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Short Communication

Identification of potential inhibitors of protein-protein interaction useful to fight against Ebola and other highly pathogenic viruses

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ABSTRACT

Despite the efforts to develop new treatments against Ebola virus (EBOV) there is currently no antiviral drug licensed to treat patients with Ebola virus disease (EVD). Therefore, there is still an urgent need to find new drugs to fight against EBOV. In order to do this, a virtual screening was done on the druggable interaction between the EBOV glycoprotein (GP) and the host receptor NPC1 with a subsequent selection of compounds for further validation. This screening led to the identification of new small organic molecules with potent inhibitory action against EBOV infection using lentiviral EBOV-GP-pseudotype viruses. Moreover, some of these compounds have shown their ability to interfere with the intracellular cholesterol transport receptor NPC1 using an ELISA-based assay. These preliminary results pave the way to hit to lead optimization programs that lead to successful candidates.

Infectious diseases represent one of the major concerns for human and veterinary public health and, as a consequence, for the global economy. Due to climate and environmental changes and travel and trade globalization, infectious agents spread more rapidly and widely than in the last century. This is shown by the number of emerging and reemerging viral infections appeared since 2000, such as the 2009 pandemic H1N1 influenza virus, the widespread epidemic of Ebola virus (EBOV) from West Africa that generated cases in Europe and America and the unprecedented pandemic caused by a novel coronavirus (SARS-CoV-2/COVID-19) in December 2019. Moreover, additional emerging viruses have been identified for the first time in Spain, most of them causing potentially lethal infections in humans, such as Crimean-Congo hemorrhagic fever, Dengue or West Nile viruses. Due to these sporadic outbreaks, these emerging viruses require a continuous surveillance activity that needs a significant effort of the National Health Systems and of the International Authorities (Wolfe et al., 2007; Zhou et al., 2020).

In December 2013, an outbreak caused by EBOV began in West Africa. The epidemic lasted until 2016 with 28,616 cases and 11,301 deaths (WHO Ebola Response Team et al., 2016). This was the largest and most complex outbreak so far, being declared by the WHO as a public health emergency of international concern. There were more cases and deaths in this outbreak than in all the others combined. In addition, it also spread between countries, starting in Guinea then moving across land borders to Sierra Leone and Liberia. For the first time, EBOV infected individuals appeared and were subsequently treated in the US and some European countries. Spain was the first country to ever have a case of EBOV transmission out of Africa (López et al., 2015). The 2014 outbreak highlighted the scarcity of antivirals and vaccines against this highly pathogenic virus which precludes not only medical care but also epidemic control. In 2016, the largest epidemic of Ebola virus disease finally ended. However, the risk of re-emergences was made evident by the 2018–2020 outbreak in the Democratic Republic of the Congo (DRC) and the current situation in this country (Rohan and McKay, 2020). Currently, despite the 280,000 persons have been vaccinated (Saphire, 2020), the epidemics continue...
and it seems necessary to concentrate research efforts in antivirals. Consequently, the WHO is trying to accelerate trials with experimental drugs in the expectation of reducing the number of deaths and protecting those who are in contact with the patients. Actually, the first clinical trials with the nucleoside analogue remdesivir (GS-5734), the single monoclonal antibody MAb 114 or combinations of monoclonal antibodies such as ZMapp (the control group) or the triple monoclonal antibody REGN-EB3 have been performed (Malvy et al., 2019; Mulangu et al., 2019).

Antibody REGN-EB3 have been performed (Malvy et al., 2019; Mulangu et al., 2019). EBOV-GPcl binding to NPC1 is the key in the lock that mediates fusion with the endosomal membrane and allows the viral nucleic acid to be delivered in the cytoplasm (Miller et al., 2012). Of note, this sequence of events is similar in a number of highly pathogenic emerging viruses affecting humans and animals (Del Campo et al., 2012; Cuesta-Geijo et al., 2016).

