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Ibuprofen as a template molecule for drug design against Ebola virus

Slobodan Paessler[1], Cheng Huang[1], Milan Sencanski[2], Nevena Veljkovic[2], Vladimir Perovic[2], Sanja Glisic[2], Veljko Veljkovic[3]

[1]Department of Pathology, Galveston National Laboratory, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX, USA, [2]Center for Multidisciplinary Research, Institute of Nuclear Sciences Vinca, University of Belgrade, Mihajla Petrovica 12–14, 11001 Belgrade, Serbia, [3]Biomed Protection, Galveston, TX, USA

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1. ABSTRACT

The Ebola virus outbreak in West Africa 2015 and Congo 2017, point out an urgent need for development of drugs against this important pathogen. Previously, by repurposing virtual screening of 6438 drugs from DrugBank, ibuprofen was selected as a possible inhibitor of the Ebola virus infection. The results of an additional docking analysis as well as experimental results showing measurable anti-Ebola effect of ibuprofen in cell culture suggest ibuprofen as a promising molecular template for the development of drugs for treatment of the infection by Ebola virus.

2. INTRODUCTION

Since March 2014, West Africa has experienced the largest outbreak of Ebola in history, with multiple countries affected and with more than 25,000 people infected and more than 12,000 deaths (1). Lack of vaccine and drugs for prevention and treatment of the Ebola hemorrhagic fever clearly demonstrated that the world was not prepared for an outbreak of this nature. A new outbreak of Ebola in Congo shows that three years later the situation is not completely under control. Despite recent headlines and scientific articles declaring the success of an experimental Ebola vaccine and drugs, the world still is not prepared for future Ebola epidemics and is not in position to prevent another deadly outbreak due to limited therapeutic and /or prophylactic options.

From the point of view of drug development, this was expected. Development of drugs is an expensive and time-consuming process, which takes more than ten years and costs more than US$ 2 billion (2). Drug development is also risky because the estimated final success rates at which new drugs receive approval from regulatory agencies is less than 35% (3).

Drug repositioning (also referred to as drug repurposing, re-profiling, therapeutic switching and drug re-tasking), is the identification of new therapeutic indications for known drugs. One motivation behind drug repositioning is the treatment of rare or neglected diseases as limited drug development exists in this field. Therefore, testing of safe and active molecules already developed for other indications, deemed suitable and attractive for this purpose.

2.1. Repurposing of approved drugs for the treatment of Ebola disease

In response to the Ebola virus outbreak in West Africa 2015, drug repositioning was considered as a possible short-cut to Ebola drugs, because

conventional time and money consuming approach of drug development (> 10 years; > 2 billion $) does not meet urgent need for these therapeutics. Although this approach does not remove the need for certain preclinical and clinical studies, it is promising in terms of acceleration of discovery of urgently needed drugs for the treatment of Ebola disease.

The first large repurposing screening of the Food and Drug Administration (FDA)-approved drugs has been reported in 2013 . In this study, Madrid and co-workers performed *in vitro* and *in vivo* screening of 1012 FDA-approved drugs and selected 24 candidate entry inhibitors for Ebola virus (4). The most noteworthy antiviral compound identified was chloroquine, which disrupted entry and replication of the virus *in vitro* and protected mice against Ebola virus challenge *in vivo*.

In the second large study, 53 inhibitors of Ebola virus infection with IC_{50} <10 μM and selectivity index SI >10-fold have been identified by *in vitro* screening of 2816 FDA-approved drugs (5). These 53 active compounds were divided into different functional categories including microtubule inhibitors, estrogen receptor modulators, antihistamines, antipsychotics, pump/channel antagonists, and anticancer/antibiotics. In the same study, additional 95 drugs active against Ebola virus infection with IC_{50} > 10 μM and SI <10-fold were also reported (5).

