

Inhibitors of Tick-Borne Flavivirus Reproduction from Structure-Based Virtual Screening

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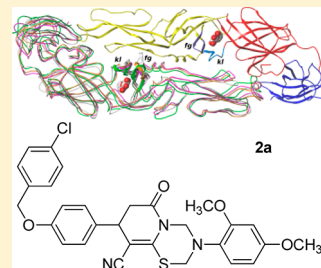
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Supporting Information

ABSTRACT: Flaviviruses form a large family of enveloped viruses affecting millions of people over the world. To date, no specific therapy was suggested for the infected people, making the treatment exclusively symptomatic. Several attempts were performed earlier for the design of fusion inhibitors for mosquito-borne flaviviruses, whereas for the tick-borne flaviviruses such design had not been performed. We have constructed homology models of envelope glycoproteins of tick-transmitted flaviviruses with the detergent binding pocket in the open state. Molecular docking of substituted 1,4-dihydropyridines and pyrido[2,1-*b*][1,3,5]-thiadiazines was made against these models, and 89 hits were selected for the in vitro experimental evaluation. Seventeen compounds showed significant inhibition against tick-borne encephalitis virus, Powassan virus, or Omsk hemorrhagic fever virus in the 50% plaque reduction test in PEK cells. These compounds identified through rational design are the first ones possessing reproduction inhibition activity against tick-borne flaviviruses.

KEYWORDS: Antiviral compounds, flavivirus, 1,4-dihydropyridines, pyrido[2,1-*b*][1,3,5]thiadiazines



The *Flavivirus* genus includes a range of viral pathogens causing severe diseases in humans.^{1–4} These viruses are transmitted by arthropod vectors to mammalian hosts. Apparently the most studied among flaviviruses are the mosquito-borne viruses such as dengue virus (DENV), West Nile virus (WNV), yellow fever virus (YFV), and Japanese encephalitis virus (JEV). Tick-borne flaviviral diseases such as tick-borne encephalitis, being a serious health concern in Russia and Europe,^{4,5} and Omsk hemorrhagic fever^{4,6} and Powassan encephalitis,^{4,7,8} showing low incidence and local importance in Russia, US, and Canada, are associated with severe symptoms. Nevertheless, their causing agents are less studied. The situation is additionally complicated by unavailability of specific drugs affecting flaviviruses. Despite that vaccines are available against tick-borne encephalitis virus (TBEV), YFV, and JEV,⁹ they can be used successfully only as prophylaxis measure before the infection. Other treatment options include symptomatic treatment schemes and immunoglobulin therapy of tick-borne encephalitis cases,¹⁰ several immunomodulating agents (e.g., jodantipyrin¹¹ and luromarin¹²) are on a preclinical stage, but their applicability in the course of flaviviral infection is limited. Moreover, in the case of Omsk hemorrhagic fever virus (OHFV), Powassan virus (POWV), and other locally

important flaviviruses, specific vaccines development would never be done due to a small number of cases. Thus, other more specific and effective ways of treatment of tick-borne flaviviral infections are urgently needed.

Inhibition of the virus entry and replication is a widely accepted strategy in the design of antivirals.^{13–15} The entry of flavivirus particle into the host cell via receptor-mediated endocytosis requires the fusion of viral and cellular membranes. This process is ruled by pH decrease in the endosome and mediated mostly by envelope glycoprotein E. During the fusion E protein dimers initially arranged in a herringbone pattern on the virion surface undergo a large-scale conformational rearrangement leading to the formation of protein spikes, which attack the host cell membrane.¹⁶ A detergent *n*-octyl- β -D-glucoside (β -OG) binding pocket has been identified in one of DENV E protein ectodomain structures (PDB ID 1OKE¹⁷), whereas in the other structures this pocket is closed by the *kl* loop.¹⁸ It was assumed that this pocket can be exploited as a putative site for DENV fusion inhibitors. As far as flaviviral E

