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High-throughput docking for the identification of new influenza A virus polymerase inhibitors targeting the PA–PB1 protein–protein interaction

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ABSTRACT

A high-throughput molecular docking approach was successfully applied for the selection of potential inhibitors of the Influenza RNA-polymerase which act by targeting the PA–PB1 protein–protein interaction. Commercially available compounds were purchased and biologically evaluated in vitro using an ELISA-based assay. As a result, some compounds possessing a 3-cyano-4,6-diphenyl-pyridine nucleus emerged as effective inhibitors with the best ones showing IC_{50} values in the micromolar range.

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Influenza, usually known as flu, is an infectious disease of birds and mammals caused by ribonucleic acid (RNA) viruses of the family Orthomyxoviridae. Influenza viruses are classified as type A, B, and C in accordance with antigenic differences among their nucleoprotein and matrix protein components.[1] Among these viruses, influenza A and B are similar in their morphology, genome structure, and protein function. Influenza A and B viruses cause severe upper respiratory diseases in humans, which occur seasonally in epidemic and sometimes in pandemic proportions. As the impact of influenza pandemic is enormous, a renewed drug discovery effort worldwide is essential to counteract the disease more efficiently. Presently vaccination is the mainstay, along with anti-influenza drugs, to control the spread and treatment of the disease. Every season a new vaccine is developed through the identification of the proper combination of antigenic strains. However, as there is a time gap of 9–12 months from the recommendation to the actual use of vaccine, this could still result in mismatch between the virus and the vaccine, which could result in ineffectiveness.[2,3] Because of these and other complicating factors influencing the development of an all effective flu vaccine, additional weapons in the form of effective anti-influenza agents are a must in our fight against seasonal or pandemic influenza that may arise in the future.[4,5] Adamantane derivatives **1** (amantadine) and **2** (rimantadine) are the first two antiviral drugs used for the treatment of influenza, which target the M2 ion channels (Fig. 1). Orally administered **3**

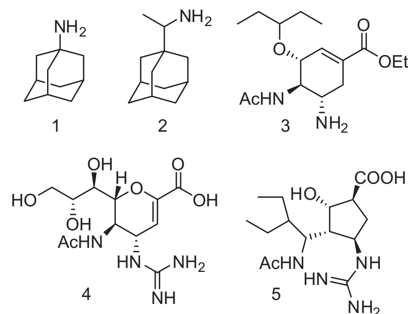

Figure 1. Anti-influenza agents.

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(oseltamivir) and inhaled **4** (zanamivir) are approved for use as anti-influenza treatments, while **5** (peramivir) is under clinical investigation; all the three compounds target the neuraminidase (NA).[6–8]

However, alternative therapeutics are needed because the development of resistance strains to these drugs targeting NA enzyme, particularly **3**, has been reported as an increasing clinical problem.[9,10] Thus there is an urgent need to identify novel compounds with inhibitory activity against influenza virus, which is the aim of this study.

The influenza RNA-dependent RNA polymerase (RdRp) heterotrimer has a crucial role in the viral RNA replication and transcription. It is constituted by three subunits: PA, PB1 and PB2. PB1 is responsible for the polymerase and endonuclease activities, while PB2 is implicated in the process of cap-binding. PA is an important protein in the heterotrimer and may be required for replication and transcription of viral RNA (vRNA) and endonuclease cleavage of the cap RNA primer. The structure of the trimeric complex at the atomic level is not yet resolved. There are only two crystal structures[11,12] available in Protein Data Bank of the C-terminal region (PAC) in complex with a peptide from PB1 (PB1N, residues 1-25). PAC consists of 13 α-helices, one short 310 helix, nine β-strands and several loops/turns. PAC resembles the head of a dragon which can be divided into two parts: domain I, the ‘brain’, and domain II, the ‘mouth’. PB1N binds PAC with its N-terminus pointing towards the back of the mouth and its C-terminus extending outwards. Residues from 5 to 11 of PB1N fold into a short α-helix. Since the association of these subunits is essential for the viral replication, and since the sequence of this domain is highly conserved, this interaction represents an attractive target for the development of antiviral drugs. In this regard, we recently reported the identification of new anti-influenza agents through an high-throughput screening (HTS) approach.[13,14] Nitrobenzofurazan derivatives showed micromolar potency of inhibition against A/WSN/33 H1N1 viral replication and were proved to act through the disruption of the PA–PB1 subunits.

