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Article

Fast computational chemistry methods applied to new anti-Ebola virus entry drugs - application for new therapeutic targets

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ABSTRACT

Ebola virus is responsible for severe symptoms and has fatality rates up to 90%. Some approved drugs among antipsychotics and Selective Serotonin Reuptake Inhibitors (SSRI) antidepressants appear to be efficient inhibitors, with fewer secondary effects. There is an immense pressure to use fast research methods to discover new antivirus-drugs or, detect new antivirus applications of clinically used drugs. We have generated quantitative structure–activity relationship (QSAR) models on drugs used to treat genetic disorders, with various inhibitor concentrations (IC50) on Ebola virus. We evaluated the predicted affinity at Ebola virus glycoproteins of other efficient SSRI antidepressants, antipsychotics and anticancer drugs.

Keywords: QSAR, Ebola virus, Antipsychotic, Computational Chemistry.

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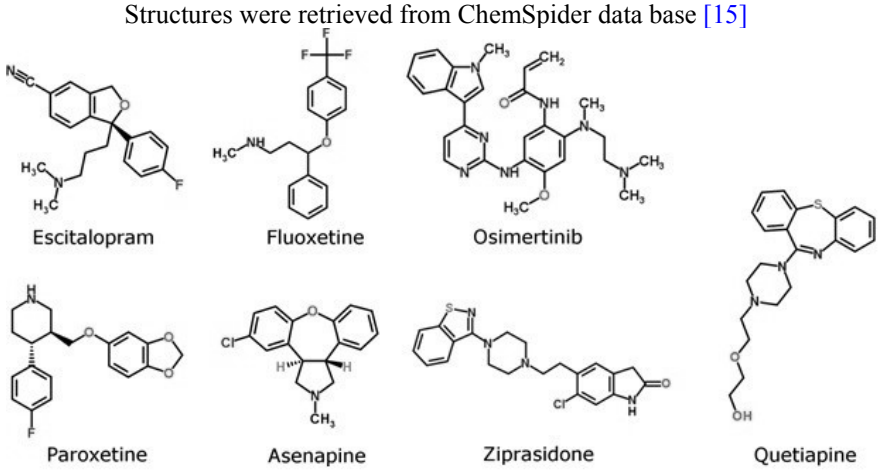
1. INTRODUCTION

Ebola virus (Ebov), belonging to Mononegavirales Order, Filoviridae family, causes a critical hemorrhagic fever disease with fatality rates up to 90% [1]. Typical symptoms occurring in the 21st day after Ebov infection include fever, fatigue, diarrhea, headache, abdominal pain, cramping, nausea and vomiting [2]. The very first Ebov outbreak was in 1976, in Democratic Republic of Congo [3]. The virus flourished in Africa due to the general instability of the region and poor levels of healthcare.

In spite of the high mortality due to Ebov infections, there is no viable treatment available [4].Previous attempts to fight the infections included: (i) whole blood from surviving Ebov patients [5]; (ii) IgGs isolated from horses hypervaccinated with Ebov [6]; (iii) humanized-mouse antibodies [7]; (iv) inhibitors of protein production based on RNA -polymerase inhibitors and interfering RNA nanoparticles; (v) gene-silencing using small interfering RNAs [8]. A promising approach in identifying an Ebov treatment is based on the repurposing of approved drugs. In this direction, Kouznetsova et al [9] identified 53 compounds with the capacity to block Ebola virus-like particle entry which included commonly used antidepressants and antipsychotics [9]. Ebola virus is composed from: a glycoprotein (subdivided in sGP, GP1, GP2 and ssGP), a protein matrix (VP24, VP40, VP35, and VP30), and a nucleoprotein [10]. In their study Kouznetsova et al. and Johansen [9,11] assessed the effect of 2816 compounds from a NCATS-approved drug collection assembled for drug repurposing tests .[9] on inhibiting the entry of Ebola virus - like particles (VLP), composed of a glycoprotein and the matrix VP40 protein fused to a beta-lactamase reporter enzyme into cultured HeLa cells. In the end, 53 compounds were identified to have the potential to block Ebola VLP entry into cells. These compounds belong to different drug classes, including microtubule inhibitors, estrogen receptor modulators, antihistaminics, antipsychotics, pump/channel antagonist and anticancer/antibiotics.

