserű GM. Virtual fragment screening on GPCRs: a case study on dopamine D3 and histamine H4 receptors. European journal of medicinal chemistry. 2014;77:38-46.
28. Pulla VK, Sriram DS, Viswanadha S, Dharmarajan S, Perumal Y. Energy-Based Pharmacophore and Three-Dimensional Quantitative Structure–Activity Relationship (3D-QSAR) Modeling Combined with Virtual Screening To Identify Novel Small-Molecule Inhibitors of Silent Mating-Type Information Regulation 2 Homologue 1 (SIRT1). Journal of chemical information and modeling. 2016;56(1):173–87.
29. Poland GA, Jacobson RM, Ovsyannikova IG. Influenza Virus Resistance to Antiviral Agents: A Plea for Rational Use. Clinical Infectious Diseases. 2009;48(9):1254-6.
30. Stu B. Flu virus proton channel analyzed. Chemical and engineering news. 2008;86(6):53-54.
31. Vyas R, Karthikeyan M, Nainaru G, Muthukrishnan M. Pharmacophore and Docking Based Virtual Screening of Validated Mycobacterium tuberculosis Targets. Combinatorial Chemistry and High Throughput Screening. 2015;18(7):624-37.
32. Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, *et al*. DrugBank: a comprehensive resource for *in silico* drug discovery and exploration. Nucleic Acids Research. 2006;34(1):D668–D72.
33. Therese PJ, Manvar D, Kondepudi S, Battu MB, Sriram D, Basu A, *et al*. 3D-QSAR, and high-throughput virtual screening of hepatitis C virus NS5B polymerase inhibitors. Journal of chemical information and modeling. 2014;54(2):539-52.
34. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies. 2004;1(4):337-41.
35. Schnell JR, Chou JJ. Structure and Mechanism of the M2 Proton Channel of Influenza A Virus. Nature. 2008;451(7178):591-5.
36. Smee DF, Huffman JH, Morrison AC, Barnard DL, Sidwell RW. Cyclopentane neuraminidase inhibitors with potent *in vitro* Anti-influenza virus activities. Antimicrobial agents and chemotherapy. 2001;45(3):743-48.
37. Balzarini J. Metabolism and mechanism of antiretroviral action of purine and pyrimidine derivatives. Pharmacy World and Science. 1994;16(2):113–26.
38. Balzarini J, Meier FH, De Clercq E, Meier C. Antiviral Activity of Cyclosaligenyl Prodrugs of Acyclovir, Carbovir and Abacavir. Antiviral Chemistry and Chemotherapy. 2001;12(5):301-6.
39. Harris A, Sha B, Luo M. Structural similarities between influenza virus matrix protein M1 and human immunodeficiency virus matrix and capsid proteins: an evolutionary link between negative-stranded RNA viruses and retroviruses. Journal of General Virology. 1999;80(4):863-9.
40. Beach LB, Rawson JM, Kim B, Patterson SE, Mansky LM. Novel inhibitors of human immunodeficiency virus type 2 infectivity. Journal of General Virology. 2014;95(12):2778-83.
41. Lanyu J, Peiwu Q, Hongliu Y, Zhengli Y, Mingzhou Z. Synthesis and antitumor activity of novel 2′,3′-dideoxy-2′,3′-diethanethionucleosides bearing 1,2,3-triazole residues. Bioorganic and Medicinal Chemistry Letters. 2010;20(1):240-3.
42. Bennett LL, Shannon WM, Allan PW, Arnett G. Studies on the biochemical basis for the antiviral activities of some nucleoside analogs. Annals of the New York Academy of Sciences. 1975;255(1):342-58.
43. Vince R, Hua M. Synthesis and anti-HIV activity of carbocyclic 2',3'-didehydro-2',3'-dideoxy 2,6-disubstituted purine nucleosides. Journal of medicinal chemistry. 1990;33(1):17–21.
44. Clercq ED, Balzarini J , Descamps J , Eckstein F. Antiviral. Antimetabolic and antineoplastic activities of 2′-or 3′-amino or -azido-substituted deoxyribonucleosides. Biochemical Pharmacology. 1980;29(12):1849-51.
45. Mansuri MM, Starrett JE, Ghazzouli I, Hitchcock MJ, Sterzycki RZ, Brankovan V, *et al*. 1-(2,3-Dideoxy-.beta.-D-glycero-pent-2-enofuranosyl) thymine-A highly potent and selective anti-HIV agent. Journal of medicinal chemistry. 1989;32(2):461–6.
46. Aerschot AAV, Mamos P, Weyns NJ, Ikeda S, Clercq ED, Herdewijn PA. Antiviral activity of C-alkylated purine nucleosides obtained by cross-coupling with tetraalkyltin reagent. Journal of medicinal chemistry. 1993;36(20):2938–42.
47. Fyfe JA, Keller PM, Furman PA, Miller RL, Elion GB. Thymidine Kinase from Herpes Simplex Virus Phosphorylates the New Antiviral Compound, 9-(2-Hydroxyethoxymethyl) guanine. *The Journal of Biological Chemistry.* 1978;253(24):8721-7.
48. Van Draanen NA, Freeman GA, Short SA, Harvey R, Jansen R, *et al*. Synthesis and Antiviral Activity of 2'-Deoxy-4'-thio Purine Nucleosides. Journal of medicinal chemistry. 1996;39(2):538–42.
49. Clercq ED, Sakuma T, Baba M, Pauwels R, Balzarini J, Rosenberg I, *et al*. Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. Antiviral Research. 1987;8(5-6):261-72.
