SMALL MOLECULE H5N1 INHIBITORS: A REVIEW

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Abstract: Highly Pathogenic Asian Avian Influenza A (HPAI) H5 or more commonly called as "Bird flu" is a severe contagious respiratory disease in birds caused by influenza A virus subtype that has an HA (hemagglutinin) 5 protein and an NA (neuraminidase) 1 protein (H5N1). H5N1 infect the respiratory and gastrointestinal tracts of birds leading to large scale deaths and imminent loss to the poultry industry. Though the fatality rate of humans infected with Avian Influenza is low, there is a huge concern regarding the mutations of the virus and its resistance to the antiviral drugs. As of now, Oseltamivir (Tamiflu) is the drug of choice, but the virus has gained resistance over it and Zanamivir, Peramivir, and long-acting Laninamivir are the other approved drugs amongst other drugs used for the treatment and prophylaxis of influenza virus. An overall review of H5N1 viral as well as host cellular mechanisms as targets and exclusive H5N1 novel small molecule inhibitors developed during the recent past are discussed in the below sections.

Index Terms - Highly Pathogenic Asian Avian Influenza, H5N1, Influenza virus, Hemagglutinin, Neuraminidase, Small molecule, Inhibitor, Oseltamivir, Zanamivir, Anti-viral drug.

I. INTRODUCTION

Influenza virus is an RNA virus belonging to the family Orthomyxoviridae with four main viral species A, B, C and D infecting birds and mammals. Avian influenza A virus has its roots in China and outbreaks occur from time to time at many parts of the world, gaining the ability to infect huge numbers causing a widespread pandemic. Influenza A virus has eight genomic segments encoding proteins namely, hemagglutinin (HA), neuraminidase (NA), matrix proteins M2 and M1, non-structural (NS) proteins-NS1 and NS2, polymerases and other proteins. HA and NA are the antigenic surface glycoproteins present on the viral envelope and they are classified based on these into different subtypes which are recognized in various biological species. H5N1 subtype of HPAI was detected in chicken, turkey, quail terrestrial birds. H5N1 is transferred to humans from infected poultry and mammals which are considered as hosts and intermediate hosts for Influenza A virus respectively [1]. HA of avian influenza virus binds to the sialic acid present on avian epithelial cells thus infecting the birds in large numbers. However, infection to humans by avian influenza virus is via mutations in the virus at various levels, but its extent of virulence is still explored.

Infection with Highly Pathogenic Asian Avian Influenza, H5N1 subtype, to humans causes acute lung injury by inducing autophagic alveolar epithelial cell death [2]. H5N1 infection to humans is characterized by hyper induction of serum cytokine levels in the body which is due to H5N1viral mediation of host's innate immune responses [3]. This prompts the researchers to develop antiviral, as well as immunomodulatory agents to contribute to the anti H5N1 therapy.

H5N1 TARGETS AND THERAPY

Virus Life cycle:

Influenza A virus has eight genomic segments that encode ten viral proteins-HA, NA, M1, M2, the nucleocapsid, Non-structural proteins-NS1, NS2, polymerases (PB1 polymerase basic-1, PB2, and polymerase acidic, PA) (Fig.1).

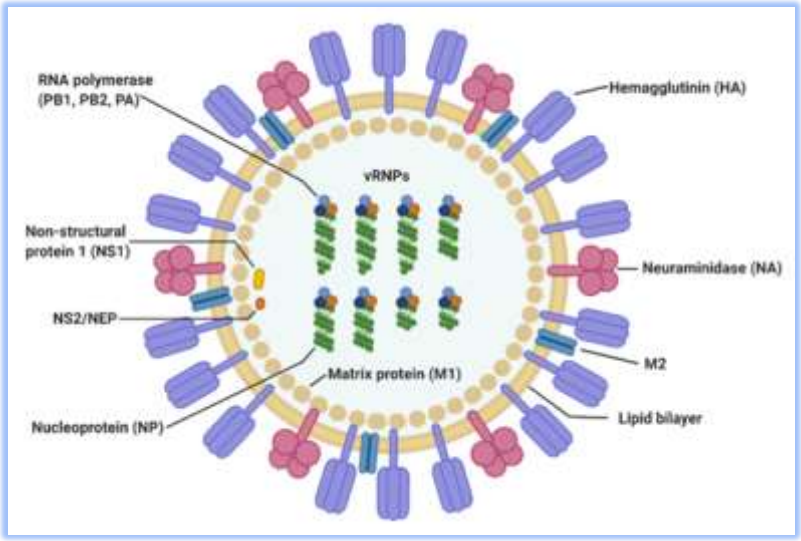

Fig. 1: Structure of Influenza A Virus. (Image courtesy: Creative Commons, Wikipedia)

