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Synthesis, thymidine phosphorylase inhibition and molecular modeling studies of 1,3,4-oxadiazole-2-thione derivatives

Sohail Anjum Shahzad [a,*], Muhammad Yar [b,*], Marek Bajda [c], Lubna Shahzadi [b], Zulfiqar Ali Khan [d], Syed Ali Raza Naqvi [d], Sadaf Mutahir [e], Nasir Mahmood [f], Khalid Mohammed Khan [g]

[a] Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan
[b] Interdisciplinary Research Centre in Biomedical Materials, COMSATS Institute of Information Technology, Lahore 54000, Pakistan
[c] Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Cracow, Poland
[d] Department of Chemistry, Government College University, Faisalabad 38000, Pakistan
[e] Department of Chemistry, GC University, Lahore 54000, Pakistan
[f] Department of Allied Health Sciences and Chemical Pathology, Department of Human Genetics and Molecular Biology, University of Health Sciences, Lahore, Pakistan
[g] Hussain Ibrahim Jamal Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

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ABSTRACT

Thymidine phosphorylase (TP) inhibitors have attracted great attention due to their ability to suppress the tumors formation. In our ongoing research, a series of 1,3,4-oxadiazole-2-thione (**1**–**12**) has been synthesized under simple reaction conditions in good to excellent yields (86–98%) and their TP inhibition potential has also been evaluated. The majority of synthesized compounds showed moderate thymidine phosphorylase inhibitory activity with IC50 values ranging from 38.24 ± 1.28 to 258.43 ± 0.43 μM, and 7-deazaxanthine (7DX) was used as a reference compound (IC50 38.68 ± 4.42). The TP activity was very much dependent on the C-5 substituents; among this series the compound 6 bearing 4-hydroxyphenyl group was found to be the most active with IC50 38.24 ± 1.28 μM. Molecular docking studies revealed their binding mode.

1. Introduction

Although angiogenesis is a very critical process in a repairing of tissues and organs it is a highly undesirable phenomenon during the tumor formation. It is believed that, tumor growth could be blocked by stopping angiogenesis [1,2]. Regarding the mechanism of the action of thymidine phosphorylase (TP), it triggers the reversible phosphorolysis of thymidine to produce thymine and 2′-deoxy-D-ribose 1-phosphate [3,4]. Subsequently, 2′-deoxy-D-ribose 1-phosphate shows dephosphorylation reaction and as a result 2′-deoxy-D-ribose is produced. It is possible that the 2′-deoxy-D-ribose stimulates the production of vascular endothelial growth factor (VEGF), which initiates a number of processes, for example, invites endothelial cells for secretion of *matrix metalloproteinases*, proliferation, and also migration to tumor tissue. All these actions help in the formation of new blood vessels, which could cause cancer metastasis [5]. This is the reason why anti-angiogenic substances are highly desirable.

The 2′-deoxy-D-ribose is considered a valuable target to suppress the tumor growth, and TP inhibitors are able to reduce the production of 2′-deoxy-D-ribose [5,6]. In such scenario, one can easily understand the advantages of TP inhibitors in the control of cancer and that is the reason recently a number of efforts have been reported on the development of TP inhibitors [5,7,8]. One of the leading candidate in this field is the 5-chloro-6-[1-(2-iminopyrrolidinyl) methyl] uracil hydrochloride (TPI). It is a pyrimidine based compound and the most active human TP inhibitor, whereas, 7-deazaxanthine (7DX) is the first known TP inhibitor as shown in Fig. 1 [9–12].

Oxadiazole motif is well known due to its huge importance in medicinal chemistry [13–16]. We have recently identified and reported the TP inhibitory potential of 1,3,4-oxadiazole-2-thiones Mannich base derivatives [17], derived from the compounds listed in the current paper. There it was observed that the oxadiazole motif played a crucial role in the inhibitory process along with the contributions from the various substituents around the molecules. In that effort we synthesized the Mannich base derivatives of 1,3,4-oxadiazole-2-thiones, which indeed required an extra synthetic step and additional chemicals. As part of our ongoing medicinal chemistry interests [17–23], in current research, we

* Corresponding authors.
E-mail addresses: sashahzad@ciit.net.pk (S.A. Shahzad), drmyar@ciitlahore.edu.pk (M. Yar).

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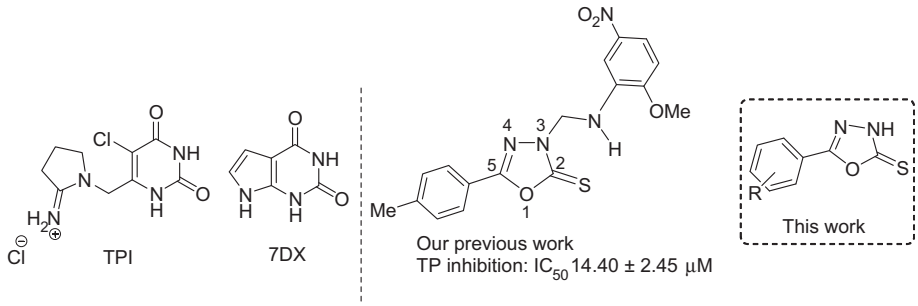


Fig. 1. Chemical structure of known TP inhibitors; TPI, 7DX, our previously reported potent TP inhibitor and newly proposed TP inhibitors.

have synthesized the simple 1,3,4-oxadiazole-2-thione derivatives [24,25] and analyzed their TP inhibition potential. We have also performed molecular modeling studies for all the synthesized compounds to rationalize their binding modes with the TP.