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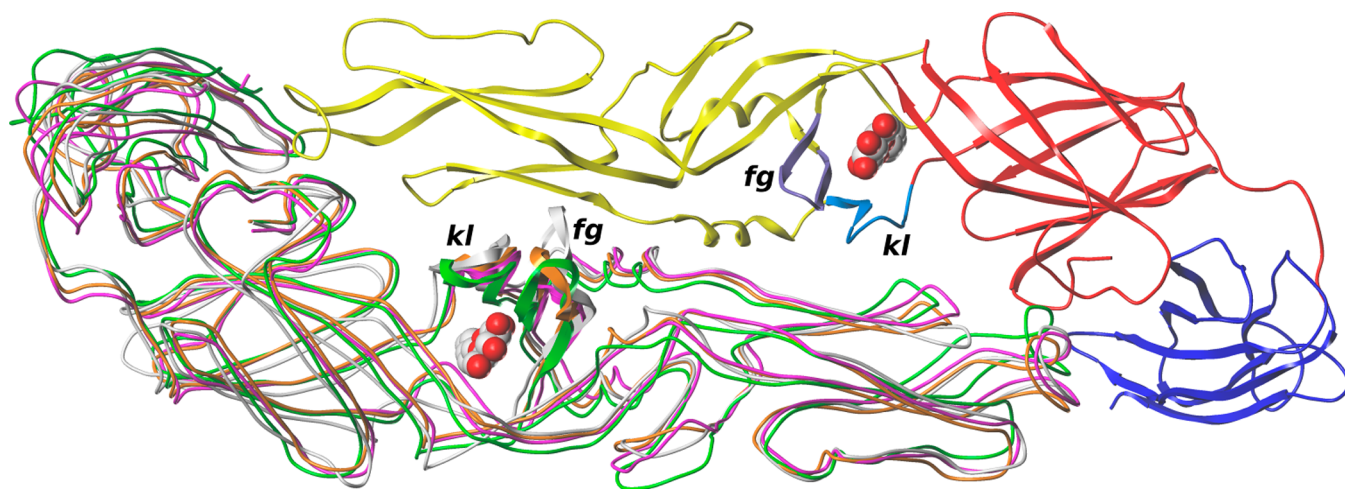
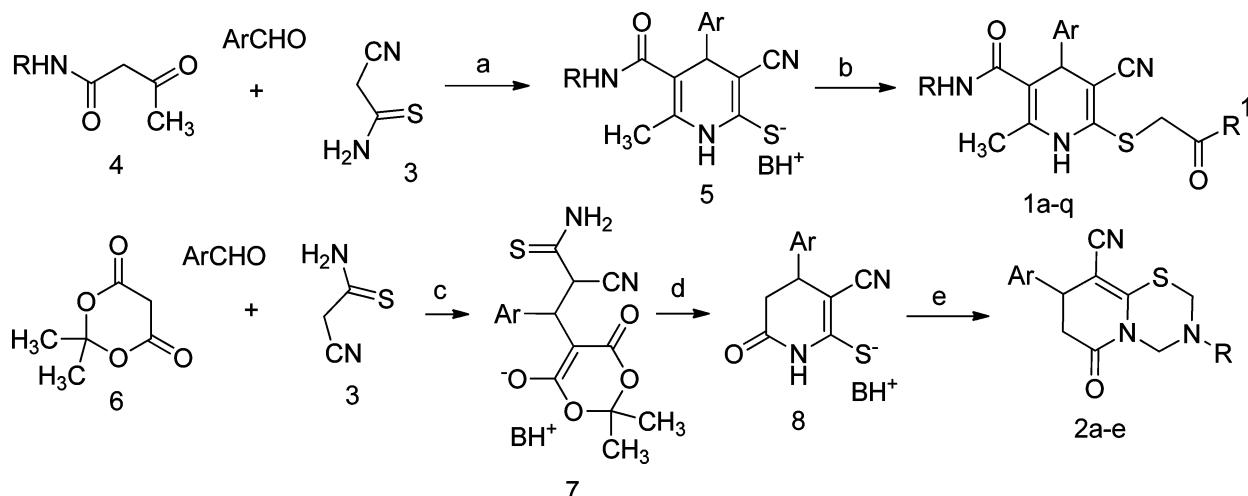


Figure 1. Superposition of E protein structures and models for DENV (subunit 1 colored by domains (red, domain I; yellow, domain II; blue, domain III), subunit 2 colored magenta), TBEV (white), POWV (green), and OHFV (orange). Loops *kl* and *fg* are colored in subunit 1 and shown as ribbons in subunit 2. β -OG molecules are shown spacefilled.

