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Chemometric modeling, docking and *in silico* design of triazolopyrimidine-based dihydroorotate dehydrogenase inhibitors as antimalarials

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ABSTRACT

In the present work, QSAR and molecular docking studies have been performed on triazolopyrimidine-based dihydroorotate dehydrogenase (DHODH) inhibitors as antimalarial agents. The QSAR studies have been carried out using classical QSAR (physicochemical) approach using linear free energy related (LFER) model and molecular shape analysis using shape, spatial, electronic, thermodynamic and structural descriptors. Docking studies suggest that the 2-methyltriazolopyrimidine ring interacts with some polar and some nonpolar amino acids whereas the substituted phenyl ring binds with a hydrophobic pocket of the enzyme formed by some nonpolar amino acid residues. According to QSAR and molecular docking studies, we have designed some new compounds which showed good *in silico* predicted activity as per the developed QSAR models.

1. Introduction

Malaria continues to represent a major threat to world health infecting between 300 and 500 million people annually and causing 1.5–2.7 million deaths [1]. The disease results from infection by parasites belonging to the *Plasmodium* species and is transmitted by the female mosquitoes of the *Anopheles* genus. Of the four species of parasite (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*) that infect humans, *P. falciparum* is responsible for the majority (95%) of fatalities [2]. The global expansion of the disease has been attributed mainly to the failure of vector control programs and spread of resistance to chloroquine and other known antimalarial drugs [3]. As a result, discovering and developing novel antimalarial agents is one of the greatest challenges. Pyrimidine biosynthesis provides a significant opportunity for the development of new chemotherapeutic agents against the malaria parasite. While mammalian cells contain enzymes for both de novo biosynthesis and salvage of preformed pyrimidine bases and nucleosides, the parasite relies entirely on de novo synthesis [4,5]. *Plasmodium* dihydroorotate dehydrogenase (DHODH) is an essential mitochondrial enzyme that catalyzes the flavin mononucleotide-dependent formation of orotic acid, a key step in de novo pyrimidine biosynthesis [6,7]. The primary function of the mitochondrial electron transport chain in the parasite appears to provide oxidized ubiquinone (CoQ) as the physiological oxidant in the DHODH reaction, further demonstrating the importance of DHODH functions to parasite growth [8].

Quantitative structure–activity relationships are the most important applications of chemometrics, giving information useful for the design of new compounds acting on a specific target. QSAR attempts to find a consistent relationship between biological activity and molecular properties. Thus, QSAR models can be used to predict the activity of new compounds. Molecular docking is used to study how a ligand is interacting with its biological target and to further support the conclusions of QSAR studies [9]. Only a few groups of researchers have reported so far QSAR and docking studies of dihydroorotate dehydrogenase inhibitors [10,11].

In the present paper, QSAR and molecular docking studies have been performed on triazolopyrimidine-based dihydroorotate dehydrogenase (DHODH) inhibitors [12] to explore important molecular properties as well as the interaction patterns between the enzyme (i.e., DHODH) and ligands at the molecular level for design of new potent DHODH inhibitors. We have tried to justify the conclusions drawn from the QSAR study by docking analysis.

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2. Results and discussion

2.1. QSAR with physicochemical descriptors

Initially we have performed QSAR study with physicochemical descriptors (classical QSAR). Though both GFA and G/PLS techniques were tried, the latter generated better quality equations two of which are reported here.

Using the G/PLS spline technique, the following equation was obtained with acceptable cross-validated predictive variance (Q^2) and external predicted variance (R^2_{pred}).

$$\begin{aligned}\mathrm{pIC50} &= 7.95575 + 3.84989\langle \mathrm{B1_p} - 1.7\rangle - 1.28315 L_o \\ &\quad -0.99756\langle 0.633 - \mathrm{MR_p}\rangle + 0.744606\langle \mathrm{MR_m} - 0.605\rangle \\ &\quad -1.58856\langle 0.14 - \pi_m\rangle \\ n_{training} &= 22,\ R^2 = 0.872,\ R^2_a = 0.859,\ F = 64.87\ (\mathrm{df}\ 2,19), \\ s &= 0.266,\ \mathrm{PRESS} = 2.456,\ Q^2 = 0.767,\ r^2_{m(LOO)} = 0.740, \\ n_{Test} &= 7,\ R^2_{pred} = 0.767,\ r^2_{m(test)} = 0.719,\ r^2_{m(overall)} = 0.733. \end{aligned} \quad (1)$$

The above model could explain 85.9% of the variance (adjusted coefficient of variation). The cross-validated predictive variance (Q^2) was found to be 76.7%. The predictive ability of the model was evaluated by means of predictive R^2 (R^2_{pred}) for the test set compounds and the resulting R^2_{pred} value of 0.767 shows the good predictive ability of the model. Using the standardized variable matrix for regression, the significance level of the descriptors was found to be in the following order: $\mathrm{B1_p}$, L_o, $\mathrm{MR_p}$, $\mathrm{MR_m}$ and π_m.

The Sterimol width parameter B1 is defined as the smallest width along the Z axis. The positive regression coefficient of the spline term $\langle \mathrm{B1_p} - 1.7\rangle$ indicates that the numerical value of B1 of the *para* substituent of the phenyl ring should be greater than 1.7 for better DHODH inhibitory activity. It has been observed that presence of substitutions like CF_3 and Br at *para* position of the phenyl ring (e.g., **9** and **7**) leads to better DHODH inhibitory activity due to corresponding higher B1 values than compounds with substitutions like OCH_3 and OCF_3 (e.g., **10** and **11**).