50. Chu CK, Schinazi RF, Ahn MK, Ullas GV, Gu ZP. Structure-activity relationships of pyrimidine nucleosides as antiviral agents for human immunodeficiency virus type 1 in peripheral blood mononuclear cells. Journal of medicinal chemistry. 1989;32(3):612-7.
51. Clouser CL, Holtz CM, Mullett M, Crankshaw DL, Briggs JE, O'Sullivan MG, *et al*. Activity of a Novel Combined Antiretroviral Therapy of Gemcitabine and Decitabine in a Mouse Model for HIV-1. Antimicrobial agents and chemotherapy. 2012;56(4):1942-8.
52. Clouser CL, Chauhan J, Bess MA, van Oploo JL, Zhou D, Gray SD, Mansky LM, Patterson SE. Anti-HIV-1 activity of resveratrol derivatives and synergistic inhibition of HIV-1 by the combination of resveratrol and Decitabine. Bioorganic and Medicinal Chemistry Letters. 2012;22:642-46.
53. Clouser CL, Patterson SE, Mansky LM. Exploiting Drug Repositioning for Discovery of a Novel HIV Combination Therapy. Journal of Virology. 2010;84(18):9301-9.
54. Tai-Shun L, Mei-Zhen L, Mao-Chin L, Pai SB, Dutschman GE, Yung-Chi C. Antiviral activity of 2′,3′-dideoxy-β-l-5-fluorocytidine (β-l-fddc) and 2′,3′-dideoxy-β-l-cytidine (β-l-ddc) against Hepatitis B virus and Human Immunodeficiency virus type 1 *in vitro.* Biochemical pharmacology. 1994;47(2):171-4.
55. Lin TS, Luo MZ, Liu MC, Zhu YL, Gullen E, Dutschman GE, *et al*. Design and Synthesis of 2',3'-Dideoxy- 2',3'-didehydro-β-l-cytidine (β-l-d4C) and 2',3'-Dideoxy-2',3'-didehydro-β-l-5- fluorocytidine (β-l-Fd4C), Two Exceptionally Potent Inhibitors of Human Hepatitis B Virus (HBV) and Potent Inhibitors of Human Immunodeficiency Virus (HIV) *in vitro*. Journal of medicinal chemistry. 1996;39(9):1757–9.
56. Ulfertsa R, Boera MD, Lindenb LVD, Bauera L, Lyooa HR, Matéc MJ, *et al*. Screening of a library of FDA-Approved Drugs Identifies Several Enterovirus Replication Inhibitors That Target Viral Protein 2c. Antimicrobial agents and chemotherapy. 2016;60(5):2627-38.
57. Burlandy FM, Rebello MA. Inhibition of Mayaro Virus Replication by Prostaglandin A_1 in Vero Cells. Intervirology. 2001;44(6):344–9.
58. Ricciotti E, FitzGerald GA. Prostaglandins and Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;31(5):986-1000.
59. Pica F, Palamara AT, Rossi A, Marco AD, Amici C, Santoro MG. Δ^{12}-Prostaglandin J_2 Is a Potent Inhibitor of Influenza A Virus Replication . Antimicrobial agents and chemotherapy. 2000;44(1):200-4.
60. O'Brien WJ, Taylor JL, Ankel H, Sitenga G. Assessment of antiviral activity, efficacy, and toxicity of prostaglandin A2 in a rabbit model of herpetic keratitis. Antimicrobial agents and chemotherapy. 1996;40(10):2327-31.
61. Santoro MG, Jaffe BM, Garaci E, Esteban M. Antiviral Effect of Prostaglandins of the A Series: Inhibition of Vaccinia Virus Replication in Cultured Cells. Journal of General Virology. 1982;63:435-40.
62. Ankel H, Mittnacht S, Jacobsen H. Antiviral Activity of Prostaglandin A on Encephalomyocarditis Virus-infected Cells: A Unique Effect Unrelated to Interferon. Journal of General Virology. 1985;66(11):2355-64.
63. Hui C. Alprostadil in the Treatment of 42 Patients with Severe Viral Hepatitis. Herald of Medicine. 2003;2003-4.
64. Xie Y, Huang B, Yu K, Shi F, Liu T, Xu W. Caffeic acid derivatives. A new type of influenza neuraminidase inhibitors. Bioorganic and Medicinal Chemistry Letters. 2013;23(12):3556-60.

65. Kumar S, Bawa S, Drabu S, Kumar R, Gupta H. Biological Activities of Pyrazoline Derivatives -A Recent Development. Recent Patents on Anti-Infective Drug Discovery. 2009;4(3):154-63.
66. Shahar YM, Bakht MA, Siddiqui AA, Abdullah MM, Clercq ED. Synthesis And Evaluation Of *In vitro* Antiviral Activity Of Novel Phenoxy Acetic Acid Derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry. 2009;24(3):876-82.
67. Hosoda S, Aoyama H , Goto Y, Salim MTA, Okamoto M, Hashimoto M, *et al*. Discovery of diphenylmethane analogs as anti-bovine diarrhea viral agents. Bioorganic and Medicinal Chemistry Letters. 2009;19(12):3157-61.
68. Cavallini G. New Antiviral Compounds with Considerable Activity *in vivo*. IV. Aromatic α-Keto Aldehydes. Journal of medicinal chemistry. 1964;7(3):255–8.

PICTORIAL ABSTRACT



SUMMARY

- We were successfully able to screen 9 molecules as potent inhibitors of M2 protein thus attempting to overcome the drug resistance caused by mutant influenza A virus.
- The approach used to acquire the above was a combination of e- pharmacophore and docking based virtual screening.

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