HA present on the surface of the viral protein recognizes and binds to the terminal sialic acid that is attached to the underlying sugar residues present on the host's cell surface. The process of receptor mediated endocytosis is triggered by this binding and lowers the endosomal pH. This acidification process [4] via intake of protons through M2 protein causes confirmational change in the HA protein which in turn exposes the viral hydrophobic fusion peptide that aids in the fusion of the viral and endosomal membrane. The viral M2 matrix protein acts as an ion channel and causes influx of hydrogen atoms into the viral core and subsequent release of viral ribonucleoprotein particles (vRNPs) from M1 matrix proteins into cytoplasm (uncoating). The vRNPs enters the host nucleus by translocation and undergoes RNA replication and transcription processes by means of PB1, PB2, and PA components of RNA polymerase enzyme followed by viral mRNA translation into host cell cytoplasm by M1 and NS2 proteins. After post-translational modifications of vRNPs, budding of new viral proteins occur at the cytoplasmic membrane with the help of M1 and NS2 proteins [5] (Fig. 2).

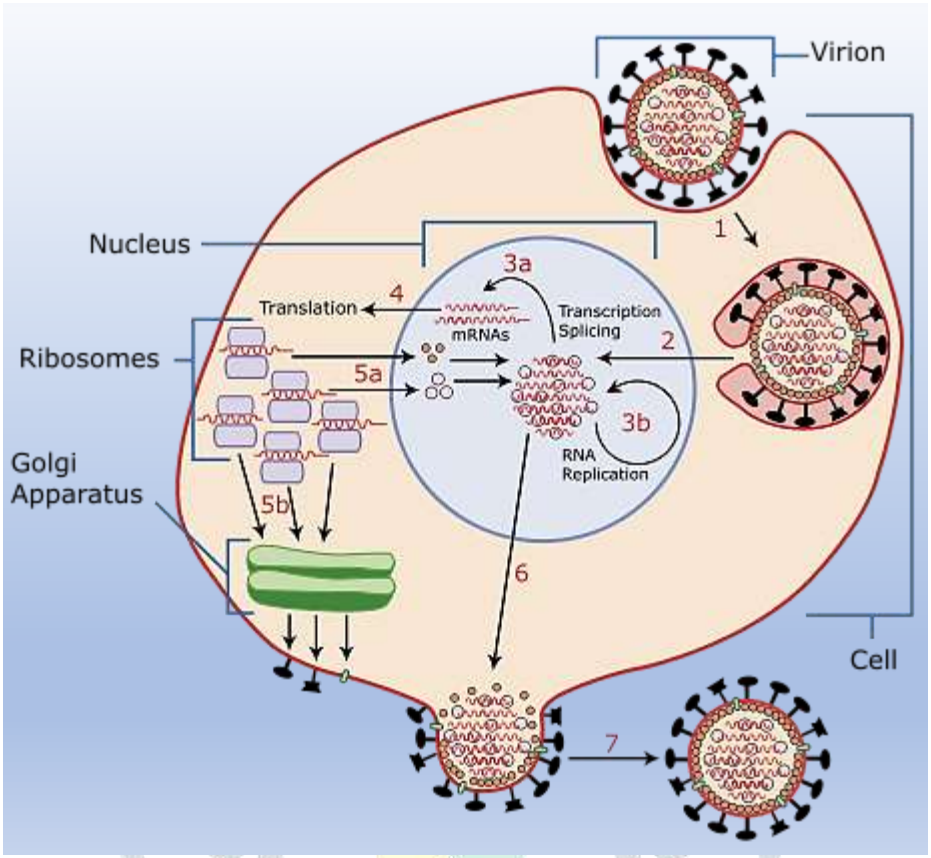

Fig. 2: Virus Life Cycle: 1: Receptor-mediated endocytosis. 2: Entry of vRNPs into host nucleus by Translocation. 3: RNA replication and transcription by PB1, PB2, and PA components of RNA polymerase enzyme. 4: Migration of newly synthesized mRNAs by M1 and NS2 proteins. 5: Posttranslational modifications of vRNAs. 6: Migration of newly formed nucleocapsids into the cytoplasm by M1 and NS2 proteins. 7: Budding of newly synthesized virions from infected cell.

Viral NA removes sialic acids from the sialylated HA and NA of new viral proteins which blocks viral aggregation and viral binding back to the host cell via HA. NA also removes sialic acid components that are present on the host cell membrane and thus allows the new viral proteins to escape from host membrane [6].

H5N1 Viral Targets and Host Cellular Mechanisms:

The H5N1 influenza viral targets are determined based on the intensive study of viral replication cycle. HA protein is essential for the viral entry into the host cell and it is considered as major target for developing anti H5N1 drugs. Several HA inhibitors are developed. Targeting the head domain vestigial esterase (VE) subdomains of HA results in the development of novel VE-binding-anti H5N1 mAbs that can be used in combinatorial approaches for H5N1 therapeutic treatment [7]. Viral matrix ion channel proteins-M1 and M2 proteins participates in viral uncoating process and viral M1 and NS2 proteins helps in viral entry into the host nucleus. The viral matrix proteins are targeted to inhibit the viral uncoating and viral entry into cytoplasm. PB1, PB2, and PA components of RNA polymerase enzyme causes viral replication and transcription processes. It is estimated that over 400 host proteins are related to the RNA polymerase of H5N1 influenza virus which could be potential targets to inhibit H5N1[8]. New virions are exited from the host cell membrane by means of viral NA protein. NA inhibitors majorly inhibit the action of NA and thus prevents the entry of new virions from infected host cell membrane [9].