Scheme 1.^a



^aReagents and conditions: (a) *N*-methylmorpholine, EtOH, 20 °C, 70–93%. (b) 10% aq. KOH, $R^1C(O)CH_2Cl$, EtOH, 20–40 °C, 56–87%. (c) *N*-Methylmorpholine, EtOH, 20 °C, 1 h. (d) EtOH, reflux, 4 h, 68–78%. (e) One equiv of RNH_2 , 37% aq. HCHO, EtOH, reflux, 51–70%. BH^+ = *N*-methylmorpholinium.

proteins are rather similar,¹ this hypothesis has been extended to the other genus members. Blind docking studies against this pocket led to a successful identification of DENV, WNV, and YFV replication inhibitors.^{19–24} In all these cases, docking was performed into 1OKE structure, whereas activity was tested with different assays. Binding of the inhibitors in the β -OG pocket was shown by NMR.²⁰ According to a Monte Carlo simulation, the open state of β -OG pocket may be preferred over the closed one in the dynamic native conditions.²⁵ Alternative binding sites were also searched for on the surface of the DENV E ectodomain dimer, and several inhibitors were identified.²⁶ A teicoplanin analogue LCTA-949 has been shown to prevent cell entry of various flaviviruses, being the most effective against TBEV, but no data is available on the mode of action of this molecule.²⁷

We tried to identify small molecule compounds able to inhibit the reproduction of tick-borne flaviviruses (namely, TBEV, POWV, and OHFV) via interaction with the envelope protein E in the β -OG pocket. To achieve this goal, we

constructed homology models of E proteins for the selected viruses with β -OG pocket in the open state. Subsequently, a virtual screening campaign of a compound library was performed, and hit compounds were selected according to the scores and visual analysis of the binding modes. In vitro assays of the hits revealed several compounds that had shown inhibition of viral reproduction. These data confirmed the validity of the chosen methodology and could be used for further design and development of novel antiviral drugs.

Homology models of E proteins were constructed for the representative strains of TBEV (Absettarov), POWV (Powsan24), and OHFV (Nikitina) (Figure 1). The homology models were built in Modeller 9.10²⁸ based on the TBEV E protein dimer template structure (PDB ID 1SVB¹⁸) for the whole sequence except the *kl* loop, which was modeled based on 1OKE template.¹⁷ All models built possessed good stereochemical quality.

Given the absence of known compounds targeting the TBEV, POWV, and OHFV E proteins, we chose a library of

Table 1. Structures and Antiviral Activity of the Compounds That Passed Preliminary Assays

| compd | Ar | R | R ¹ | CC ₅₀ , μ M (acute) | CC ₅₀ , μ M (chronic) | IC ₅₀ , μ M | | |
|-------|--|---|---|---------------------------------------|---|----------------------------|---------------|---------------|
| | | | | | | TBEV | POWV | OHFV |
| 1a | 2-furyl | 4-EtOC ₆ H ₄ | 4-nBuC ₆ H ₄ NH | 64 | 14 | 2.5 \pm 0.5 | >10 | >10 |
| 1b | 2-furyl | 4-H ₂ NSO ₂ C ₆ H ₄ | 4-EtC ₆ H ₄ NH | >250 ^a | 153 | >10 | >10 | 10 \pm 7.8 |
| 1c | 2-furyl | 4-ClC ₆ H ₄ | 4-EtC ₆ H ₄ NH | 26 | 27 | >10 | >10 | >10 |
| 1d | 2-furyl | 4-EtC ₆ H ₄ | 4-EtC ₆ H ₄ NH | 57 | 33 | >10 | >10 | >10 |
| 1e | 2-furyl | 2-MeC ₆ H ₄ | 2-naphthyl-NH | >250 | >250 | >10 | >10 | 5.3 \pm 0.1 |
| 1f | 2-furyl | Ph | 3,4-Me ₂ C ₆ H ₃ | >250 | 19 | >10 | >10 | 3.2 \pm 0.8 |
| 1g | 2-furyl | 2,6-Me ₂ C ₆ H ₃ | 2-benzothiazolyl-NH | >250 | 7 | >10 | >10 | 7.1 \pm 0.1 |
| 1h | 2-furyl | 2-benzothiazolyl | 2-benzothiazolyl-NH | >250 | 52 | >10 | >10 | 2.5 \pm 0.9 |
| 1i | 2-furyl | 2-benzothiazolyl | 4- <i>i</i> PrC ₆ H ₄ NH | >250 | 29 | >10 | >10 | 2.5 \pm 0.1 |
| 1j | 5-Me-2-furyl | 2-MeOC ₆ H ₄ | 4-BrC ₆ H ₄ NH | 248 | 34 | >10 | >10 | 3.7 \pm 0.4 |
| 1k | 2-thienyl | Ph | Ph | >250 | 17 | >10 | >10 | >10 |
| 1l | 2-thienyl | 2-MeOC ₆ H ₄ | 4-MeC ₆ H ₄ NH | >250 | 97 | >10 | >10 | 5.5 \pm 0.9 |
| 1m | Ph | 4-ClC ₆ H ₄ | 3-MeC ₆ H ₄ NH | >250 | 41 | 2.0 \pm 0.4 | >10 | >10 |
| 1n | Ph | 4-ClC ₆ H ₄ | 4-PhOC ₆ H ₄ NH | >250 | 38 | 2.8 \pm 0.6 | >10 | >10 |
| 1o | Ph | 4-ClC ₆ H ₄ | 2-naphthyl-NH | 111 | 20 | >10 | >10 | >10 |
| 1p | 2-FC ₆ H ₄ | 2-MeC ₆ H ₄ | 4-MeOC ₆ H ₄ NH | >250 | 89 | >10 | >10 | 7.2 \pm 0.5 |
| 1q | 4-HO-3-MeOC ₆ H ₃ | Ph | 4-EtC ₆ H ₄ NH | 114 | 31 | >10 | >10 | 1.8 \pm 0.4 |
| 2a | 4-(4-ClC ₆ H ₄ CH ₂ O)C ₆ H ₄ | 2,4-(MeO) ₂ C ₆ H ₃ | | 109 | 39 | 0.07 \pm 0.02 | 1.3 \pm 0.1 | >10 |
| 2b | 3-BnOC ₆ H ₄ | 2-EtOC ₆ H ₄ | | >250 | 116 | 2.6 \pm 0.4 | 2.2 \pm 0.3 | >10 |
| 2c | 3-BnOC ₆ H ₄ | 4-EtC ₆ H ₄ | | >250 | 236 | >10 | >10 | >11.9 |
| 2d | 4-BnOC ₆ H ₄ | 4- <i>n</i> BuC ₆ H ₄ | | >250 | 35 | 1.9 \pm 0.4 | >10 | >10 |
| 2e | 4-BnO-3-MeOC ₆ H ₃ | 4-MeOC ₆ H ₄ | | >250 | 53 | 0.09 \pm 0.01 | >10 | >10 |

