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Graphical Abstract

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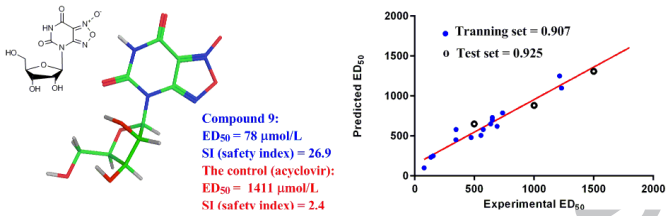

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Synthesis and quantitative structure-activity relationship (QSAR) analysis of some novel oxadiazolo[3,4-*d*]pyrimidine nucleosides derivatives as antiviral agents

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ABSTRACT

We have synthesized a series of *4H,6H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide nucleoside and their anti-vesicular stomatitis virus (VSV) activities in Wish cell were also investigated *in vitro*. It was found that most compounds showed obvious anti-VSV activities and compound **9** with ribofuranoside improved the anti-VSV activity by approximately 10 times and 18 times compared to didanosine (DDI) and acyclovir, respectively. A quantitative structure-activity relationship (QSAR) study of these compounds as well as previous reported oxdiazolo [3,4-*d*]pyrimidine nucleoside derivatives indicated that compounds with high activity should have small values of log *P*(*o/w*), vsurf_G and a large log *S* value. These findings and results provide a base for further investigations.

Nitric oxide (NO) is an important gas messenger molecular in organisms, which can modulate many physiological.[1] Endogenous NO is synthesized from L-arginine under the action of a family of the enzyme called NO synthase (NOS).[2] It has been implicated in various regulatory mechanisms ranging from wasodilation and blood-pressure control to immune response, and it acts as neuro transmission in central and peripheral nervous systems.[3] NO is also involved in non-specific host defense, which may inhibit an early stage in viral replication and thus prevent viral spread, promote viral clearance and recovery of the host.[4] As NO in-depth study, more and more results complicated that activated macrophage cells have an extensive killing effect on tumor cells while during which NO is the toxicity molecular.[5] Due to the comprehensive effects in organisms of NO, it attracts more and more interest in the past years. There is much interest today in drugs related to NO, especially in structures able to release NO. These products are collectively called NO donors, which are widely used in biological, physical and pharmaceutical fields.[6]

Among NO donors, derivatives of a minor heterocycle system, the oxadiazolo[3,4-*d*]purimidine, are also included (Fig. 1). By the interaction of [3,4-*d*]pyrimidine-5,7-dione 1-oxide derivatives with thiol such as *N*-acetylcysteamine, cysteine and glutathione *in vivo*, they can produce uracil derivatives and release NO.[5] These compounds have been proved to be a sort of effective NO donor, which can generate NO and three kinds of non-nature nucleoside-pyrimidine-base in physiological conditions.[5] The antivirus and anticancer activities of non-nature nucleosides have been suggested by many evidences. So it is obvious that oxadiazolo[3,4-*d*]pyrimidine combined with modified sugars will possess antivirus and antitumor activities, for they can release both NO and non-nature nucleosides in physiological conditions.

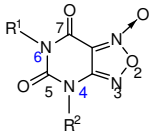

Fig. 1 The structure of oxadiazolo[3,4-*d*]purimidine ring.

Although some pyrimidofuroxan-nucleoside compounds have been reported by our collaborative laboratory,[5] these studies did not investigate comprehensively and, overall, they were limited to relatively simple compounds. In this work, we have concluded the reported 14 derivatives and synthesized another 11 novel oxdiazolo[3,4-*d*]pyrimidine nucleoside derivatives. In addition, the antivirus activities and toxicities of all these compounds were also studied (Table 1). As is known to us, the biological activity correlates greatly with the structure of the compound. For a detailed understanding of the structure-activity relationship of all reported and synthesized pyrimidofuroxan-nucleoside

