IN SILICO STUDY AND *IN VITRO* ACTIVITY OF NOVEL OXAZOLE DERIVATIVES AGAINST HUMAN CYTOMEGALOVIRUS

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Human cytomegalovirus (HCMV) is a ubiquitous pathogen affected humans with impaired or immature immune-defence functions [1-3]. Since HCMV infects approximately 60% of people in the developed world and over 99% in developing countries, the risk of morbidity due to HCMV disease is a significant problem for public health [4]. At present, there are several anti-HCMV drugs (Ganciclovir, Valganciclovir, Cidofovir, Maribavir, Letermovir), however, these ones have poor bioavailability and many shortcomings, including associated toxicities, and the emergence of drug resistant viruses [5,6]. Hence, this warrants the need for urgent development of novel antiviral substances.

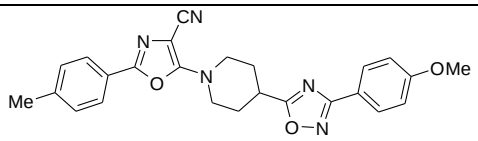
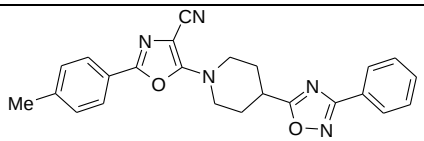
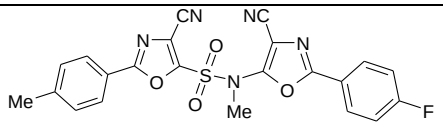
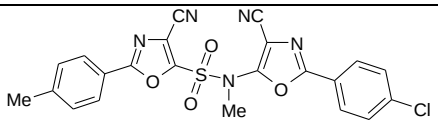
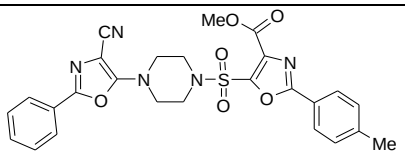
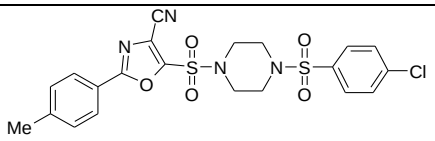
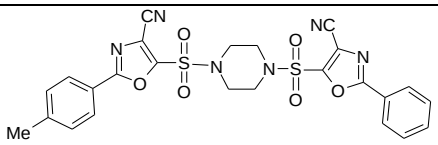
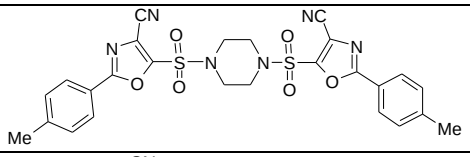
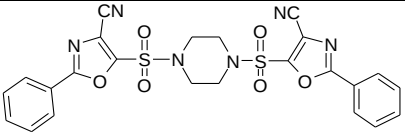
1,3-Oxazole derivatives are among the most useful heterocyclic compounds from both synthetic and medicinal chemistry aspects [7]. Recently, we have synthesized novel 1,3-oxazole-4-carboxylates and 1,3-oxazole-4-carbonitriles and their antiviral activities against the human cytomegalovirus (HCMV) were evaluated *in vitro*. These compounds exhibited considerably higher antiviral activity (EC_{50}: < 0.05 μM) against a normal laboratory HCMV strain (AD-169) than Ganciclovir (EC_{50} = 0.32 μM), an anti-HCMV agent in clinical use. Additionally, the HCMV resistant isolate (GDGr K_{17}) was tested for sensitivity to 1,3-oxazole derivatives with most antiviral potency against the strain AD169. A one of them (5-((2-hydroxyethyl)(methyl)amino)-2-(4-methylphenyl)-1,3-oxazole-4-carbonitrile) showed very high potency (EC_{50}: < 0.05; CC_{50}: >150 μM and SI_{50} = 3125) towards the resistant isolate compared to standard drugs Cidofovir (EC_{50} = 0.10 μM, CC_{50}: >30 μM and SI_{50}:<4) [8]. These data indicate that 1,3-oxazole derivatives are promising compounds in the search for design of new anti-HCMV drugs.