^aThe 250 μ M concentration is the highest concentration studied.

elongated heterocyclic compounds synthesized in ChemEx laboratory for further virtual screening (VS) studies. The molecular docking of the library containing 5886 compounds was performed with FRED 2.2.5.²⁹ The predicted binding modes for POWV E protein were scored with six scoring functions implemented in FRED. Top 500 hit lists were analyzed for each scoring function, and the compounds included into at least three hit lists were selected for further analysis along with top compounds selected by each scoring function; the total number of compounds was 201. These compounds were then docked into TBEV and OHFV E protein models. Predicted binding modes of the compounds were analyzed visually, and compounds with unacceptable binding modes (e.g., forming hydrogen bond donor–donor or acceptor–acceptor interactions) were excluded along with compounds belonging to scarcely represented classes. The final hit list consisted of 89 compounds belonging to two related classes, **1** and **2**.

The Hantzsch-type 1,4-dihydropyridines **1a–q** were synthesized in two steps by analogy with known procedures.^{30,31} First, the reaction of cyanothioacetamide **3** with aromatic aldehydes and acetoacetanilides **4** in the presence of 1.5-fold excess of *N*-methylmorpholine gave 1,4-dihydropyridine-2-thiolates **5**. The next step included regioselective S-alkylation by treatment with alkyl chlorides to give target compounds **1a–q** (Scheme 1.^a).

Pyridothiadiazines **2a–e** were obtained in three steps starting from cyanothioacetamide **3** and Meldrum's acid **6** by analogy with the known procedure.³² The stable adducts **7** were cyclized in ethanol under reflux to furnish 4,5-dihydropyridone-2-thiolates **8**.^{33,34} Double Mannich-type aminomethylation of these intermediates was achieved by treatment with 1 equiv of a primary amine and an excess of formaldehyde under short-term heating to form compounds **2** as sole products.

Prior to *in vitro* studies, cytotoxicity of the compounds was assessed in porcine embryo kidney (PEK) cell line (Table 1); acute (24 h) and chronic (7 days) toxicities were evaluated. For the majority of compounds, CC₅₀ values were much over 10 μ M. It was considered as generally acceptable for the first compounds in the class.