This whole life cycle events of viral replication in the host cell triggers various host innate immune signaling pathways that causes vigorous release of pro-inflammatory cytokines and chemokines often called as “cytokine-storm” [10] which is fatal to susceptible population. Many host cellular kinases like tyrosine kinases, serine/threonine kinases and lipid kinases regulate influenza A viral protein phosphorylation thereby playing role at various stages of viral life cycle right from viral entry, uncoating, vRNP synthesis, protein translocation, budding to release of virions. Several co-receptors like annexin V, sulfa sialyl Lewis X receptors, C-type lectin receptors, receptor tyrosine kinases (RTKs), epidermal growth factor receptor and c-Met kinase are involved in the process of receptor mediated endocytosis that allows the virus to enter into the cell. Na+/H+-transporter in host system aids in viral internalization into the cell [11]. Host’s cellular vacuolar-type ATPase has a role in endosomal acidification that helps in viral fusion with endosomal membrane. Phosphorylation sites are identified in the viral proteins and kinase residues are identified in the PB1, PB2, and PA components of RNA polymerase enzyme. The MEK signaling pathway of the host and CRM1 (Chromosomal Maintenance 1) protein are involved in the nuclear export of vRNPs into cytoplasm from the nucleus. Similarly, influenza virus is known to influence host’s PI3 kinase pathway, JAK, IKK/NFkB pathways during viral entry, viral uncoating and RNA synthesis. Inhibiting MPSL/TMPRSS13 pathway inhibits the replication of H5N1 influenza virus with high pathogenicity [12]. Host’s cellular factors are a serious target concern and development of small-molecule kinase inhibitors (SMKI) for targeting pro-inflammatory cytokines thereby targeting the influenza induced pneumonia in immune compromised patients is a necessary thing. Also, repurposing of drugs that target the above said pathways paves long run successful ways in short time to deal with H5N1 like HPAI pandemics.

Apart from targeting viral genes and proteins, focusing on host cellular pathways involved in viral pathogenesis like lipid metabolites/metabolic pathways seems to be an attractive novel strategy to develop novel anti H5N1 therapeutics. Omega-3 PUFA-derived protectins are shown to have in vivo counter regulation of pro-inflammatory chemokines and cytokines. Now that these protectins are also reported to block the viral mRNA export and thus suppressing influenza viral replication. Further study on the development of metabolic pathway-based targets, biomarkers and anti influenza drugs for treating H5N1 infections is an interesting concept [13].

Avian influenza A H5N1 virus infections induces autophagic alveolar epithelial cell death thereby causing acute lung injury. Thus, targeting autophagy by repurposing clinically available autophagy inhibitors might help treating H5N1 infections. A brief listing of H5N1 viral targets, host cellular pathway targets and their role in viral infection was given in the following table 1 and table 2.

Table 1: List of H5N1 influenza viral targets and their functions

Viral Targets	Function
Hemagglutinin protein (HA)	Attachment to host sialic acid receptors, helps in viral entry into host
Neuraminidase (NA)	Removes sialic acid during virus budding, Eliminate aggregation of budding virions on the cell surface
Nucleocapsid protein (NP)	Formation of viral ribonucleoproteins (vRNPs), helps in viral RNA replication
M2 protein	Viral uncoating
M1 protein	Assembly of viral components, formation of new virions; Viral budding
NS2 protein	Exports newly synthesized viral RNPs from the nucleus to the cytoplasm
NS1A	Multi-functional protein; Suppresses host antiviral responses
RNA dependent RNA polymerase (RdRp) (PB1 polymerase basic-1, PB2, and polymerase acidic, PA)	Viral replication

Table 2: List of host cellular targets of H5N1 influenza virus

Host cellular pathways	Role
Tyrosine kinases, serine/threonine kinases and lipid kinases	Viral entry, uncoating, vRNP synthesis, protein translocation, budding and release of virions
Epidermal growth receptors, sulfo sialyl lewis X receptors, C-type lectin receptors	Receptor mediated endocytosis; viral entry into host
Na^+/H^+-transporter	Internalization of virus into the cell
Vacuolar-type ATPase	Endosomal acidification
MEK signalling pathway and CRM1 protein	Exports newly synthesized viral RNPs from the nucleus to the cytoplasm
PI3 kinase pathway, JAK, IKK/NFkB pathways	Viral entry, viral uncoating and RNA synthesis
MPSL/TMPRSS13 pathway	Viral replication

H5N1 Inhibitors:

A cluster of viral proteins involved in the viral lifecycle and host proteins that help virus to propagate in the host are the common targets to develop anti H5N1 drugs. The anti H5N1 inhibitors are classified into different categories:

Entry Inhibitors: Entry Inhibitors are the drugs that inhibit the viral entry into the host body. Natural compound derivatives like Glycyrrhizin, Glycyrrhizic acid, Saponins with 3-O-β-chacotriosyl residues [14], chlorogenin 3-O-b-chacotrioside showed effective anti H5N1 activity by inhibiting viral entry.