The Sterimol length parameter L is defined as the maximum length along the X axis. The term L_o with negative regression coefficient indicates that to avoid detrimental interactions, the value of L_o should be less. L_o is the length of the substitutions at the *ortho* positions of the phenyl ring. It has been observed that compounds with fluoro substitution at the *ortho* position of the phenyl ring showed lower range of activity due to high L values than other compounds with unsubstituted *ortho* position (e.g., **16** vs. **1**; **19** vs. **8** etc.).

The negative regression coefficient of the spline term $\langle 0.633 - \mathrm{MR_p}\rangle$ indicates that molar refractivity has a negative impact in the *para* position of the phenyl ring when the value of MR is lower than 0.633. For example, compound nos. **13** and **24** having MR values higher than 0.633 have high DHODH inhibitory activity and compound nos. **5**, **17** and **18** having MR values lower than 0.633 have less DHODH inhibitory activity.

The positive regression coefficient of the spline term $\langle \mathrm{MR_m} - 0.605\rangle$ indicates that molar refractivity has a positive impact in the *meta* positions of the phenyl ring if the value of MR is higher than 0.605. For example, compound nos. **24** and **26** having MR values higher than 0.605 show high DHODH inhibitory activity and compound nos. **16**, **17** and **18** having MR values lower than 0.605 show less DHODH inhibitory activity.

The parameter π is the lipophilicity substitution constant which is a very important parameter in modeling studies. The spline term $\langle 0.14 - \pi_m\rangle$ has negative regression coefficient. The negative regression coefficient of the spline term indicates that the value of lipophilic substituent constant at the *meta* positions of the phenyl ring should be more than 0.14 for better DHODH inhibitory activity. Compounds with hydrophobic substituent (like CF_3 and CH_3) at the *meta* position of the phenyl ring show better inhibitory potency than compounds unsubstituted at *meta* positions (e.g., **23** vs. **6**; **26** vs.**8**, etc.). The observed and calculated/predicted values according to Eq. (1) are given in Table 1.

Another equation of good statistical quality was obtained using the G/PLS spline technique.

$$\begin{aligned}\mathrm{pIC50} &= 7.47868 + 2.75262\langle \mathrm{B1_p} - 1.7\rangle - 2.22133 \mathrm{B5_o} \\ &\quad -0.96816\langle 0.502 - \mathrm{MR_p}\rangle - 0.78873\langle 2.52 - \mathrm{B1_m}\rangle \\ &\quad -0.46286\langle 0.71 - \pi_p\rangle \\ n_{training} &= 22,\ R^2 = 0.849,\ R^2_a = 0.833,\ F = 53.50\ (\mathrm{df}\ 2,19), \\ s &= 0.289,\ \mathrm{PRESS} = 2.647,\ Q^2 = 0.749,\ r^2_{m(LOO)} = 0.711, \\ n_{Test} &= 7,\ R^2_{pred} = 0.824,\ r^2_{m(test)} = 0.788,\ r^2_{m(overall)} = 0.727. \end{aligned} \quad (2)$$

The above model could explain 83.3% of the variance (adjusted coefficient of variation). The cross-validated predictive variance (Q^2) was found to be 74.9%. The predictive ability of the model was evaluated by means of predictive R^2 (R^2_{pred}) for the test set compounds and the resulting R^2_{pred} value of 0.824 shows the good predictive ability of the model. Using the standardized variable matrix for regression, the significance level of the descriptors was found to be in the following order: $\mathrm{B1_p}$, $\mathrm{B5_o}$, $\mathrm{MR_p}$, $\mathrm{B1_m}$ and π_p.

The positive regression coefficient of the spline term $\langle \mathrm{B1_p} - 1.7\rangle$ indicates that the numerical value of B1 for the *para* substituent of the phenyl ring should be greater than 1.7 as discussed earlier in case of eq. (1).

The Sterimol width parameter B5 is defined as the maximum width (i.e. the maximum distance from X axis) of the substituent in the Z–Y plane (perpendicular to the X axis). The negative regression coefficient of the parameter B5 at the *ortho* positions indicates that it has negative impact to the DHODH inhibitory activity. It has been observed that a substitution like fluoro at *ortho* position of the phenyl ring leads to lower range of DHODH inhibitory activity due to its high B5 value in comparison to compounds with unsubstituted *ortho* positions (e.g., **16** vs. **1**; **19** vs. **8** etc.).

The negative regression coefficient of the spline term $\langle 0.502 - \mathrm{MR_p}\rangle$ indicates that molar refractivity has a negative impact in the *para* position of the phenyl ring when the value of MR is lower than 0.502. So the MR value should be more than 0.502 for better DHODH inhibitory activity. For example, compound nos. **7** and **24** having MR values higher than 0.502 show high DHODH inhibitory activity and compound nos. **5**, **16**, **17** and **18** having MR values lower than 0.502 show lower DHODH inhibitory activity.

The negative regression coefficient of the spline term $\langle 2.52 - \mathrm{B1_m}\rangle$ indicates that the numerical value of B1 of the *meta* substituents of the phenyl ring should be more than 2.52 for better DHODH inhibitory activity. It has been observed that presence of a CF_3 substitution at *meta* position of the phenyl ring (e.g., **23**, **24** and **25**) leads to better DHODH inhibitory activity due to the corresponding high value of B1 in comparison to other compounds without any substituent at *meta* position of the phenyl ring (e.g., **6**, **7** and **8**).

The spline term $\langle 0.71 - \pi_p\rangle$ has a negative regression coefficient. The negative regression coefficient of the spline term indicates that the value of lipophilic substituent constant for the *para* substituent of the phenyl ring should be more than 0.71 for better DHODH inhibitory activity. Compounds with hydrophobic substituents (like CF_3 and Br) at *para* position of the phenyl ring (e.g., **29** and **24**) show