Preliminary assessment of antiviral activity of hit compounds in 10 μ M concentration was studied in 50% plaque reduction test (PRT₅₀) in PEK cell line against selected strains of TBEV, POWV, or OHFV. Twenty-two compounds showing over 50% antiviral activity in the preliminary tests were promoted for the IC₅₀ determination stage performed as the same assay with sequential dilutions of the studied compound starting from 10 μ M. Tested compounds showed dose-dependent inhibition of viral reproduction. PRT₅₀ IC₅₀ values are given in Table 1. Detailed computational and experimental procedures and compound characterization data are provided in the Supporting Information.

Compounds from 1,4-dihydropyridine series **1** have shown the inhibitory activity against OHFV or TBEV (Table 1). These inhibitors were ranked high in the hit lists for the corresponding viruses, showing high selectivity despite 93% sequence identity between E protein ectodomains. Compound **1q**, the most active against OHFV, was the only one in the series **1** containing hydroxy and methoxy groups in the Ar substituent. According to the docking results, this moiety occupied a cavity between Tyr49 and the *kl* loop (Figure 2). The presence of many aromatic and/or hydroxyl bearing residues in this region (Ser47, Tyr49, Thr279, and Tyr281) suggested that binding of such moiety is favored there. The presence of hydrogen bond acceptors in the variable moieties was the only requirement that seems to be important for the

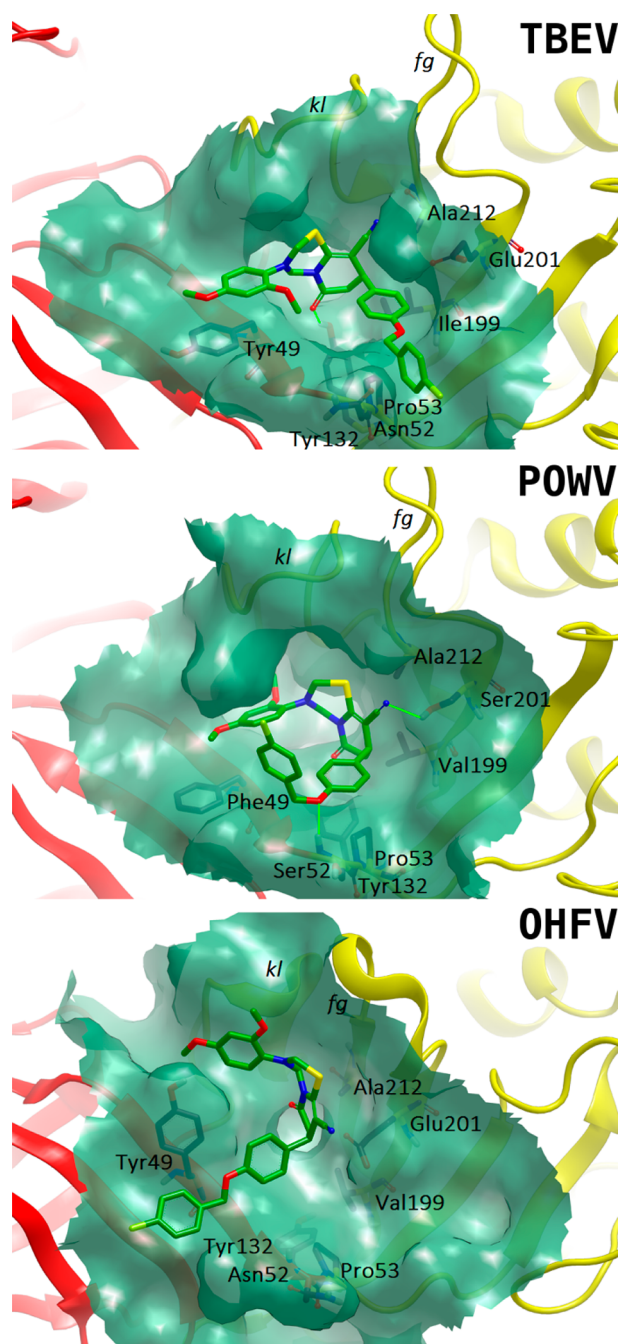


Figure 2. Putative binding modes of **2a** in the β -OG pockets according to the docking results. Hydrogen bonds are shown as green lines.

compound potency; the only active compound in the series **1** without such groups was **1m**.

Pyridothiadiazines **2** showed inhibitory activity against TBEV and POWV (Table 1); they were generally more potent despite their lower abundance in the hit lists compared to 1,4-dihydropyridines. Compounds **2a** and **2b** showed significant activity against both TBEV and POWV despite the limited similarity between these viruses. The only inactive compound in this series, **2c**, was ranked high only against the OHFV E protein. Subtle differences in the **2e** structure compared to **2a** led to the elimination of anti-POWV activity in **2e** but did not affect submicromolar anti-TBEV activity. Such ambiguities could be attributed to the fact that racemic mixtures were studied because molecular docking simulations showed some

preference for a specific enantiomer over another one: (*R*)-isomers were usually ranked higher for series **2**.