Hemagglutinin (HA) Inhibitors: The viral envelope spike glycoprotein hemagglutinin (HA) is important for viral entry via receptor binding and membrane fusion. Hence it is a potential target for the development of anti-H5N1drugs. Plant natural products, namely, Pentacyclic triterpenoids (PTs), especially Oleanane acid (OA) triterpenes are well known for their wide spectrum of antiviral activities. OA derivatives having β-chacotriosyl residues exhibited excellent H5N1 inhibitory activity by acting on HA protein [15]. Extract of *Echinacea purpurea* (Echinaforce®, EF) inhibited the receptor binding activity of the virus and interferes with the viral entry into cells as proved with Hemagglutination assays [16]. Small molecule inhibitor derived from Andrographolide, a labdane diterpenoid, isolated from the stem and leaves of *Andrographis paniculate*, showed significant anti H5N1 activity by targeting HA. Arbidol (Umifenovir), an indole-based, hydrophobic, dual-acting agent is a licensed hemagglutinin inhibitor. It has a wide spectrum antiviral activity and inhibits the reproduction of H5N1 and H9N2 subtypes [17].

Neuraminidase (NA) Inhibitors: NA inhibitors bind to the newly formed virus particle NA surface glycoprotein and prevent its release from the host cell. Zanamivir (RelenzaTM) and Oseltamivir (TamifluTM) are the licensed NA inhibitors [18]. Two new drugs, Peramivir and Laninamivir are also effective H5N1 NA inhibitors. Biochanin A and baicalein, flavonoids, showed effective interference with H5N1 replication in lung epithelial cells and baicalein showed inhibitory action on H5N1 replication in primary human monocyte derived macrophages [19].

RNA-dependent RNA polymerase (RdRp) Inhibitors: Favipiravir-RTP, an active form of Favipiravir which is a purine nucleotide, inhibits the RdRp enzyme activity both in vitro and in vivo [20]. Triazavirin is a guanine nucleotide analog that inhibits RNA synthesis. Triazavirine surpassed the inhibitory action of Rimantadine by 4-8 times against avian influenza virus. Lycorine suppress viral RNA replication and protein synthesis of avian influenza virus H5N1 [21].

Nucleocapsid protein (NP) Inhibitors: NP is a multifunctional protein which is an important component of the vRNP and it plays crucial role in RNA packing, nuclear trafficking and vRNA transcription. By binding to PB1 and PB2 subunits of viral polymerase enzyme it helps in viral replication process. Nucleozin showed effective cellular activity in vitro with sub micromolar EC50 values against H5N1 strains. Naproxen, a well-known COX-2 inhibitor-an anti-inflammatory agent. In silico molecular docking and molecular dynamics (MD) simulation studies conducted by Nathalie Lejal et al. proved that naproxen is binding with RNA-binding groove of H1N1 NP with good binding affinity scores. Thus, naproxen can exhibit dual activity-antiviral as well as suppression of pro-inflammatory immune responses that are generated by influenza strains like H5N1 and H1N1. Based on this hypothesis, further Slama-Schwok et al. designed naproxen analogs that showed enhanced binding to H1N1 nucleoprotein [22].

M2 ion channel Inhibitors: M2 matrix ion protein channel causes influx of hydrogen atoms into the viral core and subsequent release of viral ribonucleoprotein particles (vRNPs) from M1 matrix proteins into cytoplasm (uncoating). Hence blocking of M2 ion channel will block the entry of virus. The adamantane derivatives, amantadine and rimantadine are currently used for influenza treatment. They block the M2 ion channel of the virus inhibiting the early stages of viral replication, however, these drugs initiate viral resistance [23].

H5N1 NOVEL SMALL MOLECULE INHIBITORS (SMIs)

Targeting specific receptor-pathway sequences with small molecule inhibitors using advanced research tools and techniques has great scope for research and development. They are cheap when compared to therapies involving antibody or protein preparations. Several targeted small molecules are being designed rationally and synthesized from time to time and structures of some of them are shown in Fig.3 and listed in table 3. Chemistry aspects of small molecule H5N1 inhibitors with their targets developed during the past ten years has been reviewed here with an aim to give an overall idea about the development of potential drug candidates in the near future.

