2nd International Conference on Influenza

Novel and efficacious compounds disturb influenza A virus infection

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Influenza A virus is a negative RNA stranded virus of the family *Orthomyxoviridae*, and represents a major public health threat, compounding existing disease conditions. Influenza A virus replicates rapidly within its host and the segmented nature of its genome facilitates re-assortment, whereby whole genes are exchanged between influenza virus subtypes during replication**.** Antiviral medications are important pharmacological tools in influenza virus prophylaxis and therapy. However, the use of currently available antiviral is impeded by sometimes high levels of resistance in circulating virus strains. Notably, the over use of existing antiviral drugs such as oseltamivir (Tamiflu) and zanamavir (Relenza) increases the likelihood of viral escape mutations. Here, we identified novel anti-influenza compounds through screening of chemical compounds that synthesized *de novo* and several naturally occurring products on human lung epithelial cells. Computational and experimental screening of extensive natural products and water soluble chemical compounds identified novel influenza virus inhibitors that can reduce influenza virus infection without any detectable toxic effects on host cells. Interestingly, the indicated active chemical compounds inhibit viral replication most likely via interaction with cell receptors and disturb influenza virus entry into host cells. Additionally, the selected natural product inhabits viral replication via increasing of interferon beta (IFN-β) production from infected cells. In conclusion**,** screening of new synthesis compounds and natural extractions on influenza A virus replication provides a novel and efficacious anti-influenza compounds that can inhibit viral replication and indicates that these compounds are attractive candidates for evaluation as a potential anti-influenza drugs.

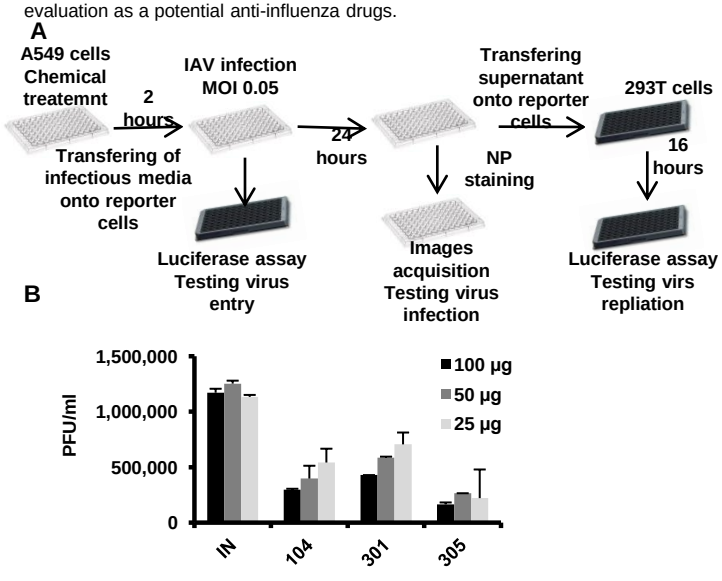

Figure 1. **Chemicals screening on influenza A virus replication**. **(A)** Schematic representation of chemical inhibitors screening on infected A549 cells using 96 well plates as primary infection. Infection buffer and infectious media were used to infected 293T cells to determine remained virus and produced viral particles, respectively, using virus dependent luciferase assay. **(B)** Plaque forming units of virus particles on MDCK cells infected with the infectious media that was collected 24 hours upon infection of A549 cells.

