Bioorganic & Medicinal Chemistry 26 (2018) 3654–3663

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Rational design of *bis*-indolylmethane-oxadiazole hybrids as inhibitors of thymidine phosphorylase

Muhammad Taha[a,*], Umer Rashid[b], Syahrul Imran[c,d], Muhammad Ali[e]

[a] Department of Clinical Pharmacy, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam 31441, Saudi Arabia
[b] Department of Chemistry, COMSATS University Islamabad, Abbottbad Campus 22060, Pakistan
[c] Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA, Puncak Alam 40450, Malaysia
[d] Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam 42300, Malaysia
[e] UoN Chair of Oman's Medicinal Plants and Marine Natural Products, University of Nizwa, P.O. Box 33, Birkat Al Mauz, Nizwa 616, Oman

ARTICLE INFO

Keywords:
Human thymidine phosphorylase
Privileged scaffolds
Structure-based drug design
Virtual screening

ABSTRACT

Inhibition of Thymidine phosphorylase (TP) is continuously studied for the design and development of new drugs for the treatment of neoplastic diseases. As a part of our effort to identify TP inhibitors, we performed a structure-based virtual screening (SBVS) of our compound collection. Based on the insights gained from structures of virtual screening hits, a scaffold was designed using 1,3,4-oxadiazole as the basic structural feature and SAR studies were carried out for the optimization of this scaffold. Twenty-five novel *bis*-indole linked 1,3,4-oxadiazoles (**7**–**31**) were designed, synthesized and tested *in vitro* against *E. coli* TP (*Ec*TP). Compound **7** emerged as potent TP inhibitor with an IC_{50} value of 3.50 ± 0.01 μM. Docking studies were carried out using GOLD software on thymidine phosphorylase from human (*h*TP) and E. coli (*Ec*TP). Various hydrogen bonding, hydrophobic interactions, and π-π stacking were observed between designed molecules and the active site amino acid residues of the studied enzymes.

1. Introduction

Thymidine phosphorylase (TP, E.C 2.4.2.4), also known as gliostin and platelet-derived endothelial cell growth factor (PD-ECGF), was discovered in 1954.[1–3] It is a key enzyme and has both physiological and pathological functions in the body. It is involved in the composition and decomposition of pyrimidine nucleotides, angiogenesis and metastasis of colorectal carcinoma1.[3] In angiogenesis, new blood vessels formed during the growth of tumor. In tumor, it has been reported that TP is 10-times over expressed in several cancerous tissues especially in platelets, solid tumors, in renal carcinoma, breast, pancreatic and ovary. Moreover, it is also expressed in a number of chronic inflammatory diseases such as rheumatoid arthritis. Therefore, TP inhibition is continuously studied for the design and development of new drugs for the treatment of neoplastic as well as non-neoplastic diseases.[4–6]

There are some classical inhibitors used as benchmark for the design of new inhibitors. Among them, 6-aminothymine (**1**, 6AT) and 6-amino-5-bromouracil (**2**, 6A5BU) were the only TP inhibitors with IC_{50} value in nano-molar range. Other synthetic compounds belong to different groups; a rationally designed purine derivative 7-DX (**3**) had emerged as potent TP inhibitor. Following with the discovery of 7-DX, another purine derivative KIN59 (**4**) with a new mechanism of TP inhibition emerged. Two uracil derivatives **5** and **6** were identified as the most potent TP inhibitors with IC_{50} values of20nM and 35 nM respectively (Fig. 1).[3]

In computer aided drug designing and development (CADD), computers are used to study chemical and biological information of ligands and/or targets for identification and optimization of new drugs. These computational tools describe the strength of interaction between a variety of ligands and targets in amalgamation with good graphic 3D visualization and are growing into important technologies to select lead molecules from the data bases.[7] Virtual screening (VS) or *in silico* screening is an increasingly important component of the computer-based search for novel lead compounds. There are many tools available for *in silico* screening and generally they can be categorized as being either ligand-based or receptor-based.[8–9] Structure-based virtual screening is most commonly implemented as the prediction of binding modes and binding affinities of each compound in the dataset by means of high throughput docking to an X-ray structure or model of the target receptor. The popularity of the technique is due to its potential for identifying novel chemotypes outside the scope of known ligands.

* Corresponding author.
E-mail address: mtaha@iau.edu.sa (M. Taha).

https://doi.org/10.1016/j.bmc.2018.05.046
Received 22 February 2018; Received in revised form 29 April 2018; Accepted 26 May 2018
Available online 26 May 2018
0968-0896/