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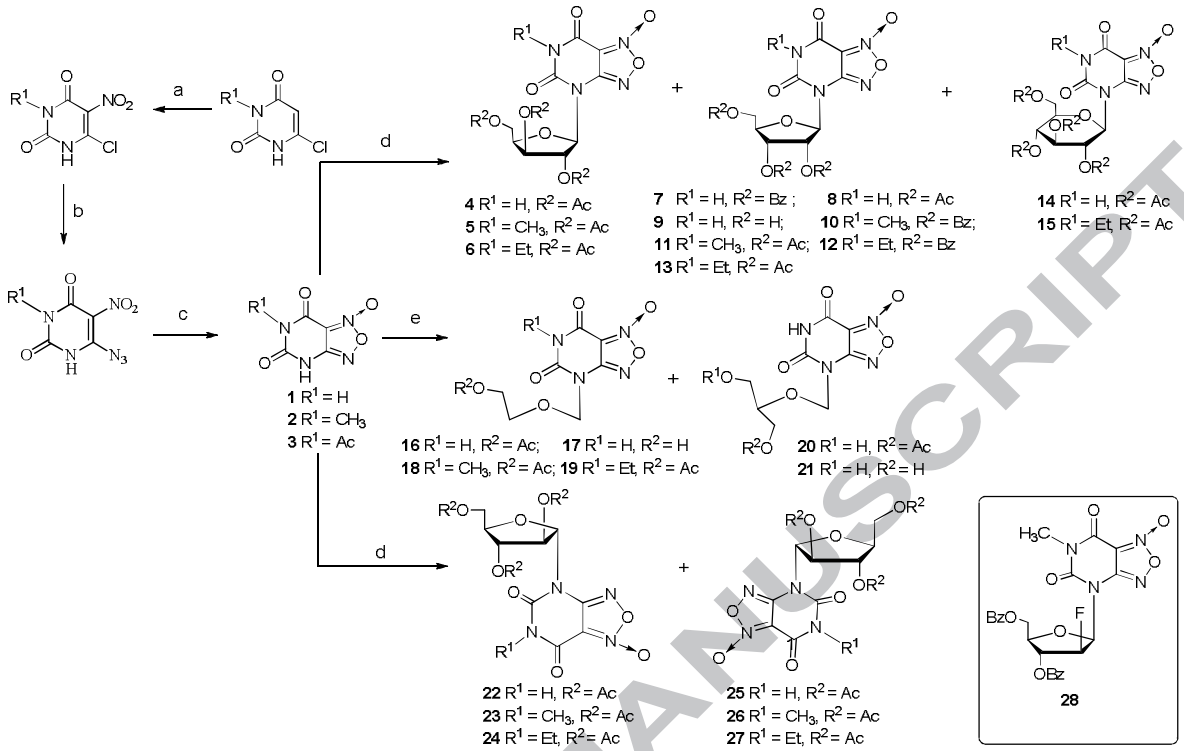
compounds, we also carried the quantitative structure-activity relationship (QSAR) studies.



Scheme 1. Reagents and conditions: (a) fuming HNO_3, H_2SO_4, 0 °C, r.t., 30 min; (b) NaN_3, THF, r.t., 3 h; (c) vacuum, 100 °C, 8 h; (d) Acetylated glycosylating agents cat. conc. H_2SO_4, 140 °C, 30 min; (e) 2-*O*-(acetoxymethyl)-1-*O*-acetylglycol or 2-*O*-(acetoxymethyl)-1,3-di-*O*-acetylglycerol, cat. conc. H_2SO_4, 140 °C, 30 min.

The starting pyrimidofuroxan derivatives **1**, **2** and **3** were obtained by the thermal decomposition of the corresponding 6-azido-5-nitrouracils, which were prepared from the 6-chloro-5-nitrouracils with sodium azide and followed by heating with loss of nitrogen.[7] The pyrimidofuroxans and fully acetylated glycosylating agents or 2-*O*-(acetoxymethyl)-1-*O*-acetylglycol or 2-*O*-(acetoxymethyl)-1,3-di-*O*-acetylglycol were heated for 30 min at 140 °C in the presence of little H_2SO_4 as catalyst under vacuum and fusion reaction condition, the products were obtained at 20-48% yields.

The toxicities of all prepared nucleosides were first measured. Then we also evaluated their antivirous activities, and the ED_{50} were obtained using the Reed-Muench method.[8] The antiviral activity of all of the pyrimidofuroxan nucleosides was tested against vesicular stomatitis virus (VSV) in Wish cell by previously reported method.[9] Acyclovir and didanosine (DDI) were picked as control compounds. The results were summarized in Table 1.

As shown in Table 1, generally, most of the compounds (**4**-**28**) possessed higher of roughly equal anti-VSV activities compared to acyclovir and DDI. Especially, the anti-VSV activity of compound **9** with ribofuranoside exhibited over 10 times and 18 times compared to DDI and acyclovir, respectively. Compounds (**14**-**21**) with either pyran glycosides or open-loop glycosylation glycosides showed much lower potency than the compound **9**. These results indicated that the ribofuranoside may be the priority substituent to obtain better anti-VSV when we modified these structures. Moreover, it was observed that most of the compounds showed more or less toxicities, demonstrating the ability of the antitumor activities. From a security point of view, many of these compounds showed higher safety index (SI = (TC0)/ED_{50}) than DDI (SI = 3.34) and acyclovir (SI = 2.4). Notably, compound **9** that showed the strongest antiviral activity also afforded the highest safety index (SI = 26.9). These results indicated that the compound **9** could be considered as a lead chemotype worth of investigations.