Pursuing this research line, computational strategies were employed herein with the aim of identifying new small molecules able to affect the PB1–PA protein–protein interaction. To this aim, we first performed a molecular dynamic simulation on the PA–PB1 complex to identify the crucial residues for the interaction between the two proteins (hot spots residues, Table 1S, Supporting material). Accordingly with experimental data previously reported by Wunderlich et al.,[15] residues Met1, Val3, Asn4, Pro5, Leu7, Leu8, Phe9 and Leu10 on the PB1 peptide seemed to be the most important for the interaction with PA, showing the lowest binding free energy. Furthermore, stable hydrogen bonds were found at the interface of the two interacting proteins (Table 2S, Supporting material). Particularly, amino acids Gln408, Trp706, Asn412, Arg673 and Gln670 in PA established hydrogen-bond contacts with Val3, Asn4, Asp2, Leu10 and Pro13 in PB1N, respectively. Noteworthy, we observed close to 100% occupancy for the polar interaction between the side chain of Gln408 and the backbone carbonyl group of Val3.

On the basis of results from molecular dynamic simulation, we focused next calculations on the wide region of the identified hotspots to discover small molecule inhibitors of PA–PB1 protein–protein interaction. Particularly, we searched for small-molecules that mimic the interactions made by the first part of PB1 (residues 1, 3–5) or established by its central portion (residues 7–10), being those the amino acids which emerged as the most effective from MM-GBSA analysis (Table 1S, Supporting material). Asinex commercial databases (consisting of 703,200 compounds) were virtually screened using an high throughput docking approach. In detail, a consensus docking protocol that combines the use of two docking programs (Glide[16] and Gold[17]) was applied in this study, accordingly with a procedure recently reported by us.[14] The docking study led to the final selection of 115 compounds (Chart 1S, Supporting information) which were chosen by considering both the overall match between the binding modes proposed by the two programs and the predicted score values. Taking advantage from the selected compounds, characterized by various scaffolds and many different types of binding modes, the whole binding site was explored. An ELISA protocol previously published[18] was then used as a biochemical method to test the virtual screening hits. Compounds **6–10**, possessing a 3-cyano-4,6-diphenyl-pyridine nucleus, were identified as weak inhibitors of the PA–PB1 interaction being endowed with IC_{50} ranging from 80 to 180 μM. Starting from the structures of the identified active compounds, a research of analogues has been performed by using the web-site eMolecules (http://www.emolecules.com/). Ten derivatives have been selected for biological studies (Chart 2S, Supporting information). Among them, **11** and **12** appear to inhibit the PA–PB1 interaction with IC_{50} of 35 and 30 μM, respectively (Fig. 2 and Table 1). Preliminary cytotoxicity assay indicates that the active derivatives are nontoxic in vitro (CC_{50} between 100 and 150 μM in MDCK cells).

Analysing the poses of the active compounds within the binding pocket, we can observe that their binding mode is stabilized by three hydrogen bonds involving the residues Gln408, Val621 and Lys643. Furthermore, three regions of hydrophobic interactions can be identified: I) consisting of residues Val621, Cys415 and Phe411; II) characterized by amino acid Val628; III) formed by residues Phe658, Phe707, Leu666 and Phe710. As an example, the binding mode of compound **11** is visualized in Figure 3.

