Journal of Molecular Structure 1143 (2017) 49–64

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

First report on the structural exploration and prediction of new BPTES analogs as glutaminase inhibitors

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ARTICLE INFO

Article history:
Received 4 January 2017
Received in revised form
7 April 2017
Accepted 8 April 2017
Available online 12 April 2017

Keywords:
Glutaminase
BPTES analog
ANN
SVM
HQSAR
Topomer CoMFA

ABSTRACT

Glutaminase is one of the important key enzymes regulating cellular metabolism, growth, and proliferation in cancer. Therefore, it is being explored as a crucial target regarding anticancer drug design and development. However, none of the potent and selective glutaminase inhibitors is available in the market though two prototype glutaminase inhibitors are reported namely DON as well as BPTES. Due to severe toxicity in clinical trials, the use of DON is restricted. However, BPTES is an allosteric glutaminase inhibitor with less toxic profile and, therefore, lead optimization of BPTES may be a good option to develop newer drug candidates. In this study, a multi-QSAR modeling is carried out on a series of BPTES analogs. A significant connection between different descriptors and the glutaminase inhibitory activities is noticed by employing multiple linear regression, artificial neural network and support vector machine techniques. The classification-based QSAR such as linear discriminant analysis and Bayesian classification modeling are also performed to search important molecular fingerprints or substructures that may help in classifying the probability of finding 'active' and 'inactive' BPTES analogs. Moreover, HQSAR and Topomer CoMFA analyses are also performed. In addition, the SAR observations are interpreted with all these validated computational models along with the structure-based contours. Finally, new twenty two compounds are designed and predicted for their probable glutaminase inhibitory activity.

1. Introduction

Profound metabolic change is an interesting hallmark of a number of tumor cells. These metabolic changes may occur due to overexpression or suppression of oncogenes including *MYC*, *PI3K*, *RAS*, *LKB1*, *p53*, *VHL* [1,2]. The proliferative cells may also display an elevated rate of glutamine catabolism to form ATP and lactate. This process is known as glutaminolysis [3] (Fig. 1).

To exhibit glutaminolysis, transformed cell depends upon glutamine. Several pathways including nucleotide synthesis, oxidation for the production of ATP in Krebs cycle as well as to serve as lipogenic and gluconeogenic precursor, glutamine is found to play a pivotal role. It is the most abundant amino acid in human system [4,5]. Glutamine is converted to glutamate by releasing the γ -amino group. Glutaminase is the key enzyme solely responsible for glutamine metabolism [6,7]. In human, three types of glutaminase enzymes are identified: (i) kidney-type glutaminase (KGA) which is predominantly expressed in kidney and brain, (ii) liver-type glutaminase (GLS2) which is highly expressed in extra-hepatic tissues and (iii) a truncated and non-catalytically competent splice variant of *KGA* gene glutaminase C (GAC) [3,6]. The KGA and GAC alleles are generally referred as GLS. Over expressive *GLS* gene is found in many tumor cell lines and primary tumors whereas *GLS2* gene is not widely expressed in tumor cell lines. The enzyme GLS is positively involved in the transformation of NIH 3T3 fibroblasts by Rho GTPases and may help in the Rho GTPase-mediated controlling of cytoskeleton and cell division [7]. As GLS has proliferative and invasive activities in cancer cells, it is given crucial attention for anticancer drug research.

Intense efforts are being taken into consideration to develop the inhibitor of GLS enzyme (Fig. 1) such as 6-diazo-5-oxo-L-norlucine