Table 1
Toxicity for Wish cells and antivirus effect of oxadiazolo[3,4-*d*]pyrimidine derivatives.

Entry	Compounds	(TC0) (μmol/L)	ED_{50} (μmol/L)[a]
1	**4**[b]	387	-
2	**5**[b]	477	-
3	**6**	356	1038
4	**7**[b]	650	736
5	**8**[b]	1459	345
6	**9**[b]	2095	78
7	**10**[b]	1055	473
8	**11**[b]	1460	346
9	**12**	1026	498
10	**13**	377	1230
11	**14**[b]	1300	-
12	**15**	1345	1214
13	**16**[b]	2083	651
14	**17**[b]	2561	636
15	**18**[b]	2077	650
16	**19**	408	-
17	**20**[b]	1475	651
18	**21**[b]	2279	555

19	**22**	398	691
20	**23**	378	-
21	**24**	1411	574
22	**25**	380	154
23	**26**	300	135
24	**27**	20	-
25	**28**[b]	10	1500
26	**Acyclovir**	3414	1411
27	**DDI**	2646	792

[a] '-' Represents no anti-VSV activities.

[b] These compounds have been previously reported by our collaborative laboratory.

In order to rationalize the observed anti-VSV activities, the QSAR study was carried out. For the QSAR study dataset, 19 compounds (the other 6 compounds acted as the reference of non-active compounds) were used to develop the models, 16 of which were selected randomly as training set and the rest 3 were used as the test set (Table 2). The structures of these compounds were sketched using the molecular builder module of MOE software (version 2013.08) and minimized for energy via steepest descent, conjugate gradient, and truncated Newton method in sequence using MMF94 as force field with energy tolerance value of root mean square gradient 0.001 kcal/mol. A conformation search of each energy-minimized structure was performed using stochastic approach. The QSAR model generation was done using AutoQSAR packed in MOE, and the QSAR models were constructed based on the partial least square method using more than 300 descriptors built in MOE. QSAR was built using the descriptor as an independent variable and ED_{50} as a dependent variable by forward stepwise regression analyses. QSAR equations were acquired according to different combinations of various descriptors. The data matrix was analyzed using the partial least squares (PLS) method.[10] The quality of each regression model was evaluated, using a squared correlation coefficient (r^2), root mean square error (RMSE), and cross validation squared correlation coefficient (q^2). The coefficient r^2 indicated how well the equation fits the data. The q^2 was considered as an indicator of the predictive performance and stability of a QSAR model.[11,12] The excellent established QSAR equations including two or three descriptors were summarized in Eqs. (1) and (2) respectively along with their statistical parameters. The descriptors used in QSAR building were defined as follows: Partial Equalization of Orbital Electronegativities (PEOE_VSA+4), Molecular refractivity (SMR_VSA6), log of the octanol/water partition coefficient (log *P*(*o*/*w*)), log of the aqueous solubility (log *S*), surface globularity (vsurf_G).

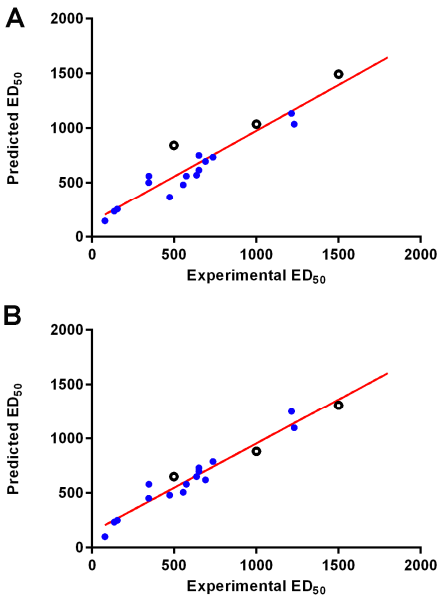


Fig. 2 Correlation plot of experimental activity versus predicted activity for QSAR models. (A) Eq. 1 QSAR model. (B) Eq. 2 QSAR model. (○) values for compounds in the test set. (●) values for compounds in the training set

The partial charge descriptor PEOE_VSA+4 represents the sum of vander Waals surface area of the molecule.[13] Mathematically, it can be defined as the sum of vander Waals surface area (v_i) such that partial charge (q_i) is in the range [0.2, 0.25) divided by the total surface area. The descriptor PEOE_VSA+4 takes a negative weight in the correlation, which suggests that increase in the molecular surface area bearing a polar positive charge will decrease the antivirus potency of these derivatives.