The criterion for *in silico* screening of molecular libraries for candidate Ebola drugs, which is based on the electron-ion interaction potential (EIIP) and the average quasi-valence number (AQVN), was proposed (6). These molecular descriptors determine specific recognition and targeting between interacting biological molecules at distances >5 Å (7). Analysis of the 152 small molecule inhibitors of Ebola virus infection, selected by *in vitro* screening of 3828 FDA approved drugs (6), show that 79% of these compounds are placed within AQVN and EIIP regions (2.3. - 2.7.) and (0.0.829 - 0.0.954 Ry), respectively. This part of the AQVN/EIIP space was designed as the “Ebola Virus Infection Inhibitors Space” (EVIIS). The AQVN region (2.3.6 - 2.5.4) and the EIIP region (0.0.912 - 0.0.924 Ry) form the part of EVIIS, which encompasses 55.5. % of all drugs from the learning set (core EVIIS, cEVIIS). Additional literature data mining revealed 49 compounds with experimentally proved activity against Ebola virus infection and most of these compounds 47 (95.9. %) were placed within EVIIS. By repurposing virtual screening of 6438 drugs from DrugBank (http://www.drugbank.ca) using EVIIS as a filter, 267 approved and 382 experimental drugs have been selected as candidates for the treatment of Ebola virus disease, including 15 anti-malarial drugs and 32 antibiotics, (6).

Recently, several studies *in silico*, *in vitro* and *in vivo* were carried out for repurposing of approved drugs for prevention and treatment of the Ebola disease (8). The AQVN and EIIP values calculated for drugs selected in these studies are given in Table 1. Sixty eight percent of these drugs are located within the EVIIS domain of the AQVN/EIIP space and we selected previously the majority of them as candidate Ebola drugs (6). These results additionally confirm efficacy of the proposed AQVN/EIIP filter for *in silico* screening of the molecular libraries for candidate Ebola drugs.

By detailed analysis of approved drugs located within EVIIS, including the docking analysis, we selected the non-steroidal anti-inflammatory drug ibuprofen as an inexpensive, widely accessible and minimally toxic candidate for prevention and treatment of Ebola virus disease (9). Later high resolution structural study confirmed binding of ibuprofen to the Ebola virus glycoprotein (GP) (10). Authors of this study hypothesized that ibuprofen binding destabilizes GP and triggers premature release of the subunit GP2 of this protein, thereby preventing fusion between the viral and endosome membranes (10).

The binding site of ibuprofen on the Ebola virus GP proposed by Zhao and co-workers (10) differs from the binding site proposed in our study (9). In this article, we performed an additional docking analysis in order to explain possible reason for this discrepancy. We also experimentally confirmed inhibitory effect of ibuprofen on the Ebola virus. In Zhao’s paper, authors used EBOV GP pseudotyped HIV virus as a surrogate to study the effect of drugs on EBOV infectivity. We used EBOV in our study.

These results, together with the results reported by Zhao and co-workers, suggest ibuprofen as the molecular template for development of drugs for treatment of the Ebola virus disease.

3. MATERIAL AND METHODS

3.1. Ligand optimization

Ligands were built in VEGAZZ (11), protonated according to physiological conditions and optimized on PM6 level of theory using MOPAC 2009.

3.2. Molecular docking

Ligand and receptor were prepared using AutoDock Tools 1.5.7. (12). The docking was carried with Autodock Vina1.1.2. (13). The whole receptor conformational space was searched, using grid box dimensions 60 × 60 × 60 Å3. After selection of conformations that were docked near recognized sequences important for binding of anti-malarials, the binding pocket was recognized, grid box centerd to occupy close amino acid residues and set to dimensions

Table 1. The AQVN and EIIP values calculated for candidate drugs against Ebola infection