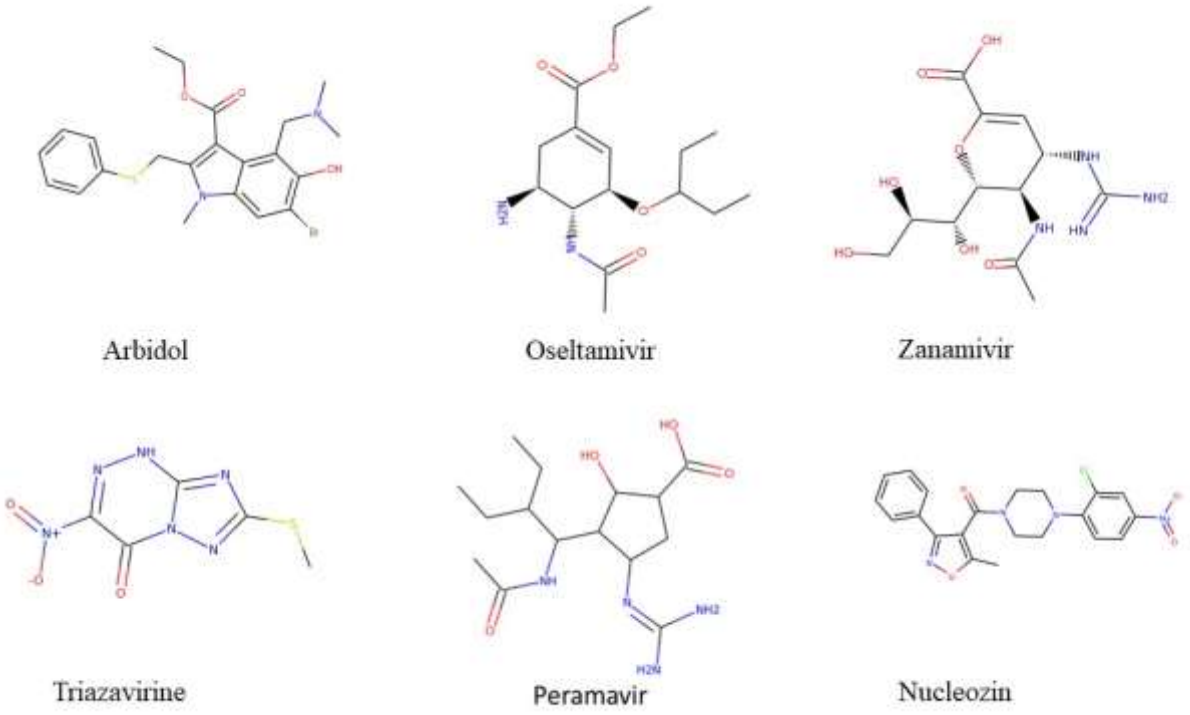

Fig. 3: Small molecule Inhibitors of Influenza Virus

Table 3: List of some H5N1 inhibitors and their role

Some H5N1 Inhibitors	Role
Glycyrrhizin	Inhibits viral entry
Glycyrrhizic acid, Arbidol Oleanane acid (OA) triterpenes, Andrographolide derivative, Methanolic extracts of Extract of Echinacea purpurea	Inhibits HA
Zanamivir, Oseltamivir, Peramivir, Laninamivir	Inhibits NA
Favipiravir, Triazavirin	Inhibits RdRp
Nucleozin	Inhibits NP
Amantadine, Rimantadine	Inhibits M2 ion channel
Ribavirin	Inhibits inosine 5′ monophosphate (IMP) dehydrogenase; suppress the biosynthesis of GTP; blocks viral RNA replication

Oseltamivir derivatives:

Modification of existing drug molecules of known activities with other substituents results in the development novel drug molecules that have desired actions. Oseltamivir is an effective oral drug that inhibits NA of viral influenza. Other derivatives of Oseltamivir include OS phosphonate congeners, N-substituted OS derivatives, acyl guanidine derivates of OSC, and guanidino-oseltamivir (GO) and its phosphonate congeners: NG-substituted GOC, acyloxy ester derivatives of GOC, and N-hydroxyamide-substituted OSC and GOC [24]. Presence of N, N diethyl amino group on the aromatic ring substituent of the amine moiety on Oseltamivir carboxylate (OSC) showed greater Neuraminidase (NA) inhibitory activity than OSC [25]. Secondary amine derivatives are found to be having good anti H5N1 activity. Introduction of biphenyl substituent on 5th position amine of Oseltamivir selectively targeted 150-cavity of NA-1 and showed IC50 values of 0.0019 µM, 0.0038 µM and 0.0067 µM against the three types of H5N1 NAs [26] (Fig. 4A). [4-(phenylsulfanyl) phenyl] methanamine derivative of OSC showed better anti H5N1 activity. Its computational studies also showed good potency against N1, N8, and N1-H274Y mutant NAs in the inhibitory assays [27]. Modification of ethyl carboxylate moiety on C-1 of OST with {[amido(carboxy)methyl] amino}-3-methylbutanoic acid resulted in safe and effective compound on par with OSC. It also exerted IC50 value of 0.088 mM and EC50 value of 4.26 mM against H5N1 strains, targeting the 430-cavity of NA [28]. Carboxyl-modified oseltamivir derivatives developed by Boyu Wang et al. showed improved lipophilicity (Log D = -0.12) than OSC (Log D = -1.69) at pH7.4, favoring good membrane permeability and oral drug absorption with high metabolic stability in human liver microsomes [29].