To identify the desired protein-protein interaction inhibitors as new antiviral agents, a virtual screening was performed initially. We used our in-house chemical library (MBC library) (Sebastian-Pérez et al., 2017) and the crystal structure of the complex EBOV-GPcl/NPC1 domain C (Wang et al., 2016) focusing the screening in the interface between both proteins. As filter, the interaction with the key residues Phe503 and Phe504 of NPC1 located in the protruding loop 2, which play a major role by contributing to the majority of the tight hydrophobic interactions with the GPcl head cavity was used (Wang et al., 2016). The druggability of this interaction was previously validated and reported as Phe503, and Phe504 (Fig. S1). Noteworthy, compounds MBX2254 y MBX2270, (Basu et al., 2015) previously reported as inhibitors of the EBOV-GPcl/NPC1 binding, such as adamantane dipeptide piperazine 3,47, (Côté et al., 2011) triazole thioether MBX2270 and aminoacetamide sulfonamide MBX2254 (Basu et al., 2015). The study here presented has allowed the selection and identification of novel candidates to be evaluated as anti-EBOV agents.

From this initial virtual screening, 34 compounds were selected to be evaluated as antivirals based on best docking scores and chemical diversity (Table S1). All selected compounds were located between the interface of the GPcl/NPC1 domain C complex, The compounds with best scoring are located between loop 1 and loop 2 of NPC1 domain C showing interactions with key residues involved in the interaction such as Phe503, and Phe504 (Fig. S1). Noteworthy, compounds MBX2254 y MBX2270, (Basu et al., 2015) previously reported as inhibitors of the target interaction were used as reference controls being near the top of the ranking. Compounds 1–34 were tested in a first step using a lentiviral EBOV-GP-pseudotyped infection assay (Table S2) using as

### Table 1

| Comp. | Chemical structure | IC₅₀ (pEBOV) | CC₅₀ (HeLa) | SI (pEBOV) (CC₅₀/IC₅₀) | IC₅₀ (ASFV) | CC₅₀ (Vero) | SI (ASFV) (CC₅₀/IC₅₀) |
|-------|--------------------|--------------|-------------|------------------------|-------------|-------------|-----------------------|
| 2     | ![Chemical Structure](image1) | 0.37 μM      | 7 μM        | 19                     | 2.54        | 16.56       | 6.5                   |
| 9     | ![Chemical Structure](image2) | 0.37 μM      | 96 μM       | 258                    | inactive    | >100        | –                     |
| 12    | ![Chemical Structure](image3) | 64.9%@10 μM  | –           | inactive               | >100        | –           | –                     |
| 14    | ![Chemical Structure](image4) | 4.69 μM      | 30 μM       | 11                     | 35.17       | >100        | >2.8                  |
| 19    | ![Chemical Structure](image5) | 70.9%@10 μM  | –           | inactive               | >100        | –           | –                     |
| 26    | ![Chemical Structure](image6) | 2.04 μM      | 60 μM       | 26                     | 15.75       | >100        | >6.34                 |
| 30    | ![Chemical Structure](image7) | 61.2%@10 μM  | –           | inactive               | >100        | –           | –                     |

- IC₅₀: 50% inhibitory concentration.
- CC₅₀: 50% cytotoxic concentration.
- SI: Selectivity Index.
μ showed an IC$_{50}$ (50% cytotoxic concentration). Benzothiazepine KZ52 was incubated with EBOV-GPcl particles. Unbound viral particles were discarded using the GraphPad Prism v6.0 software. Comparisons of the three chemical classes could be explained on basis to the binding of thermolysin-cleaved EBOV-GP to NPC1-domain C determination using ELISA-based assay. Results pointed to the fact that carbazoles and sulfides potentially act through inhibition of NPC1-GP interaction, while benzothiazepines do not affect this interaction. The two groups of chemicals able to inhibit the infection of both viruses, carbazoles and sulfides, probably share a common mechanism of action relevant for both viruses. In contrast, benzothiazepines could act against EBOV through a novel specific mechanism that is currently under study. While further studies to assess experimentally the mechanism of action of these new compounds are in progress, a hit to the work reported in this paper.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Appendix A. Supplementary data**

Supplementary data to this article: Table S1 (top-ranked compounds selection), Table S2 (biological evaluation of compounds initially selected), Figure S1 (binding poses of top-ranked compounds), Figures S2 and S3 (dose-response curves for top compounds) and Material and Methods section.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2021.105011.
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