Structure–activity relationships for the compounds seem to be rather obscure, being more complicated by the absence of a consistent binding mode of the compounds: even similar compounds are predicted to interact with the proteins in different orientations (Figure 2). The compounds, protecting the cells against OHFV, do not protect the cells against TBEV and POWV, and vice versa. It should be attributed to E protein peculiarities resulting in different cellular tropism and therefore clinical outcome. These peculiarities also reveal themselves in the docking results: usually, compounds active against certain viruses form more hydrogen bonds with the corresponding E proteins (Figure 2) and are ranked higher against them. Because of the significant similarity of OHFV and TBEV and the compounds themselves, this situation is counterintuitive and may be attributed to a rather small number of compounds assessed in this study and evaluation of activity only for racemates.

Whether the mode of interaction of the identified inhibitors with the flavivirus envelope proteins is realized as predicted, i.e., via the binding in the β -OG pocket, cannot be unambiguously established in this experiment. Even a nonspecific mode of binding cannot be unanticipated. Rigorous study of the reproduction inhibition mechanism and the binding mode will be reported elsewhere.

Our virtual screening campaign against homology models of the envelope proteins from tick-borne flaviviruses resulted in the identification of the small molecule compounds preventing TBEV, POWV, and OHFV reproduction in the host cells. These compounds belong to the series of 1,4-dihydropyridines, showing activity against TBEV or OHFV, and pyridothiadiazines, showing submicromolar activity against TBEV and micromolar against POWV. Further optimization of these compounds guided by in vitro and in vivo studies will be performed with the aim to obtain drugs for flaviviral fevers and encephalitis treatment.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures for the homology modeling, virtual screening, synthesis and purification of compounds, and antiviral assays. Preliminary experimental data for inactive compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

The study was initiated and designed by D.I.O., L.I.K., G.G.K., and V.A.P. Computational study was performed by D.I.O., E.V.D., and E.G.R. Compounds were synthesized and characterized by V.V.D., K.A.F., S.G.K., and A.S.M. Biological assessment was performed by L.I.K. and Y.V.R. The study was supervised by V.A.P., G.G.K., V.M.P., and N.S.Z. All authors discussed and approved the publication of the manuscript.

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Notes

The authors declare no competing financial interest.

[#]Vladimir M. Pentkovski deceased on December 24, 2012. We dedicate this paper to his memory.

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ABBREVIATIONS

β -OG, *n*-octyl- β -D-glucoside; DENV, dengue virus; JEV, Japanese encephalitis virus; OHFV, Omsk hemorrhagic fever virus; PEK, porcine embryo kidney; POWV, Powassan virus; TBEV, tick-borne encephalitis virus; VS, virtual screening; WNV, West Nile virus; YFV, Yellow fever virus