Peramivir Derivatives:

Peramivir is an effective neuraminidase inhibitor, approved for intravenous administration. The dehydration derivative of phosphonate compound (Fig. 4B) of Peramivir (Phosphono-Peramivir, resulted from replacement of carboxylate group in Peramivir with a phosphonate group) [30], has a rigid cyclopentene core structure and exhibited strongest inhibitory activity (IC50 = 0.3-4.1 nM against avian influenza viruses H1N1, H3N2, H5N1 and H7N9 specifically targeting NA.

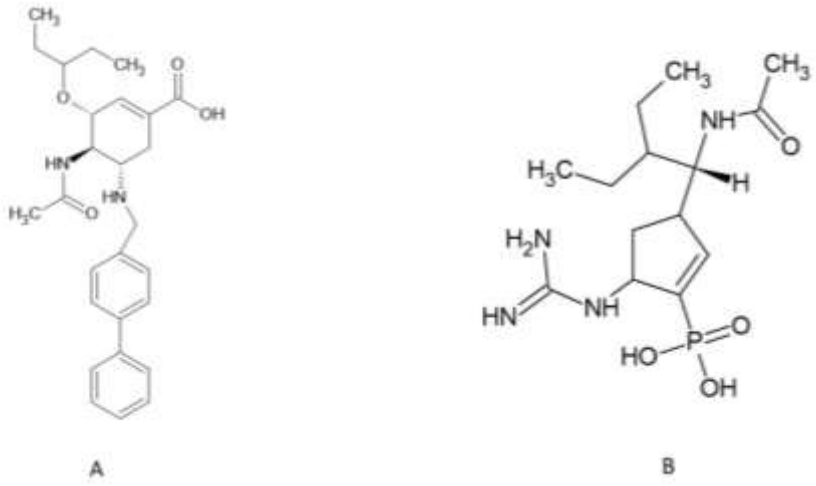

Fig. 4: H5N1 inhibitors of Oseltamivir and Peramivir derivatives

Zanamivir (ZA) analogues:

Zanamivir was the first commercially developed neuraminidase inhibitor used to treat both influenza A and B infections. Zanamivir-Caffeic acid conjugates [31] with ester linkage and Zanamivir-Caffeic acid conjugates with amide linkage showed dual targeted action of both anti-influenza-NA inhibition and anti-inflammatory-suppression of proinflammatory cytokines. These conjugates showed activity superior to Zanamivir alone and other combination therapy of ZA with anti-inflammatory agents. Based on bio isostere replacement and scaffold hopping strategies, replacement of C-4 guanidinium group of zanamivir with a-L-amino acid moiety especially with L-Asn showed good potency with IC50 values of 2.72 mM against group-1 (H5N1) NA [32]. Substitution of the C-4 guanidinium group of ZA with the ureido bio isostere and the introduction of Furan-2-ylmethyl substituent at the terminal N-position, showed more anti-AIV activity against infected MDCK cells than ZA.

Natural Product Derivatives:

Natural products are the huge repositories of polyphenols, flavonoids, alkaloids, lignans with anti-viral properties. Many herb species with their active constituents having potential anti-viral activities were reported. In vitro cell culture methods and in vivo mouse models were used frequently to report the inhibitory action of natural products and their derivatives. Nur Kusaira Khairul Ikram et al. performed virtual screening of natural products belonging to five tropical plants namely G. mangostana, E. longifolia, T. divaricata, B. javanica, and M. charantia which have anti H5N1 NA activity against H5N1 NA (PDB ID: 2HU4). Extracts from the various parts of these plants showed good to moderate anti-H5N1 NA activity, among which, G. mangostana showed the highest inhibition of 82.95% at 250 µg/mL. Significant IC50 values ranging from 89.71 to 95.49 µM were observed in rubraxanthone, α-mangostin, and garcinone C [33]. A.K. Ibrahim et al. isolated three compounds from Capparis sinaica veill, namely, quercetin, isoquercetin and rutin and using plaque inhibition assay in Madin–Darby's canine kidney cells, they confirmed that the methanolic extracts of the above three showed anti H5N1 activity with a potency greater than reference compound Zanamivir, probably inhibiting viral NA [34].

Terpenes: Molecular simulation studies confirmed that benzyl derivative of saponins, more specifically, benzyl derivative of 3-ß-chacotriosyl oleanic acid, prevented viral entry via binding to hemagglutinin (HA2) protein. 7-dehydroabietanone, a diterpene isolated from the stem bark of *Fraxinus sieboldiana*, showed inhibitory activity with IC50 value of 4.8 µM compared to positive control Zidovudine (IC50 value of 0.048 µM) against H5N1 avian influenza virus [35] (Fig. 5C).