Drug	Formula	AQVN	EIIP (Ry)
Castanospermine	C8H15NO4	2.7.14	0.0.570
Celgosivir	C12H21NO5	2.6.67	0.0.693
Ouabain	C29H44O12	2.7.29	0.0.527
17-DMAG	C32H48N4O8	2.6.52	0.0.727
BGB324	C30H34N8	2.6.94	0.0.624
Stavudine	C10H12N2O4	3.0.71	0.0.700
Abacavir	C14H18N6O	2.8.20	0.0.233
Omeprazole	C17H19N3O3S	2.9.30	0.0.173
Esomeprazole	C17H19N3O3S	2.9.30	0.0.173
Gleevec	C29H31N7O	2.7.65	0.0.420
Tasigna	C28H22F3N7O	2.9.18	0.0.126
Mitoxantrone	C22H28N4O6	2.8.67	0.0.067
Labetalol	C19H24N2O3	2.6.67	0.0.693
Tafluprost	C25H34F2O5	2.5.15	0.0.935
Misoprostol	C22H38O5	2.4.00	0.0.955
Carboplast	C21H36O5	2.4.19	0.0.961
Fosinopril	C30H46NO7P	2.5.65	0.0.883
Benzylpenicilloy Polylysine	C22H32N4O6S	2.8.00	0.0.304
Bimatoprost	C25H37NO4	2.4.78	0.0.957
Nebivolol	C22H25F2NO4	2.6.67	0.0.693
Valrubicin	C34H36F3NO13	2.9.66	0.0.308
Tamsulosin	C20H28N2O5S	2.7.50	0.0.466
Montelukast	C35H36ClNO3S	2.6.75	0.0.672
Indinavir	C36H47N5O4	2.6.09	0.0.814
Iloprost	C22H32O4	2.4.83	0.0.955
Travoprost	C26H35F3O6	2.5.43	0.0.909
Latanoprost	C26H40O5	2.4.51	0.0.964
Remikiren	C33H50N4O6S	2.5.98	0.0.836
Vitamin K1	C31H46O2	2.3.04	0.0.868
Toremiphene	C26H28ClNO	2.5.26	0.0.926
Amodiaquine	C20H22ClN3O	2.6.38	0.0.756
Chloroquine	C18H26ClN3	2.3.75	0.0.941
Amiodarone	C25H29I2NO3	2.5.67	0.0.880
Azithromycin	C38H72N2O12	2.4.68	0.0.961
Sertraline	C17H17Cl2N	2.4.86	0.0.954
Aripiprazole	C23H27Cl2N3O2	2.5.96	0.0.835
Astemizole	C28H31FN4O	2.6.15	0.0.802
Atovaquone	C22H19ClO3	2.8.00	0.0.304
Benztropine	C21H25NO	2.5.00	0.0.946
Bepridil	C24H34N2O	2.3.93	0.0.952
Clemastine	C21H26ClNO	2.4.40	0.0.964
Clomiphene	C32H36ClNO8	2.7.95	0.0.321
Clomipramine	C19H23ClN2	2.4.44	0.0.964
Dasatinib	C22H26ClN7O2S	2.8.48	0.0.137
Efavirenz	C14H9ClF3NO2	2.8.67	0.0.067

Flupentixol	C23H25F3N2OS	2.5.82	0.0.858
Fluphenazine	C22H26F3N3OS	2.5.71	0.0.874
Hycanthone	C20H24N2O2S	2.6.94	0.0.626
Lomerizine	C27H32Cl2F2N2O3	2.5.29	0.0.923
Maprotiline	C20H23N	2.4.54	0.0.963
Mycophenolate mofetil	C23H31NO7	2.7.42	0.0.490
Paroxetine	C19H21ClFNO3	2.6.52	0.0.727
Pimozide	C28H29F2N3O	2.6.03	0.0.824
Piperacetazine	C24H30N2O2S	2.6.00	0.0.811
Prochlorperazine	C20H24ClN3S	2.5.71	0.0.874
Quinacrine	C23H30ClN3O	2.4.83	0.0.955
Simvastatin	C25H38O5	2.4.71	0.0.960
Strophanthin	C29H44O12	2.7.29	0.0.527
Teicoplanin	C88H97Cl2N9O33	3.0.31	0.0.554
Terconazole	C26H31Cl2N5O3	2.6.87	0.0.644
Thioridazine	C21H26N2S2	2.5.88	0.0.848
Toremifene	C26H28ClNO	2.5.26	0.0.926
Vinorelbine	C45H54N4O8	2.7.21	0.0.552
Propafenone	C21H27NO3	2.5.77	0.0.866
Promethasine	C17H20N2S	2.6.00	0.0.829
Dilazep	C31H44N2O10	2.7.36	0.0.509
Trimipramine	C20H26N2	2.4.17	0.0.961
Cyproheptadine	C21H21N	2.5.58	0.0.891
Clomifene	C26H28ClNO	2.5.26	0.0.926
Alverine	C20H27N	2.3.33	0.0.904
Aprindine	C22H30N2	2.3.70	0.0.938
Salmeterol	C25H37NO4	2.4.78	0.0.957
Topotecan	C23H23N3O5	2.9.63	0.0.298
Deslanoside	C47H74O19	2.6.86	0.0.647
Digoxin	C41H64O14	2.6.22	0.0.789
Proglumetacin	C46H58ClN5O8	2.6.78	0.0.666
Posaconazole	C37H42F2N8O4	2.7.53	0.0.458
Raloxifene	C28H28ClNO4S	2.7.94	0.0.326
Clarithromycin	C38H69NO13	2.5.12	0.0.937
Colchicine	C22H25NO6	2.8.52	0.0.121
Tamoxifen	C26H29NO	2.5.26	0.0.926
Thiothixene	C23H29N3O2S2	2.7.12	0.0.577
Dronedarone	C31H44N2O5S	2.5.78	0.0.864
Vincristine	C46H56N4O10	2.7.59	0.0.439