REFERENCES

- (1) Lindebach, B. D.; Thiel, H.-J.; Rice, C. M. *Flaviviridae: The Viruses and Their Replication*. In *Fields Virology*, 5th ed.; Knippe, D. M., Howley, P. M., Eds.; Lippincott Williams and Wilkins and Wolters Kluwer Business Publishers: Philadelphia, PA, 2007; pp 1101–1152.
- (2) Turtle, L.; Griffiths, M. J.; Solomon, T. Encephalitis Caused by Flaviviruses. *QJM* **2012**, *105*, 219–223.
- (3) Sips, G. J.; Wilschut, J.; Smit, J. M. Neuroinvasive Flavivirus Infections. *Rev. Med. Virol.* **2011**, *22*, 69–87.
- (4) Gritsun, T. S.; Nuttall, P. A.; Gould, E. A. Tick-Borne Flaviviruses. *Adv. Virus Res.* **2003**, *61*, 317–371.
- (5) Mansfield, K. L.; Johnson, N.; Phipps, L. P.; Stephenson, J. R.; Fooks, A. R.; Solomon, T. Tick-Borne Encephalitis Virus: A Review of an Emerging Zoonosis. *J. Gen. Virol.* **2009**, *90*, 1781–1794.
- (6) Růžek, D.; Yakimenko, V. V.; Karan, L. S.; Tkachev, S. E. Omsk Haemorrhagic Fever. *Lancet*. **2010**, *376*, 2104–2113.
- (7) Gholam, B. I. A.; Pukša, S.; Provias, J. P. Powassan Encephalitis: A Case Report with Neuropathology and Literature Review. *Can. Med. Assoc. J.* **1999**, *161*, 1419–1422.
- (8) Raval, M.; Singhal, M.; Guerrero, D.; Alonto, A. Powassan Virus Infection: Case Series and Literature Review from a Single Institution. *BMC Res. Notes* **2012**, *5*, 594.
- (9) Heinz, F. X.; Stiasny, K. Flaviviruses and Flavivirus Vaccines. *Vaccine* **2012**, *30*, 4301–4306.
- (10) Pen'evskaia, N. A.; Rudakov, N. V. Efficiency of Use of Immunoglobulin Preparations for the Postexposure Prevention of Tick-Borne Encephalitis in Russia (A Review of Semi-Centennial Experience). *Med. Parazitol.* **2010**, *1*, 53–59.
- (11) Khudoley, V. N.; Saratikov, A. S.; Lepekhin, A. V.; Yavorskaya, V. E.; Evstropov, A. N.; Portnyagina, E. N.; Pomogaeva, A. D.; Beloborodova, E. L.; Vnushinkaia, M. A.; Schmidt, E. V.; Krilova, N. V.; Khunafina, D. K.; Mezenzeva, M. V.; Ershov, F. I.; Raevski, K. K.; Vlasova, E. V.; Abdulova, G. A.; Kropotkina, E. A. Antiviral Activity of Jodantipyryn: An Anti-Inflammatory Oral Therapeutic with Interferon-Inducing Properties. *Anti-Inflammatory Anti-Allergy Agents Med. Chem.* **2008**, *7*, 106–115.
- (12) Krylova, N. V.; Leonova, G. N.; Maistrovskaia, O. S.; Popov, A. M.; Artiukov, A. A.; Kozlovskaya, E. P. In Vitro Activity of Luromarin against Tick-Borne Encephalitis Virus. *Antibiot. Khimioter.* **2010**, *55*, 17–19.
- (13) De Clercq, E. Strategies in the Design of Antiviral Drugs. *Nat. Rev. Drug Discovery* **2002**, *1*, 13–25.
- (14) Stevens, A. J.; Gahan, M. E.; Mahalingam, S.; Keller, P. A. The Medicinal Chemistry of Dengue Fever. *J. Med. Chem.* **2009**, *52*, 7911–7926.
- (15) Teissier, E.; Penin, F.; Pécheur, E.-I. Targeting Cell Entry of Enveloped Viruses as an Antiviral Strategy. *Molecules* **2011**, *16*, 221–250.
- (16) Smit, J. M.; Moesker, B.; Rodenhuis-Zybert, I.; Wilschut, J. Flavivirus Cell Entry and Membrane Fusion. *Viruses* **2011**, *3*, 160–171.
- (17) Modis, Y.; Ogata, S.; Clements, D.; Harrison, S. C. A Ligand-Binding Pocket in the Dengue Virus Envelope Glycoprotein. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6986–6991.
- (18) Rey, F. A.; Heinz, F. X.; Mandl, C.; Kunz, C.; Harrison, S. C. The Envelope Glycoprotein from Tick-Borne Encephalitis Virus at 2 Å Resolution. *Nature* **1995**, *375*, 291–298.
- (19) Yang, J.-M.; Chen, Y.-F.; Tu, Y.-Y.; Yen, K.-R.; Yang, Y.-L. Combinatorial Computational Approaches to Identify Tetracycline Derivatives as Flavivirus Inhibitors. *PLoS One* **2007**, *2*, e428.