Alkaloids: Recent molecular docking studies of iso quinolone alkaloids (palmatine, berberine, jatrorrhizine, epiberberine, columbamine, and coptisine) extracted from *Coptis chinensis* rhizomes, exhibited good binding affinities to NA-1 and NA-2 proteins in both H5N1 wild type, and H5N1 H274Y mutant variety than commercial drugs like Oseltamivir and Zanamivir. Out of these six compounds jatrorrhizine formed four hydrogen bonds and one similar active receptor site [36]. However, lack of strong interaction with the protein limits their property of protein inhibition except for jatrorrhizine (Fig. 5D), which could be a promising candidate in anti-influenza A drug development, probably due to the benzylisoquinoline nucleus that allows the ligands to fit into the binding pockets.

Polyphenols: Isolated polyphenolic compounds of *Phellinus baumii* mushroom effectively inhibited H5N1 NA activity and also reduced the viral induced cytopathic effect [37]. Synthetic compounds with amino acid fragments incorporated into their structure were developed by Yuanchao Xie et al. inspired from reported flavonoid and mimosine tetrapeptides NA inhibitors. These compounds showed weak inhibitory activity against NA and moderate anti H5N1 activity in chick embryo. Chlorogenic acid derivatives of *Stemona japonica* namely methyl 3-O-feruloylquinate (Fig. 5E) and methyl 5-O-caffeyolquinate (Fig. 5F) showed moderate in vitro H5N1 inhibitory effect [38].

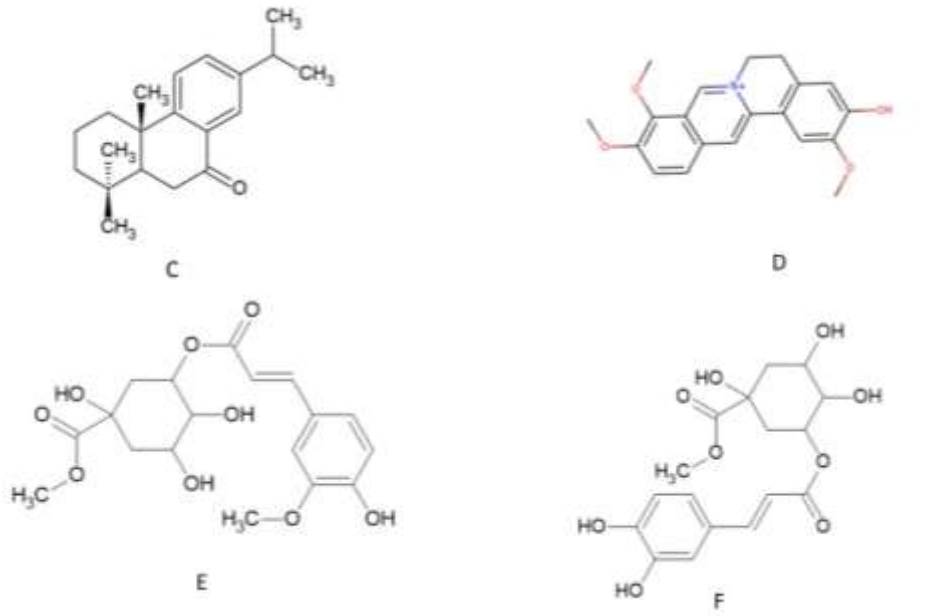

Fig. 5: Natural Product Derivatives as H5N1 inhibitors

Nitrogen containing SMIs:

Cyclohexyl carboxylic acid derivative of azaindole has successfully inhibited H5N1 strain and it showed good oral bioavailability [39] (Fig. 6G). Thiadiazole derivative of 3-hydroxyadamantane inhibited the M2 proton channel of S31 mutant avian influenza with sub micromolar range of EC50 values [40]. Benzyl derivative of amantadine is a dual inhibitor of both wild type and S31 mutant influenza variety [41] (Fig. 6H). 7-methyl-guanosine derivatives developed by Stéphane Pautus et al. targeted the Polymerse PB2 cap binding domain and inhibited viral mRNA [42]. Carbocyclic nucleoside derivatives-C-3 chloro substituted synthetic derivative developed from 5-noraristeromycin showed activity against H5N1 strains [43] (Fig. 6I).