Based on (7–12)

of 20 × 20 × 20 Å3. The docking was carried out with and without weighting of hydrophilic interactions. In case of hydrophilic weighting, the value was set to -1.2.0 (compared to default weight_hydrogen = -0.5.87439 value). The exhaustiveness was set to 250. For the rescoring process of ibuprofen crystalyzed with Ebola glycoprotein, GP structures and ibuprofen coordinates were taken from pdb structure 5JQB, and all other rescoring parameters were set to be the same as in the docking process to our GP1 model.

3.3. Inhibition of Ebola virus with ibuprofen

We incubated the virus with 100 uM ibuprofen and performed plaque assay after diluting the virus with media containing ibuprofen. Briefly,

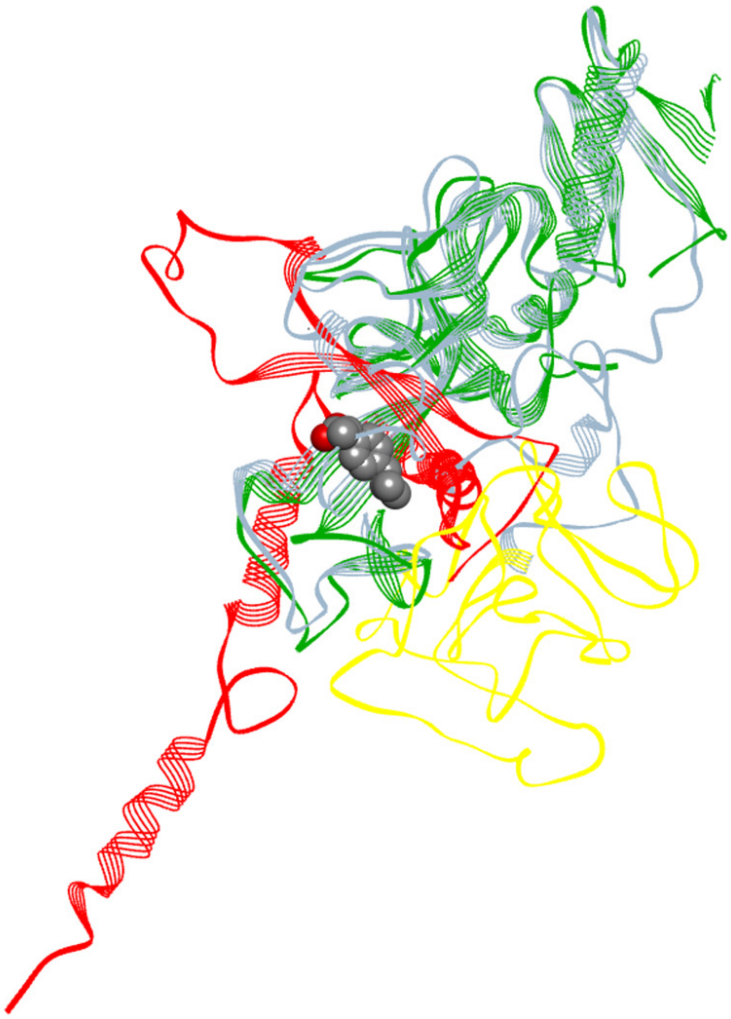

Figure 1. Crystal structure of GP1 (green) and GP2 (red) subunits with ibuprofen molecule (CPK presentation), from pdb structure 5jqb, overlayed with our GP1 model (blue). Yellow region on GP1 model is not crystalized and is considered disordered.