In this work, in order for subsequent investigation of antiviral activity of novel 1,3-oxazole derivatives we applied the machine learning technic. The prediction of the antiviral activity of the 1,3-oxazole derivatives against HCV was performed using the Online Chemical Modeling Environment (OCHEM). The predictive QSAR model was created by Transformer-CNNi method [9]. The data set consisted of 671 compounds with antiviral activity obtained from multiple publications and uploaded into the OCHEM. The EC_{50} values of the molecules in μM were converted into the -log(M) and used as the target variable to develop regression model. The 5-fold cross-validation method was used to evaluate the accuracy of the QSAR model. The optimized parameters setting of machine-learning method was used provided by OCHEM platform. The main statistical parameters of the created QSAR model such as the coefficient of determination (q^2) and the mean absolute error (MAE) were 0.71±0.03 and 0.43±0.02 respectively that demonstrated high accuracy and robustness for prediction of the antiviral activity. A set of novel 2-aryl-4-cyano-5-amino-1,3-oxazoles and 1,3-oxazole-5-sulfonylamides as potential anti-HMCV agents generated and screened using the QSAR model were synthesized in V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine and evaluated *in vitro*. The antiviral activity of the compounds **1** and **2** was predicted as 4.6 -log(M) unit, while the activity of the rest compounds was predicted in the range of 3.8-4.0 -log(M) units.

The all cytotoxicity and efficacy tests performed at UAB. The evaluation was carried out under the auspices of the National Institute of Allergy and Infectious Diseases (USA) and its Collaborative Antiviral Testing Group. The effects of the 1,3-oxazole derivatives on antiviral activity against a normal laboratory HCMV strain, AD-169, and their cytotoxicity was evaluated on HFF cells using CellTiter-Glo (Cytopathic effect/Toxicity) assay (Table 1).

Table 1

The antiviral activity and cytotoxicity of the 1,3-oxazole derivatives against AD169 strain of HCMV, in HFF cell line. Compound concentrations are in μM.

No	Compound	Strain AD169		
		EC_{50}	CC_{50}	SI_{50}
1		0.77	>150	>196
2		5.33	>150	>28
Ganciclovir		0.75	>150	>201
3		>30	85.52	<3
4		>30	64.18	<2
5		>150	>150	1
6		>150	>150	1
7		>6	21.47	<4
8		>30	115.65	<4
9		>30	63.25	<2
Ganciclovir		1.54	>150	>97

Control and drug concentrations ranges are 0.048-150 μM. Vehicle is DMSO. EC_{50} and CC_{50} are compound concentrations that reduce viral replication and cell viability, respectively, by 50% in CellTiter-Glo (Cytopathic effect/Toxicity) assay. Selectivity Index (SI_{50}) is calculated as the CC_{50} value divided by the EC_{50} value.

Compounds exhibited different antiviral activity against the HCMV strain AD169 and, with the exception of **1**, **2** and **7**, had EC_{50} values of >30 μM. Compound **1** showed the best activity with EC_{50} = 0.77 μM which appeared to be similar to control drug Ganciclovir (EC_{50} = 0.75 μM). Compounds **3, 4, 8** and **9** have shown the identical moderate antiviral activity.

Thus, only 5-amino-4-cyanooxazoles 1 and 2 with oxadiazole moiety displayed the mild biological activity among the series of 5-amino-1,3-oxazole-4-carbonitriles that was also confirmed by the QSAR model predictions. Among sulfonylamide derivatives 3-9 compound 7 showed the best result with EC50 values of >6 μM however it was lower compared to control drug.

These results provided evidence that derivatives of 1,3-oxazole could be useful for developing new anti-HCMV drugs.

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