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Identification of novel small-molecule inhibitors of Zika virus infection

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ABSTRACT

The recent re-emergence of Zika virus (ZIKV), a member of the *Flaviviridae* family, has become a global emergency and a serious public health threat worldwide. ZIKV infection causes severe neuroimmunopathology and is particularly harmful to the developing fetuses of infected pregnant women causing various developmental abnormalities. Currently, there are no effective methods of preventing or treating ZIKV infection, and new treatment options are urgently needed. Therefore, we have used an *in vitro* plaque assay to screen a limited proprietary library of small organic compounds and identified highly bioactive leads, with the most active analogs showing activity in low picomolar range. Identified "hits" possess certain common structural features that can be used in the design of the next generation(s) of ZIKV inhibitors. Collectively, our findings suggest that identified compounds represent excellent template(s) for the development of inexpensive and orally available anti-Zika drugs.

Zika virus (ZIKV) infection has recently become a major health concern in many countries,[1,2] including the United States (US), prompting the World Health Organization to declare outbreak of the ZIKV a global health emergency.[3] A strong correlation was observed between cases of ZIKV infection and a dramatic increase in microcephaly cases in Brazil,[4–7] as well as the occurrence of the Guillain-Barré syndrome.[8,9] Subsequent reports have established the ability of ZIKV to cross the human fetal-placental barrier and infect the developing central nervous system.[10–14] Recent pathology and imaging studies in cases with confirmed ZIKV infection in the prenatal brain showed devastating consequences of infection, including severe microcephaly, lissencephaly, hydrocephaly, necrosis, periventricular and cortical calcification, diffuse astrogliosis, and activated microglia[6,13] with findings of massive cell death and necrosis exceeding those occurring in many genetic forms of microcephaly. In addition, animal model studies also suggest that in adults, ZIKV infection may lead to male infertility and severe neurological and other systemic complications.[15–17] ZIKV is mainly transmitted by the mosquito vector *Aedes aegypti*,[18] but can also be spread by maternal to fetal vertical transmission[19] as well as sexual contact[20–25] stressing the need for reliable new anti-Zika drugs.

Currently, no specific anti-ZIKV therapeutic or vaccine is available, emphasizing the need for development of effective and safe antiviral drugs and vaccines for worldwide treatment and prevention of ZIKV infection. Numerous studies have identified various modalities that can inhibit ZIKV replication: (1) cytokines (IFN-α, IFN-β and IFN-γ),[26] (2) antibodies,[27] (3) peptides,[28,29] and (4) small-molecule compounds such as: 7-deaza-2′-C-methyladenosine,[30] 2′-C-methylated nucleosides,[31] (−)-epigallocatechin gallate, sofosbuvir,[32] bromocriptine and others.[33–37] Additionally, some US FDA-approved drugs used in clinics for other purposes, such as PHA-690509, niclosamide, bortezomib, mycophenolic acid, mefloquine and daptomycin, were also found to inhibit ZIKV infection.[38–40] Generally, bioactivity (IC_{50}) reported to date for small organic molecules is in the micromolar range and for most active peptides in low nanomolar range with IC_{50} values for antibodies of ~1 ng/mL, depending on antibody and the viral strain tested.[26–31,33,37–40] As mechanism of ZIKA virus entry/infection is poorly understood, mechanism/structure-based drug development studies to date have focused on either HCV-analogous NS2B-NS3 protease inhibitor system[33,34,37] or entry inhibitor(s) derived from the stem region of ZIKV envelope protein E.[29]

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Since development of inexpensive and orally available anti-Zika drugs is of critical importance, small-molecule organic compounds are preferable candidates for versatile use as a prophylactic, post-exposure prophylactic, and treatment option for Zika virus infections in general and high-risk populations, including pregnant women. Such hypothesis is strongly supported by previous positive results for numerous antivirals development studies concerning anti-HIV and anti-HCV compounds. Therefore, in our screening studies, we utilized a proprietary limited library (~250 compounds) consisting of both, commercially available and newly synthesized small-molecule analogs which we arbitrarily considered as "relevant" for our search. Tested library was not targeted toward any particular structural properties/features of its members. All compounds were screened using a viral plaque-forming assay[41] and the A549 human lung carcinoma cell line, which was previously described as highly permissive to Zika infection[42,43] reaching a virus titer almost 7 log PFU mL^{-1} within the 48 h of infection. As a result, we found 4 "hits" showing varying anti-Zika inhibitory activity, all within low nanomolar or sub-nanomolar range (Fig. 1). Newly found compounds were both proprietary (NE9) as well as commercially available entities (ASN07115854, rolipram, preladenant) with potential for further pharmaceutically-relevant modifications. As chemically quite diverse, our "hits" show certain common structural features, namely they contain an assembly of 3 aromatic/aliphatic rings (or 2 rings with rigid linker, see **2**) in *para*- or meta-configuration and with or without additional peripheral modifications (acetyl, S-allyl, O-Me, etc.) which are usually small. An additional common denominator seems to be the presence of N-phenyl-substituted piperazine or phenyl-substituted-piperazine-like moiety (4-phenyl-2-pyrrolidone in case of rolipram (**3**)). Limited molecular modeling studies have shown that at least 3 of our leading compounds which possess similar size (namely **1**, **2** and **3**) can adopt certain conformations allowing them to occupy dimmensionaly similar binding cavities (Fig. 2). All 4 compounds are very "drug-like" and conform to at least some of Lipinski's rule of five criteria.[44,45] In addition, for compound 3 and 4, numerous bioactivity and toxicity data exist to aid further their development.[46–48] Importantly, both compounds are also orally available[46–48] strongly suggesting potential for oral availability of their derivatives. Therefore, the identified analogs are excellent leads for the development of prospective anti-Zika drugs, including inexpensive and orally available candidates. Interestingly, two newly identified inhibitors of ZIKV possess known molecular targets. Rolipram (**3**) is a known selective inhibitor of phosphodiesterase 4A and 4B[49] and preladenant (4) is a highly specific antagonist of adenosine receptor A_{2A},[50] raising intriguing possibilities of their involvement in ZIKV life cycle. Further analysis of the content of our proprietary library suggests that pentafluorobenzene-sulfonyl (Pfbs) moiety is a necessary structural component to achieve high anti-Zika inhibitory activity for sulfonamide-based compounds. To this end, we tested 18 differently substituted sulfonamides (Fig. 3) of 1-phenyl-piperazine and found that only Pfbs derivative exhibits antiviral activity in sub-nanomolar range, with virtually all other compounds listed (Fig 3B) being inactive. In our initial screen, we utilized an enantiomeric mixture of rolipram which possesses the strong anti-Zika properties showing IC_{50} value of 44.6 ± 11.9 nM. Numerous structurally related inhibitors of PDE4 are commercially available from various vendors, including enantiomerically pure isomers of rolipram: (*R*)-(−)-rolipram and