SMR is the descriptor of molecular refractivity. This property is an atomic contribution model that assumes the correct protonation state. Mathematically, SMR_VSA6 can be defined as the sum of vander Waals surface area (v_i) such that molecular refractivity (R_i) is in the range [0.485, 0.56) divided by the total surface area.

The log *P*(*o*/*w*) is known as a measure of lipophilicity and log *P* has been widely used to study biological process relevant to drug action, metabolism, cellular uptake, bio-availability, and toxicity.[14]

Log *S* is log of the aqueous solubility. This property calculated from an atom contribution linear atom type model.[13] The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Normally, low solubility results in poor absorption. Aqueous solubility is among the most important characteristics in ADMET studies and the relevant physicochemical descriptors in QSAR studies.[15]

Vsurf_G is defined as the ratio of the molecular surface to the surface area of a sphere of the same volume. It is specifically designed for the prediction of pharmacokinetic properties and is also related to molecular flexibility.[16]

$$ED_{50} = +\ 16403 - 359.40\ (\text{PEOE\_VSA+4}) + 328.17\ (\text{SMR\_VSA6}) - 323.25\ (\log P(o/w))$$

$$n = 16,\ r^2 = 0.896,\ RMSE = 0.331,\ q^2 = 0.762 \qquad (1)$$

ED50 = – 999.19 – 32.562 (log*P*(*o*/*w*)) + 127.05 (log *S*) -234.17 (vsurf_G)

$n = 16$, $r^2 = 0.907$, $RMSE = 0.305$, $q^2 = 0.791$ (2)

Table 2
Experimental (ED_{50}) and predicted activity values (Model 1-2) for anti-VSV activities

Entry	compound	ED_{50}	Model 1	Model 2
1[a]	**6**	1000	1038	882
2	**7**[b]	736	735	789
3	**8**[b]	345	503	453
4	**9**[b]	78	148	100
5	**10**[b]	473	368	480
6	**11**[b]	346	563	580
7[a]	**12**	498	844	650
8	**13**	1230	1038	1099
9	**15**	1214	1135	1001
10	**16**[b]	651	753	700
11	**17**[b]	636	570	650
12	**18**[b]	650	618	790
13	**20**[b]	651	753	700
14	**21**[b]	555	483	507
15	**22**	691	697	620
16	**24**	574	563	580
17	**25**	154	256	250
18	**26**	135	235	234
19[a]	**28**[b]	1500	1494	1309

[a] Compounds were set as test set.

[b] These compounds have been previously reported by our collaborative laboratory.

As we all know, a good QSAR model is indicated by r^2 and q^2 values close to 1.0, as well as small RMSE. According to our experience, the equations with regression coefficients $r^2 > 0.80$ and $q^2 > 0.50$ are considered reasonable. As shown in Fig. 2 and Table 2, both of the two equations exhibited good regressions between the experimental and predicted activity. Especially for the Eq. (2), which had three descriptors, achieved the higher r^2 and q^2 values than Eq. (1). The Eq. (2) was selected as the example model and implied that the physical-chemical properties log *S* and log *P*(o/w), which were independent of conformation, while vsurf_G dependent on the conformation, played important roles in bioactivity. Log *P*(o/w) and log *S* showed negative and positive and negative sign in the equation respectively, which meant that low ocatanol/water partition coefficients and high solubility were favorable for activity. Moreover, vsurf_G had a negative correlation with activity. Small molecular surface reduced the values of vsurf_G, thereby, improved activity. In conclusion, the compounds that had high activity should have small values of log*P*(*o*/*w*), vsurf_G and a large log *S* value.

In summary, a series of *4H*-[1,2,5]oxadiazolo[3,4-*d*] pyrimidine-5,7-dione 1-oxide nucleosides were synthesized and concluded. The toxicities and the anti-VSV activities of these compounds were also evaluated. Especially compound **9** improved the anti-VSV activity by approximately 10 times and 18 times compared to those of DDI and acyclovir, respectively. QSAR study has been carried out to understand the structural features responsible for the activity of the compounds against virus. Six descriptors are likely to influence the anti-VSV activities of these compounds. Among them, two important ones are the SMR_VSA6 and log *S*. It is believed that the QSAR model could be applicable in the next step work, which might facilitate the discovery of improved compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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