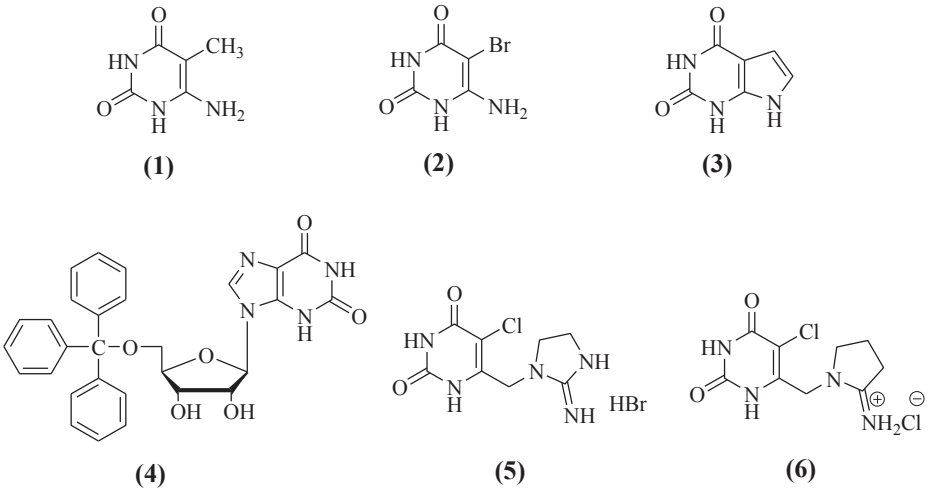


Fig. 1. Structures of some TPIs.

The experience achieved by our research group in the field of computer aided drug design and synthesis of chemical entities as putative drugs for the treatment of various diseases prompted us to further explore new inhibitors for thymidine phosphorylase.[10–14] Keeping in view the medicinal importance of TP inhibition, it was planned to identify novel TP inhibitors by means of various computational tools such as structure based virtual screening and molecular docking. Herein, we report in detail the rational design of thymidine phosphorylase inhibitors. Structure based virtual screening was carried out on the structure of human TP. The identification of hits was based on the binding modes, key interactions and GOLD score. The designed *bis*-indole linked 1,3,4-oxadiazoles (**7–31**) were synthesized and tested *in vitro* against *E. coli* TP (*Ec*TP).

2. Results and discussion

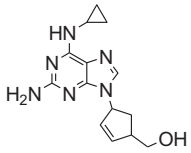
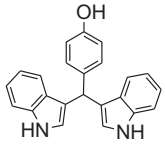
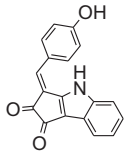
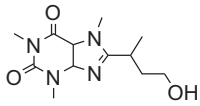
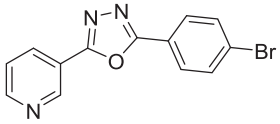
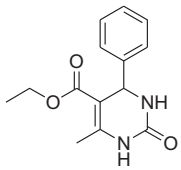
2.1. Designing rationale and methods

The structure-based design of the TP inhibitors was based on the three-dimensional (3-D) structure human TP (*h*TP) due to the conserved active site residues of *h*TP and *Ec*TP.[15–18] The three-dimensional (3-D) crystal strucure of human thymidine phosphorylase was obtained from Protein Data Bank (PDB) with code 2J0F. The active site consists of α-domain (pyrimidine binding site) and α/β domain (phosphate-binding site). The important hydrogen bond forming residue in α/β domain are Lys115, His116 and Ser117. While, Arg202, Ser217 and Ly221 are the important residues of α-domain.[17–18] His116 is a residue of key importance in Pyrimidine-nucleoside phosphorylases (PyNPs).[18] The residues of hinge region are: Leu148, Val208, Ile214 and Val241.[19]

Recently, we have developed a small in-house data base of 1000 molecular framework named as “Privileged scaffolds”. The members of this library were selected by identifying frameworks from bioactive natural products, marketed, investigational drugs and drug-like molecules. To begin with this small data base library and to identify new ligands as TP inhibitors, structure based virtual screening (SBVS) was carried out against *h*TP using GOLD docking program 5.4.1. Out of 1000 compounds, 497 compounds were found to have GOLD fitness score between 40 and 55. We only retained six compounds with GOLD fitness score value between 50 and 55. The key compound series identified from this process was based on the *bis*-indole, dihydropyrimidinone, purine, indole, and 1,3,4-oxadiazolescaffolds. These structures of newly identified scaffolds along with their therapeutic category and GOLD fitness score are given in Table 1.

In the next step, we proceeded to design a molecular framework of our new TP inhibitors. The initial task was to examine the binding orientations of the Hits (**32–37**) into the binding site of the enzyme. Except Hit-36, all the Hits identified were in close proximity to α/β-domain residues (His116 and Ser117). Interestingly, Hit-36 pointing toward thymine binding domain (α-domain) (Fig. 3a). It is interesting to note that 1,3,4-oxadiazole templates (**Hit-36,** Table 1) have been already reported as TP inhibitors (Fig. 2, **38–40**).[20–22] Therefore, we focus on an appropriate substitution point to attach at Hit-36 that can

Table 1
Structures and Gold fitness scores of initial hits.

No.	Compounds	Therapeutic Category	GOLD Fitness score
32		Antiviral	55.39
33		Anti-proliferative and apoptotic	55.08
34		Mitotic spindle poison (Anti-cancer)	54.33
35		Decongestant (Nasal)	52.70
36		Tyrosinase inhibitors	52.19
37		Antimitotic, Ca Channel Blocker	50.10