Figure 1.Chemical structures of the drugs taken into account as possible Ebola medication. Structures were retrieved from ChemSpider data base [15]



The aim of our study was to expand Kouznetsova's study by predicting the inhibitory reaction of other widely used antipsychotics, antidepressants and new anticancer drugs, with minimal side effects. Hereby, based on our expertise in computational biology[12,13,14] we conducted a Quantitative Structure-Activity Relationship (QSAR) study on the viability as Ebola medication of three potent antidepressants belong to SSRI's (Selective serotonin reuptake inhibitors), namely Escitalopram, Fluoxetine and Paroxetine; three atypical antipsychotics namely Asenapine, Quetiapine and Ziprasidone; and a new anticancer compound represented by Osimertinib (Figure 1).

2. METHODS

2.1. Dataset for Analysis

In the present study we used a set of 21 drugs identified from the literature that were clinically proven to inhibit Ebola VLP entry into HeLa cells [9]. These drugs covered large classes of pharmaceutical compounds used in depression, psychosis, viral infections and cancer. These chemical compounds are represented by 5 antidepressants: Bifemelane, Clomipramine, Imipramine, Maprotiline and Sertraline; 4 antipsychotics: Piperacetazine, Thioproperazine, Thiothixene, Trifluoperazine, 9 anticancer drugs: Bosutinib, Daunomycin, Raloxifene, Sunitinib, Topotecan, Toremifene, Vinblastine, Vincristine, Vinorelbine, one antiviral compound Tilorone, one anticholinergic compound namely Benztropine and one antiallergic compound namely Clemastine. Their inhibitory activities expressed as the half maximal inhibitory concentration (IC50) vary between 0.048 microM (Vinblastine) and 13.7 microM (Imipramine). Biological activities were evaluated as pIC50 using the following formula: -logIC50 (uM)* 10-6 Eq.(1) [16].

2.2. Molecular modeling and the minimum potential energy calculation of compounds

Molecular modeling of the compounds was performed using their 3D structures retrieved from the ChEMBL database [17] (the database code of each compound is listed in Table 3). Minimum energy evaluation of compounds was performed by AM1 semi-empirical method, Conjugate-Gradient algorithm, convergence 0.01. After energy minimization, Gasteiger partial charges were used [18].

2.3. QSAR methodology

Initially, we calculated 23 descriptors belonging to two categories: (i) 2D descriptors including physical properties: steric (subdivided van der Waals surface and volume, solvent accessible surface and volume), atom and bond counts (hydrophobic/polar, donor /acceptor

atoms, rigid and rotatable bonds) and electronic descriptors (molecular polarizability, molar refractivity, dipole moment); and (ii) 3D molecular descriptors including potential energy descriptors and globularity. Descriptors calculation was performed with MOE software.
Descriptors evaluation was followed by overlapping descriptors removal using Pearson correlations. Afterwards, the most useful 3 descriptors for the QSAR model were selected by performing a SLR (Simple Linear Regression) [19]. The selected descriptors are: (i) ASA – describing the water accessible surface induced by negative atoms, determined using a probe radius of 1.4 Å that rolls over all atoms with negative partial charges (less than 0);(ii) LogP - represents the logarithm of the octanol/water partition coefficient [20]; (iii) SMR - molecular refractivity calculated using an atomic contribution model that assumes the correct protonation state (washed) structures [21].

Table 1 Pearson correlation matrix

Pearson correlation matrix			
	LogP	ASA-	SMR
LOGP	1		
ASA	0.051	1	
SMR	0.061	0.058	1

Furthermore, the selected molecular descriptors were used for developing several QSAR models. The Pearson correlation matrix of these descriptors is presented in Table 1.

2.4. Chemometric analysis

The QSAR models developed in Volsurf software were statistically analyzed in order determine their reliability. The statistical parameters that we calculated are: correlation coefficient of the regression between the predicted and observed activities of compounds (R^2), cross-validated r^2 (Q^2), the root mean square error (RMSE) and cross-validated RMSE. In practice, a QSAR model can be validated if R^2 and Q^2 exceed 0.8 and 0.5 [17]. The following QSAR model: pIC50 = 2.85442 -0.00157 * (ASA-) +0.18857 * logP(o/w)+0.21406 * SMR Eq.(2) presented the best statistical parameters, therefore it was used to predict the biological activities of the 7 drugs that we considered as candidates for repurposing.

2.5. Training and testing sets

The composition of the training and test sets is very important, as it has the ability to influence the consistency of resulting QSAR models. Here, the molecules were randomly distributed in the training (16 molecules) and test set (5 molecules). The predictive power of

our QSAR model was used for predicting the Ebov inhibitory activity of the seven drugs we considered for repurposing.