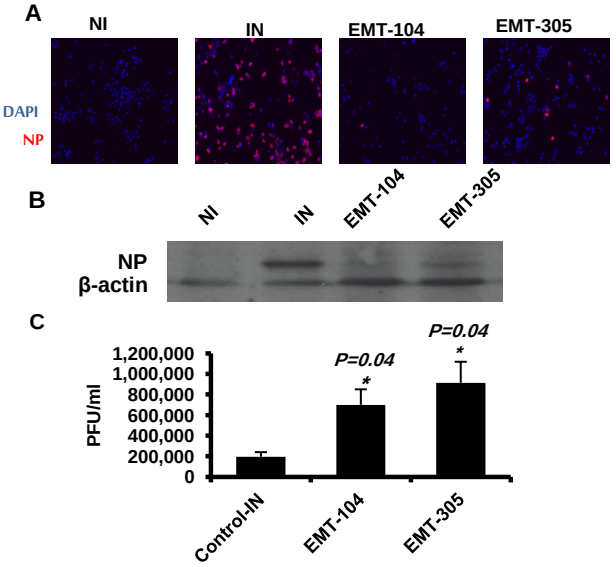

Figure 2. EMT-305 and EMT-104 compounds inhibit IAV infection via disturbing entry. (A) Representative confocal images depicting infected A549 cells revealing the expression of viral NP (red) and DNA (blue). **(B)** Western blot analysis of viral NP protein in infected and pretreated A549 cells. β-actin served as loading control. **(C)** Plaque forming units of viral particles on MDCK cells infected with infectious media that was collected 1 hour post infection of A594 cells pre-treated with the indicated inhibitors.

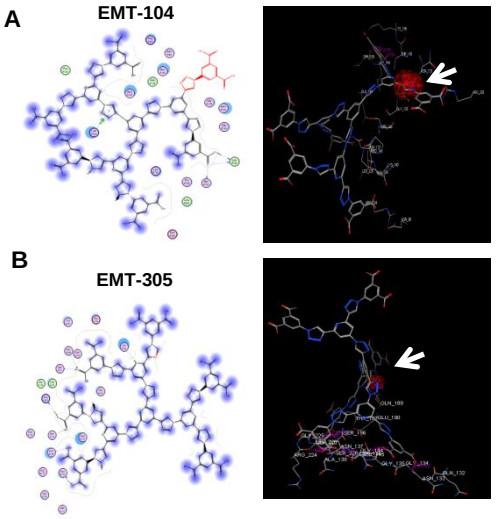

Figure 3. **Molecular docking analysis of EMT-305 and EMT-100 compounds with hemagglutinin**. **(A)** The molecular docking indicates the binding affinity of synthesized compound EMT-104 and hemagglutinin in host cell receptor and clarifies the seeding region of the possible interaction between the inhibitor EMT-104 and sialic acid. **(B)** The molecular docking of synthesized compound EMT-305 and hemagglutinin in host cell reveals the binding affinity receptor and seeding region of the possible interaction between the inhibitor EMT-305 and sialic acid.

Our findings here indicate novel and active water soluble compounds (EMT-104 and EMT-305) against IAV infection without-detectable toxic effect on cell viability and cell proliferation. These identified compounds have the ability to disturb virus entry may via binding with cell receptor and reduced the binding between viral hemagglutinin and host cell receptors. Recently, solubility of chemical compounds in aqueous buffer has become a critical issue in drug discovery to prevent several barriers in biological challenge assays. For instance, Dimethyl sulfoxide (DMSO) is an organic compound with a median lethal dose higher than ethanol usually used at concentration of 30 mM to dissolve hydrophobic compounds**.** Notably, when absorbed through skin, DMSO causes contamination and unexpected harmful cytotoxic effects in cellular function resulted in suppression of cell proliferation **.** Accordingly, the current data provide novel and active candidates that can inhibit IAV entry to host cells with high solubility in aqueous buffer. Importantly, based on the hypothesis assumes that, the numerous virus particles remained in the infectious media reveals the upset level of virus entry during primary infection on treated cells, virus particles in the rest of infectious media was quantified to investigate the possible interaction between chemical inhibitors and host cell receptors. Both luciferase assay and plaque assay showed high concentration of virus particles remained in infectious media used to infect A549 cells treated with both EMT-305 and EMT-104 compared to control treated cells.

Acknowledgments

The authors thank Prof. Dr. Thomas Meyer (Max-Planck Institute for Infection Biology, Berlin, Germany) for kindly providing Flu-Luci plasmid and facilities doing the screening experiments. The current work is supported by Young Researcher Grant Program, provided by The Egyptian Science and Technology Development Fund (STDF), Project ID: 4694.