- (20) Zhou, Z.; Khaliq, M.; Suk, J.-E.; Patkar, C.; Li, L.; Kuhn, R. J.; Post, C. B. Antiviral Compounds Discovered by Virtual Screening of Small-Molecule Libraries against Dengue Virus E Protein. *ACS Chem. Biol.* **2008**, *3*, 765–775.
- (21) Li, Z.; Khaliq, M.; Zhou, Z.; Post, C. B.; Kuhn, R. J.; Cushman, M. Design, Synthesis and Biological Evaluation of Antiviral Agents Targeting Flavivirus Envelope Proteins. *J. Med. Chem.* **2008**, *51*, 4660–4671.
- (22) Kampmann, T.; Yennamalli, R.; Campbell, P.; Stoermer, M. J.; Fairlie, D. P.; Kobe, B.; Young, P. R. In Silico Screening of Small Molecule Libraries Using the Dengue Virus Envelope E Protein Has Identified Compounds with Antiviral Activity against Multiple Flaviviruses. *Antiviral Res.* **2009**, *84*, 234–241.
- (23) Poh, M. K.; Yip, A.; Zhang, S.; Priestle, J. P.; Ma, N. L.; Smit, J. M.; Wilschut, J.; Shi, P.-Y.; Wenk, M. R.; Schul, W. A Small Molecule Fusion Inhibitor of Dengue Virus. *Antiviral Res.* **2009**, *84*, 260–266.
- (24) Mayhoub, A. S.; Khaliq, M.; Botting, C.; Li, Z.; Kuhn, R. J.; Cushman, M. An Investigation of Phenylthiazole Antiflaviviral Agents. *Bioorg. Med. Chem.* **2011**, *19*, 3845–3854.
- (25) Osolodkin, D. I.; Kozlovskaya, L. I.; Palyulin, V. A.; Pentkovski, V. M.; Karganova, G. G.; Zefirov, N. S. A Molecular Model and Monte Carlo Simulation of Flavivirus Envelope Building Block. *Biochem. Biophys. Res. Commun.* **2012**, *425*, 207–211.
- (26) Yennamalli, R.; Subbarao, N.; Kampmann, T.; McGeary, R. P.; Young, P. R.; Kobe, B. Identification of Novel Target Sites and an Inhibitor of the Dengue Virus E Protein. *J. Comput.-Aided Mol. Des.* **2009**, *23*, 333–341.
- (27) De Burghgraeve, T.; Kaptein, S. J. F.; Ayala-Nunez, N. V.; Mondotte, J. A.; Pastorino, B.; Printsevskaya, S. S.; de Lamballerie, X.; Jacobs, M.; Preobrazhenskaya, M.; Gamarnik, A. V.; Smit, J. M.; Neyts, J. An Analogue of Antibiotic Teicoplanin Prevents Flavivirus Entry In Vitro. *PLoS One* **2012**, *7*, e37244.
- (28) Šali, A.; Blundell, T. L. Comparative Protein Modelling by Satisfaction of Spatial Restraints. *J. Mol. Biol.* **1993**, *234*, 779–815.
- (29) McGann, M. FRED Pose Prediction and Virtual Screening Accuracy. *J. Chem. Inf. Model.* **2011**, *51*, 578–596.
- (30) Dyachenko, V. D.; Krivokolysko, S. G.; Nesterov, V. N.; Litvinov, V. P. Synthesis and Properties of *N*-Methylmorpholinium 4-Aryl-6-methyl-5-phenylcarbamoyl-3-cyano-1,4-dihydropyridine-2-thiolates. Molecular and Crystalline Structure of 2-Allylthio-6-methyl-5-phenylcarbamoyl-4-(2-chlorophenyl)-3-cyano-1,4-dihydropyridine. *Chem. Heterocycl. Compd.* **1996**, *32*, 1066–1074.
- (31) Dyachenko, V. D.; Krivokolysko, S. G.; Litvinov, V. P. Synthesis of *N*-Methylmorpholinium 6-Methyl-4-(2-thienyl)-5-phenylcarbamoyl-3-cyano-1,4-dihydropyridine-2-thiolate and Its Reaction with Various Functionally Substituted Methyl Halides. *Chem. Heterocycl. Compd.* **1997**, *33*, 577–582.
- (32) Dotsenko, V. V.; Krivokolysko, S. G.; Chernega, A. N.; Litvinov, V. P. Synthesis and Structure of Pyrido[2,1-*b*][1,3,5]thiadiazine Derivatives. *Dokl. Chem.* **2003**, *389* (No. 4–6), 92–96.
- (33) Nesterov, V. N.; Krivokolysko, S. G.; Dyachenko, V. D.; Dotsenko, V. V.; Litvinov, V. P. Synthesis, Properties, and Structures

of Ammonium 4-Aryl-5-cyano-2-oxo-1,2,3,4-tetrahydropyridine-6-thiolates. *Russ. Chem. Bull.* **1997**, 46, 990–996.

(34) Krivokolysko, S. G.; Dyachenko, V. D.; Litvinov, V. P. A New Method for the Synthesis of *N*-Methylmorpholinium 4-Aryl-5-cyano-2-oxo-1,2,3,4-tetrahydropyridine-6-thiolates and Their Properties. *Russ. Chem. Bull.* **1997**, 46, 1758–1762.