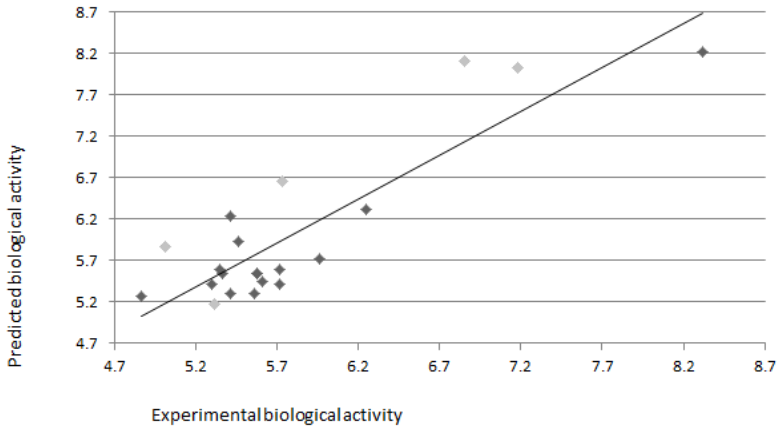
3. RESULTS AND DISCUSSIONS

In the present study, we generated a QSAR model that associates the molecular descriptors of several drugs proved to inhibit Ebov VLP entry into cultured cells with their inhibitory activities. The dataset we considered includes antidepressant, antipsychotic, anticancer, antiallergic, anticholinergic and antiviral drugs. The power of prediction of the developed model was used to predict the Ebov antagonistic activity of 7 compounds already used in clinics for different purposes: 3 antidepressants, 3 antipsychotics and 1 anticancer. The advantages of identifying Ebov inhibitors among these drugs are that they are already used in clinics and have minimal side effects.

Table 2. Summary of QSAR statistical parameters for the best model

ROOT MEAN SQUARE ERROR (RMSE)	0.22
CORRELATION COEFFICIENT (R^2)	0.90
CROSS-VALIDATED RMSE	0.42
CROSS-VALIDATED R^2 (Q^2)	0.73

Figure 2. Correlation between predicted and experimental values of the pIC50 obtained by QSAR model (q^2=0.73 r^2 =0.90). Dark grey – Values from the training set. Light grey – Values from test set.



Initially, several QSAR models were developed based on the three molecular descriptors we identified as being significant (non-overlapping and non-redundant) for the inhibition of Ebov VLP particles entry into the cells. From these, we selected the model that presented the best statistical parameters (Table 2) for predicting the anti-Ebov activity of the repurposing candidate drugs.

The QSAR model has a good predictive power, as showed by the predicted pIC50 values (pIC50 pred) of the 21 drugs taken in testing and training sets and by the residuals calculated as experimental pIC50 values (pIC50exp) minus the predicted pIC50 values (Table 3).The good correlation between predicted biological activities and experimental biological activities is presented in Figure 2.

Table 3. Comparison between observed and predicted activities (pIC50) and the residual values differences between experimental and predicted biological activities for training and test (Bolded) sets.

Approved indication	Drugs	ChEMBL code	pIC50 exp	pIC50 pred	Residual
Antidepressants	**Bifemelane**	**CHEMBL1192517**	**5.31**	**5.17**	**-0.13**
	Clomipramine	CHEMBL415	5.30	5.42	0.11
	Imipramine	CHEMBL11	4.86	5.27	0.41
	Maprotiline	CHEMBL21731	5.61	5.44	-0.16
	Sertraline	CHEMBL809	5.56	5.30	-0.25
Antipsychotics	**Piperacetazine**	**CHEMBL1584**	**5.01**	**5.87**	**0.85**
	Thioproperazine	CHEMBL609109	5.36	5.54	0.18
	Thiothixene	CHEMBL1201	5.71	5.59	-0.11
	Trifluoperazine	CHEMBL422	5.34	5.59	0.24
Anticancer	Bosutinib	CHEMBL288441	5.41	6.24	0.82
	Daunomycin	CHEMBL178	5.58	5.53	-0.04
	Raloxifene	**CHEMBL81**	**5.73**	**6.64**	**0.91**
	Sunitinib	CHEMBL535	5.71	5.41	-0.30
	Topotecan	CHEMBL84	5.41	5.30	-0.11
	Toremifene	CHEMBL1655	6.24	6.31	0.07
	Vinblastine	**CHEBI:27375**	**8.31**	**8.21**	**-0.10**
	Vincristine	**CHEBI:28445**	**6.85**	**8.10**	**1.25**
	Vinorelbine	**DB00361(*)**	7.18	8.03	0.84
Antiallergic	Clemastine	CHEMBL1626	5.95	5.72	-0.23
Anticholinergic	Benztropine	**CHEBI:3048**	5.57	5.54	-0.03
Antiviral	Tilorone	CHEMBL47298	5.46	5.93	0.47