we incubated either 20 or 4 µl of Ebola (Zaire) virus (containing 1.3.6x10^5 PFU or 0.2.7x10^5 PFU virus respectively) with 180 µl of DMEM+2% FBS containing 100 uM ibuprofen in a 96-well plate in triplicates. As control, we incubated 20 µl of Ebola virus (1.3.6x10^5 PFU) or 4 µl virus (0.2.7 x10^5 PFU) with 180 µl of DMEM+2% FBS containing 0.5.% DMSO in a 96-well plate in triplicates. Samples are than diluted 1:10 in corresponding media for 4 times. After that we infected Vero cells grown on 12-well plates with 100 µl of above diluted samples for 1 hr with shaking performed for every 15 mins. Upon incubation time, we perform plaque assay in which we fixed the cells and counted plaques at 11 dpi.

4. RESULTS AND DISCUSSION

Rescoring the position of the ibuprofen molecule in pdb structure 5jqb yielded binding energy value of -5.8.2 kcal/mol. Comparing to our previous results, which gave binding energy value of -9.0. kcal/mol in our predicted region, it is significantly weaker, but again, closer to the experimentally determined Kd of 6 mM (-5.8.2 kcal/mol corresponds to Kd=78µM). This can be explained as a matter of protein-ligand interactions type between interacting atoms of amino acid residues and the ibuprofen molecule. In the case of the crystal structure 5jqb, ibuprofen is placed in the fusion region between GP1 and GP2 subunits, forming interactions with Arg64 (salt bridge), Val66 and Leu184 from GP1 and Met548 and Leu554 from GP2 (hydrophobic interactions) (10). The binding region is far from the one we previously predicted, and still not crystalographically isolated (9). Also, our homology modelling of GP1 suggested disordered structure of the binding region (Figure 1). The reported intermolecular interactions from docking were with Thr 338, Ser 340, Gln 344 (hydrophilic interactions) and with Ala415 (hydrophobic interactions) (9). Therefore, binding of ibuprofen to this region is favoured by more

Table 2. Inhbition of the EBOV infection with ibuprofen

Author Please provide title???	S1	S2	S3	Ave
Mock 1 (20 µl virus)	920000	8.0.0E+05	8.7.0E+05	8.6.3E+05
Mock 2 (4 µl virus)	290000	2.5.0E+05	2.6.0E+05	2.6.7E+05
100 µM Ibu I (20 µl virus)	450000	4.0.0E+05	5.4.0E+05	4.6.3E+05
100 µM Ibu 2 (4 µl virus)	150000	1.6.0E+05	1.7.0E+05	1.6.0E+05

hydrophilic interactions and a disordered structure that could be more prone to finer structural tuning of the binding site to ibuprofen than the one in the 5jqb structure suggested by Zhao and co-workers (10). However, binding energy calculations are limited by the docking software forcefield. Structural data reported by Zhang and co-workers indicate that the GP fusion is hindered by ibuprofen binding to fusion regions (10). Our results suggest that binding of ibuprofen to GP1 modulates the long-range interaction between virus and the receptor. This does not exclude the possible existence of two different binding sites for ibuprofen on the Ebola GP, which modulates virus/host interaction in different ways.

The experiments with infectious Ebola virus have shown that 100 µM ibuprofen can reduce the virus titer in Vero cells by 50% (Table 2), however, the biological significance of this reduction is not established yet.

In conclusion, results presented here, together with data reported by Zhao and co-workers (10), suggest that ibuprofen, although a weak inhibitor of the Ebola virus infection, represents a promising molecular template for further development of drugs against this important pathogen.

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Send correspondence to: Veljko Veljkovic, Biomed Protection, Galveston, TX, USA, Tel: 381116453686, Fax: 381113408361, E-mail: vv@vinca.rs