2. Results and discussion

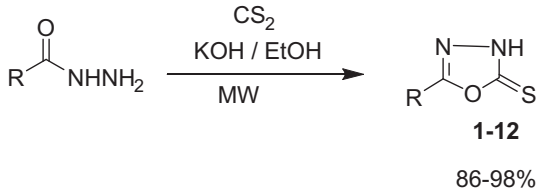
2.1. Chemistry

In our current research, a series of 1,3,4-oxadiazoline-2-thione derivatives **1**–**12** [17] bearing different level of C-5 substituents was prepared by condensing respective hydrazides with carbon disulfide in the presence of potassium hydroxide and ethanol on alumina as a solid support as shown in Scheme 1 [24]. Neutral alumina oxide was used as solid support which does not affect the yield and speed of reaction [25]. The reaction proceeded and completed efficiently under microwave irradiation within 7 min. The pure solid products were isolated as precipitates, which were washed with 50% aqueous ethanol and needed no further chromatographic techniques for purification. Also, it can be seen from percentage yield that different substituents influence the conversion rate and shorten the reaction time (please see Table 1). All final compounds were structurally characterized by IR, NMR, EIMS and elemental analysis.

2.2. Thymidine phosphorylase inhibition activities

In this study, to develop and understand structure–activity relationship (SAR) twelve derivatives of oxadiazole-2-thione were synthesized bearing different degree of aryl substituents at C-5 position. Among the tested compounds, compound **6** having 4-hydroxyphenyl at C-5 position was found to be the most active with an IC_{50} value 38.24 ± 1.28 μM (entry 6, Table 1), which was decreased to IC_{50} 68.37 ± 1.23 μM in the case of its analogue **2** having 2-hydroxyl group at C-5 (entry 2 and 6, Table 1). Similarly, compound **7**, having methoxy group at *para* position showed IC_{50} 72.43 ± 0.48 μM while its trimethoxy analogue **8** exhibited slightly enhanced enzyme inhibition activity (IC_{50} 63.43 ± 0.92), this is may be due to combined greater inductive effect by three methoxy substituents.

Again very interesting results have been seen on the basis of position of substituents. For example, compound **10** and **11**, they



Scheme 1. Synthetic protocol of 1,3,4-oxadiazole-2-thione derivatives **1**–**12**.

both contain chloro substituents in phenyl ring but IC_{50} value of compound **11** having chloro group at *para* position was 63.97 ± 0.73 μM, and the IC_{50} value dramatically increased to 258.43 ± 0.43 μM in case of compound **10** having chloro group at *meta* position. The results also revealed the electron-withdrawing polar group like nitro completely hinders the enzyme inhibition activity and resulted as precipitates.

2.3. Molecular modeling studies

Analysis of the binding mode for the novel derivatives **1**–**12** was performed according to the previously described method [17]. Thymidine phophorylase (TP) from *Escherichia coli* of high resolution (1.50 A; PDB code: 4EAD) was used for docking studies upon initial preparation. This enzyme structure represents the most closed form, characteristic for the most complexes with inhibitors, bound with 3′-azido-2′-fluoro-dideoxyuridine (ONP). Docking validation was based on two reference compounds from crystal structures – ONP and TPI (5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]-uracil) and it was shown that docking runs were able to reproduce original arrangement of the ligand with low rmsd (root mean square deviation) value below 1. Further, 7-deazaxanthine (7DX) – assay reference compound – was docked to TP. It was observed that NH and CO groups formed hydrogen bonds with Lys190, Ser186 and Arg171 while the whole molecule created π–π stacking with Tyr168 residue.

Initial calculations revealed that all compounds **1**–**12** occurred in physiological conditions in ionized form II (Fig. 2) and therefore such form was docked into thymidine phosphorylase. It was noted that the binding mode of novel compounds was highly dependent on the substituents in the phenyl ring (Fig. 3). In case of the most active compound **6**, phenyl ring created CH-π interactions with Phe210. The hydroxyl group in position 4 formed hydrogen bond with Arg171. The oxadiazole moiety was engaged in two hydrogen bonds. The oxygen atom interacted with water molecule while nitrogen atom in position 4 created H-bond with hydroxyl group of Tyr168. The orientation of the inactive compound **5** was reversed. The phenyl ring created π–π stacking with Tyr168 while the oxygen atom from oxadiazole ring formed hydrogen bond with Ser186. Comparing both compounds **5** and **6**, it is worth to note that inhibitor **6** provided more interactions of greater importance within active center than inactive derivative **5**. This could explain the observed differences in the activity. Moreover, other derivatives **1**–**4** and **7**–**12** presented variable binding mode. It seems that even the most potent compound **6** is not specific inhibitor of thymidine phosphorylase but it remains a good starting point for further optimization to obtain the highly active derivatives.

3. Conclusion

We synthesized 5-substituted-1,3,4-oxadiazole-2-thione derivatives via a procedure which is much more efficient and has