*Vinorebline mol file was acquired from drugbank.ca database [22]

After validating the QSAR model, we used it for predicting the Ebov infection inhibitory activity of the seven drugs we considered for repurposing. Their predicted biological activities are presented in Table 4. As can be seen, the highest pIC50 value that should correlate to a higher biological activity was obtained in the case of Osimertinib, an anticancer compound. This result is in agreement with the observation that, in the dataset we considered, experimental pIC50 values of some anticancer compounds are higher and even significantly higher than that of antipsychotic and antidepressant drugs (Table 3). According to its predicted pIC50 value (6.41), Osimertinib should have an Ebov inhibitory activity higher than that of Toremifene (6.24) and lower than that of Vincristine (6.85). The following activities are those of antipsychotic drugs Ziprasidone (5.72) and Quetiapine (5.37). Zisprasidone has a similar predicted pIC50 value to that of the antipsychotic drug Thiothixene (5.71) and the anticancer compounds Raloxifene (5.73) and Sunitinib (5.71). Quetiapine should have similar inhibitory activities with Thioproperazine (5.36) and Trifluoperazine (5.34), two antipsychotic drugs.

Table 4: Predicted biological activities of the drugs with potentially inhibitory effect on Ebola VLP virus

Approved indication	Drugs	ChEMBL code	Predicted pIC50
Antidepressant	Escitalopram	CHEMBL1508	5.27
	Fluoxetine	CHEMBL41	5.04
	Paroxetine	CHEMBL490	5.11
Anticancer	Osimertinib	31042598 (**)	6.4
Antipsychotic	Asenapine	CHEMBL1201756	4.91
	Quetiapine	CHEMBL716	5.37
	Ziprasidone	CHEMBL708	5.72

**Osimertinib file was acquired from Chemspider.com database

The three antidepressants that we considered for repurposing, namely Escitalopram, Paroxetine, Fluoxetine, have predicted pIC50 values of 5.27, 5.11, 5.04. By comparing these values with the experimental pIC50 values of the compounds in training and testing sets, we identified that: (i) Escitalopram should have an Ebov inhibitory activity similar with the antidepressants Bifemelane (5.31) and Clomipramine (5.30); (ii) Paroxetine and Fluoxetine might inhibit Ebov entry into cells in a similar manner with the antipsychotic Piperacetazine (5.01), Paroxetine being slightly more active. The lowest predicted pIC50 value was calculated for the

antipshychotic Asenapine (4.91). This value is similar to the experimentally determined pIC 50 value for Imipramine (4.86)

Results presented above show that the compounds we considered for repurposing should exert a moderate effect of inhibiting Ebov VLP entry into host cells. The limitations of our study are those raised by the measurements performed by Kouznetsova et al, 2014.: experiments were performed using Ebov VLP, therefore their results should be further confirmed in Ebov infection assays and in animal models. Nevertheless, our study brings new information on the potential of antipsychotics and antidepressants to be repurposed even for fighting serious infections, such Ebov infections.

4. CONCLUSION

Here we investigated the possibility of repurposing three antidepressants (Escitalopram, Fluoxetine and Paroxetine), three antipsychotics (Asenapine, Quetiapine and Ziprasidone) and one anticancer compound (Osimertinib) as anti Ebov medication. Our idea is supported by the fact that in screening for anti-Ebov compounds, Kouznetsova et al. identified antipsychotic and antidepressant drugs that could inhibit Ebov entry into host cells. The effectiveness of our molecules of interest was predicted using QSAR. A reliable QSAR model (Q^2=0.73,R^2=0.90) was obtained by considering three non-overlapping and non-redundant molecular descriptors: ASA, LogP (including implicit hydrogen atoms) and SMR (including implicit hydrogen atoms). By applying the derived QSAR equation in the case of our target molecules, we identified that considered antipsychotic and antidepressant drugs should present a medium anti-Ebov effect, comparable with Thiothixene, Thioproperazine, Trifluoperazine, Bifemelane, Clomipramine, Piperacetazine or Imipramine. In the case of the anticancer compound considered here, we observed that it should have a higher anti-Ebov activity than the considered antipsychotic and antidepressant drugs, higher than that of the anticancer drug Toremifene and lower than that of Vincristine.

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