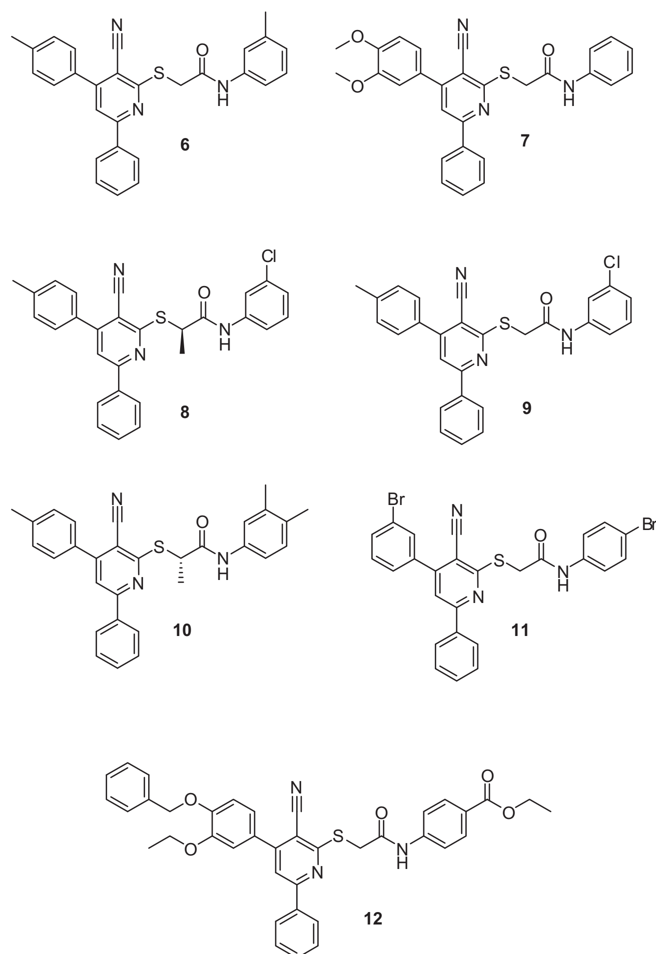

Figure 2. Active compounds selected by virtual screening.

Table 1
IC_{50} values of the hit compounds obtained by the ELISA assay previously set-up to test PA–PB1 interaction inhibitors[18]

Name	Commercial code	ELISA IC_{50} (μM)
6	BAS 01157570	80
7	BAS 00801667	125
8	ASN 01157620	125
9	ASN 01213501	180
10	ASN 01054154	180
11	STK670561	35
12	AG-690/40750299	30

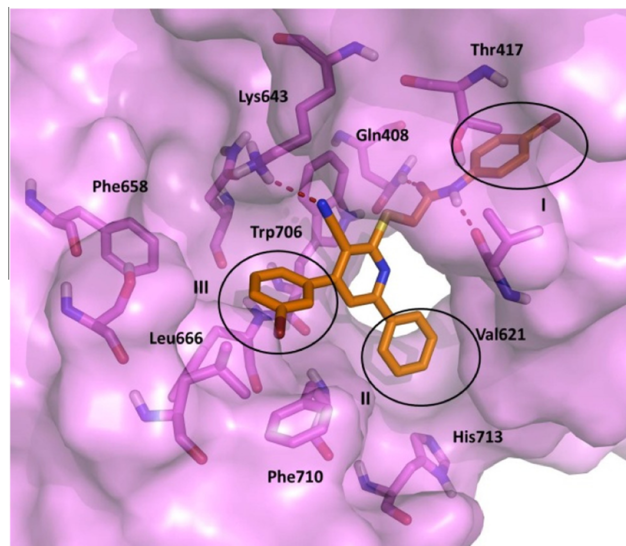

Figure 3. Binding mode of compound **11** (orange stick) as representative of the 3-cyano-4,6-diphenyl-pyridine family.

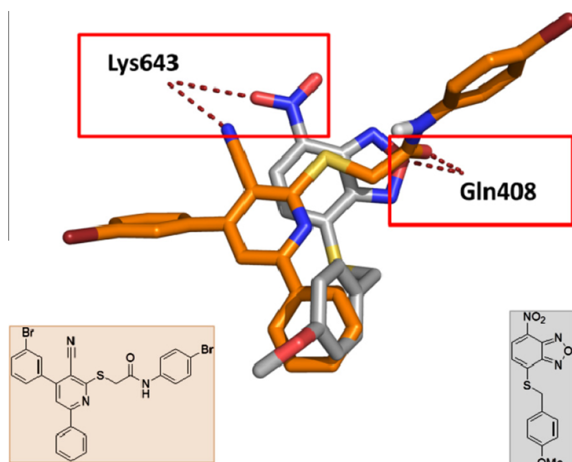

Figure 4. Superimposition of 3-cyano-4,6-diphenyl-pyridine and nitrobenzofurazan scaffolds highlight the regions of common interactions.

Remarkably, comparing the binding orientation of compound **11** with the pose previously identified for nitrobenzofurazan inhibitors, some common interactions were identified (Fig. 4): an hydrogen bond with Gln408, an electrostatic interaction with Lys643 and hydrophobic contacts with Val628 in the region usually occupied by Leu7 of PB1N. Interestingly, while this work was ongoing, another research group published an article in which some of these residues were described as important for the development of PA–PB1 inhibitors.[19,20]

In conclusion, some compounds possessing a 3-cyano-4,6-diphenyl-pyridine nucleus were identified as new influenza A virus polymerase inhibitors targeting the PA–PB1 protein–protein interactions. Moreover, some key interactions emerged as crucial in determining the binding of potential inhibitors to PB1 binding site on PA. Further biological studies on the PA–PB1 inhibitors reported herein are currently underway and will be published in due course.

Acknowledgments

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Supplementary data

Supplementary data (molecular modeling procedures, and compounds selection and ELISA assay) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.11.019.

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