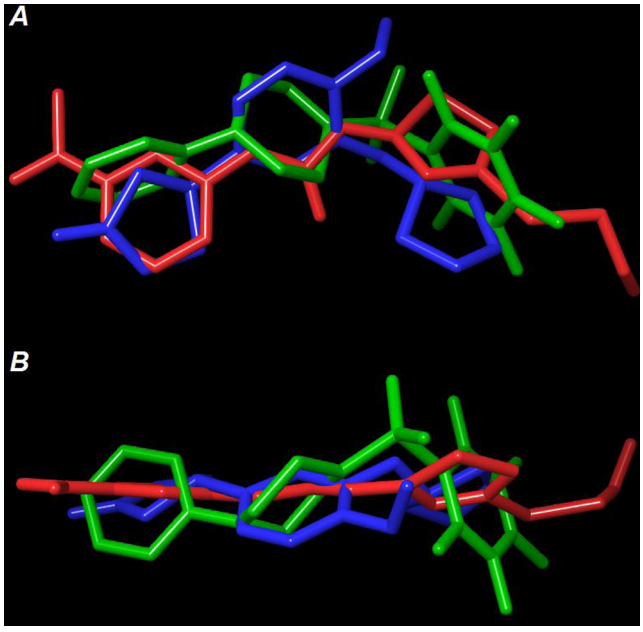
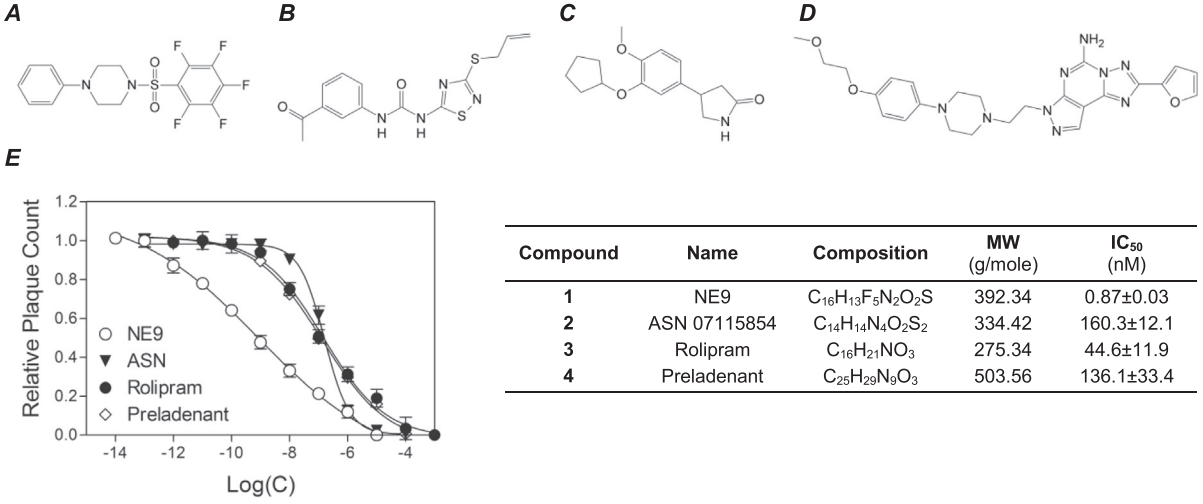


Fig. 2. An overlay of minimized structures of NE9 (green), ASN 07115854 (red) and (*S*)-(+)-rolipram (blue). (A) front view, (B) top view.



Compound	Name	Composition	MW (g/mole)	IC_{50} (nM)
1	NE9	$C_{16}H_{13}F_5N_2O_2S$	392.34	0.87±0.03
2	ASN 07115854	$C_{14}H_{14}N_4O_2S_2$	334.42	160.3±12.1
3	Rolipram	$C_{16}H_{21}NO_3$	275.34	44.6±11.9
4	Preladenant	$C_{25}H_{29}N_9O_3$	503.56	136.1±33.4

Fig. 1. The structures of highly active anti-Zika compounds found in the initial screen: (A) NE9 (1), (B) ASN 07115854 (2), (C) rolipram (3), (D) preladenant (4) and their corresponding dose response curves (E).