Jagadeeshwar R. Rao et al. successfully synthesized the cyclopentyl cytosine (-)-carbodine via cylopentanol key intermediate derived from D-ribose and in vitro comparison studies of anti-influenza activity of (+)-carbodine, (±)-carbodine and ribavirin was performed against various H5N1 strains. (-)-carbodine showed significant anti-influenza activity [44]. 1H-1,2,3-triazole-4-carboxamide derivatives of Nucleozin showed good anti influenza activity by inhibiting viral nucleoproteins [45] (Fig. 6J). Thienopyramidine derivatives developed by Aymn E. Rashad et al. which have structural resemblance to thiouracil, showed effective anti H5N1 activity determined by plaque reduction assay on MDCK cells [46]. Benzamide-based 5-aminopyrazoles having ethylthio, pyrazolo[1,5-a] pyrimidine scaffold and amino-, cyano- and methoxy phenyl substituents showed significant anti H5N1 activity determined by MTT cytotoxicity and plaque reduction assays. These compounds showed viral reduction in the range of 85−65% [47]. Another small molecule CL385319 potently targeted a cavity in HA2 stem region near FP domain thereby inhibiting H5N1 influenza virus (Fig. 6K). Yiwu Yan et al. showed that Chloroquine, an anti-malarial drug and an autophagy inhibitor effectively treated avian influenza A H5N1 virus infection in a mouse model [48]. Isocyanides as Influenza A Virus Subtype H5N1 Wild-Type M2 Channel Inhibitors [49].

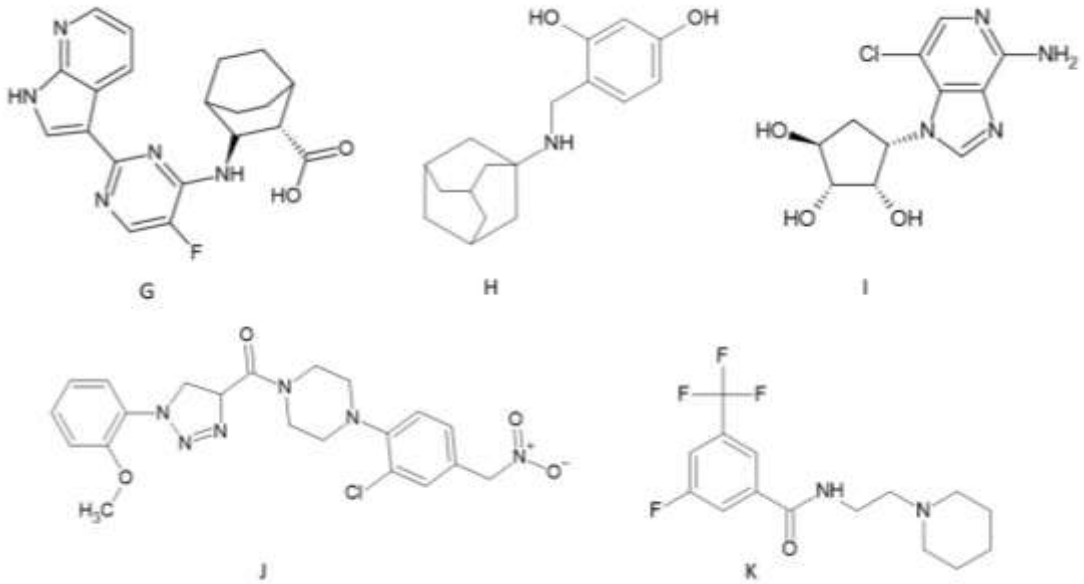

Fig. 6: Some of the nitrogen containing compounds acting as H5N1 inhibitors

Sulphur SMIs:

A series of aryl sulphonamide derivatives [50] inspired from previously developed furan-carboxamide analogues with potent H5N1 viral M2 proton channel inhibition was developed by Yongshi Yu, Qi Tang et al. The compound with cyano and thienyl groups as substituents have showed potent inhibitory action with EC50 value of 0.006µM and CC50 value of 201.26 µM. Compounds with thiophene chromophore [51] having strong pi-pi interactions with the viral protein were developed by Zhibo Zhu et al. Amino group substituents with 3-4 carbon linkage between thiophene and amine group showed good viral entry inhibitory activity with IC50s 0.029 µM against H5N1 influenza pseudo virus.

CONCLUSION

Very few drugs are really effective towards influenza virus which constantly undergoes strain mutations and acquires drug resistance. Development of SMIs that can inhibit resistant strains is a challenging task. Oseltamivir is an effective NA inhibitor but the virus gained resistance over it. Modification of the amine and carboxylate moieties of Oseltamivir with other substituents like biphenyls resulted in drug candidates with improved potency, oral absorptivity and lipophilicity. Peramivir phosphonates, Zanamivir conjugates showed improved efficacy. However, modification of guanidino groups in Zanamivir with furan containing ureido bio-isosteres also resulted in improved anti H5N1 activity as proved by bio-assay experiments in infected MDCK cells. Many natural product derivatives in the recent past containing alkaloids, polyphenols, terpenes studied via in silico molecular docking studies, various in vitro and in vivo assays showed moderate to good anti H5N1 activity. Similarly, many nitrogen and Sulphur containing SMIs that exhibit H5N1 inhibitory activity were developed. Apart from SMIs, several other therapeutic approaches like vaccine therapy, mAb therapy, peptide therapy are also present, but still SMIs always remains as one of the meteoric approaches to ever challenging drug resistant viral infections.

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