THE ALGORITHM OF VIRTUAL SCREENING AND PREDICTION OF THE PROMISING ANTICONVULSANTS' MECHANISM OF ACTION

Severina H. I., El Kayal W. M., Georgiyants V. A.
National University of Pharmacy,
Kharkiv, Ukraine
severina.ai@ukr.net

Despite both the availability of well-known, and successful development of new antiepileptic drugs (AEDs), the search for new promising compounds for treatment of seizures and epilepsy, which are, according to preclinical and clinical trials results, more efficient and better tolerated, is still being used. The algorithm of the search for new AEDs consists of the complex use of screening models of seizures with different pathogenesis. However, efficient search for anticonvulsants is limited by high and painful mortality in animals during pharmacological experiment. The use of modern targeted virtual screening allows rationalizing the search for new AEDs, predicting possible mechanism of action, and selecting the screening model correctly. The mentioned screening is based on scientific knowledge concerning molecular changes generating epileptic seizures, anticonvulsant action mechanisms, target proteins stuctures and amino acid composition of receptor active sites, as well as it is based on the arsenal of computer analysis methods and the protein affinity with ligand evaluation. The developed by us algorithm consists of the stepwise use of virtual targeted docking and pharmacological screening models.

On the first stage we carry out primary virtual screening using Scigress Explorer 7.7 software for the formed from logical and structural analysis of the substances base. The mentioned software has high speed data processing, which allows evaluating the affinity for a large library of substances in the minimum period. For the primary receptor-oriented screening we chose $GABA_A$ (PDB 4COF) receptor – a ligand-dependent ionotropic channel at the central nervous system synapses, which inhibits the transmission of nervous excitement and is a target for many AEDs.

The substances, which have shown low binding energy values with $GABA_A$ receptor, are selected for the further screening on PTZ-induced seizures, because its proconvulsant action is caused by inhibition of $GABA_A$-site of benzodiazepine receptor complex and decrease in intensity of GABA-ergic inhibition processes in the CNS.

We used AutoDockTools1.5.6rc3 software to carry out virtual prediction of less number of substances. The software is widely used for molecular docking and, according the literature data, demonstrates the maximal ligand positioning accuracy in biotarget, allows evaluating nature and length of the chemical bounds and amino acid residues, interacting with the ligand. The next stage of the docking was determination of the ligands affinity with GABA-aminotransferase active site (PDB 1OHW). Native $GABA_{AT}$ ligand Vigabatrin was chosen as a reference drug. Results of the studied ligands binding with $GABA_A$ receptor and $GABA_{AT}$ enzyme give an idea of the possible GABA-ergic anticonvulsant activity mechanism.

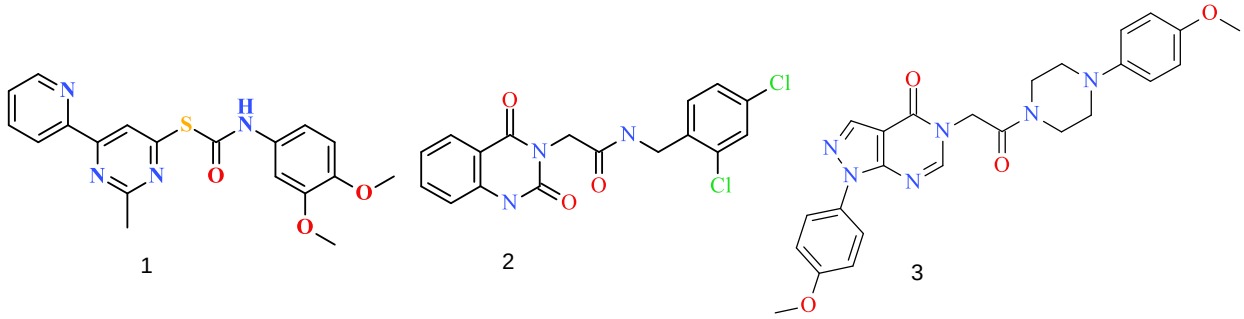
The next stage was estimation of expediency of the further pharmacological screening on the model of primary-generalized clonic-tonic MES-induced seizures.

For this purpose, molecular docking in the active sites of the known biotargets comparing with AEDs, which are effective on MES model of seizures, is carried out. Modern felbamate and parampanel, which are native ligands of ionotropic glutamate NMDA receptor (PDB 5PT9) and AMPA-receptor (PDB 5L1F), respectively, are among them. Low binding energy values with the given active sites substantiates pharmacological screening of determined ligands on the MES model and allows to evaluate the possibility of glutamatergic mechanism of anticonvulsant activity realization. Besides, if low scoring function is the result of the studied ligand docking into the active site of AMPA-receptor (PDB 5L1F), this is an indicator of possible activity on the model of audiogenic psychomotor seizures in mice and, thereafter, possible efficiency of the studied compounds in resistant epilepsy.

Then the docking into the active sites of carbonic anhydrase II (PDB 1 EOU and 3IEO) and the affinity estimation of the studied ligand comparing with the modern AEDs – topiramate and lacosamide, respectively, are carried out. Affinity estimation for gabapentin active site in branched side-chain amino acid aminotransferase (hBCAT, PDB 2COI) is also possible. Gabapentin has multifactorial mechanism of anticonvulsant activity, and increasing of GABA concentration due to influence on leucine transport is one of them, as well as it increases glutamate decarboxylase activity. The obtained results allow to estimate the possibility of realization of multifactorial mechanism of anticonvulsant activity.

The final stage is the feasibility estimation of the study on secondary epileptogenesis models characterizing chronic epileptogenesis (kindling models). Complex estimation of the obtained docking results into the active sites of the following AEDs: topiramate, lacosamide, felbamate and parampanel, and satisfactory results majority will allow to conclude on the possibility of chronic study and to choose a kindling promising model (PTZ, picrotoxin, etc.)

The given algorithm was successfully realized by us on pyrimidine-4(*3H*)-on derivatives (1), its annelated derivative – quinazoline-4(*3H*)-on (2), and condensed derivative – pyrazolo[3,4-d]pyrimidine-4(*3H*)-on (3).



1 2 3

In every of the mentioned groups compounds having a pronounced anticonvulsant activity on different models of seizures were found.