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http://dx.doi.org/10.1016/j.molstruc.2017.04.020
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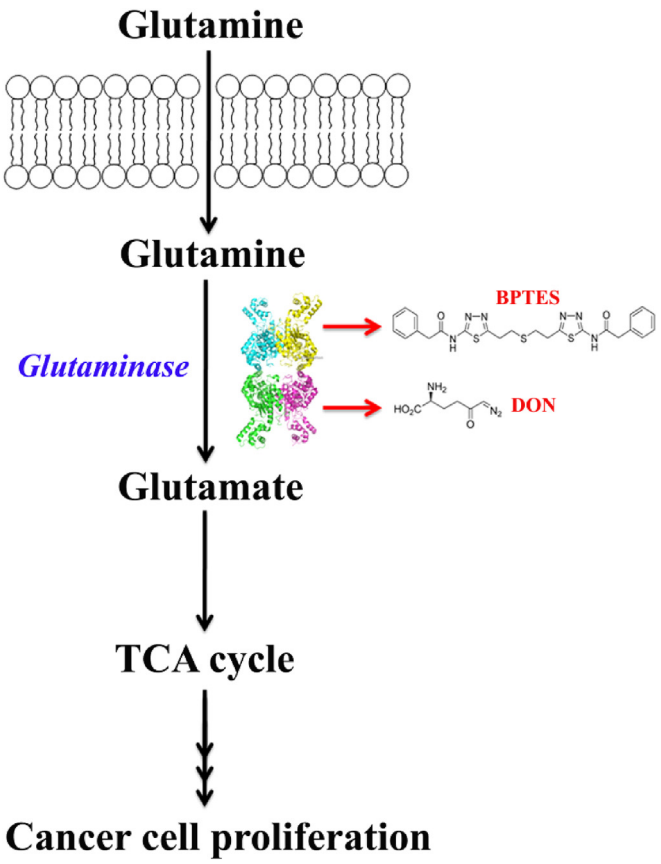


Fig. 1. Schematic model of the glutaminolysis (BPTES: Bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide; DON: 6-diazo-5-oxo-L-norlucine).

(DON) which has structural similarity with glutamine and it successfully inhibits the kidney-type glutaminase through binding to the enzyme active site [4–7]. However, due to nonspecific and toxic issues, DON fails to come out as a potent anticancer drug [8]. Nevertheless, benzophenanthridinone 968 is also claimed to inhibit this enzyme and BPTES [bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide] has been identified as an allosteric inhibitor of glutaminase [9,10]. During 2011, the first full-length crystal structure of GAC protein in the presence and absence of BPTES molecule was reported [4]. It was observed that the GAC protein comprises four tetramers. Interestingly, two BPTES molecules are found to interact with the GAC protein at the interface area [4]. BPTES is also reported to bind at the allosteric pocket of the dimeric interface of glutaminase involving a dramatic conformational change in the loop (from Glu312 to Pro329) near to the catalytic site [6].

A renewed interest in the glutamine metabolism as a potential target for anticancer drug discovery may attract not only the synthetic and computational chemists but also the biologists to find new BPTES analogs with improved activity profile. In this regard, integrated multi-QSAR chemometric modeling approaches (such as regression-based linear and non-linear QSARs, classification-based QSARs, hologram-based QSAR and three-dimensional topomer CoMFA technique) were performed to correlate the structural and physicochemical properties of forty BPTES analogs with their glutaminase inhibitory activity. Depending on the important features, twenty two new molecules were designed and predicted for their biological potency as per chemometric models.

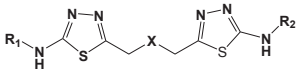
2. Materials and methods

2.1. Data preparation

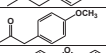
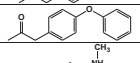
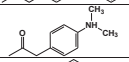
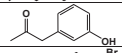
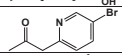
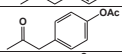
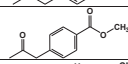
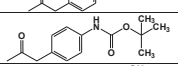
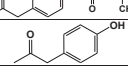
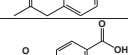
A dataset containing forty novel bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulphide (BPTES) analogs were collected from the published work [11,12] to perform the molecular modeling studies. The BPTES analogs include aliphatic and aromatic hydrocarbons, heteroaromatic, amide, trifluoromethyl, ester, keto, sulphonyl compounds, etc. (Table 1).

The GLS inhibitory activities [IC_{50} (μM)] were converted into the logarithmic scale [$pIC_{50} = -\log (IC_{50}/10^6)$] prior to model development and considered as the dependent variable [13–15]. The

Table 1
The kidney-type glutaminase (GLS) inhibitory activity of BPTES analoges.



Compound **1 – 11, 21 – 40**

Cpd[a]	R_1	X	R_2	IC_{50} (μM)
1		$H_2C—S—CH_2$		3.3
2	H—	$H_2C—S—CH_2$	—H	100.0
3		$H_2C—S—CH_2$	—H	2.7
4		$H_2C—\overset{O}{\overset{\|}{S}}—CH_2$	—H	61.0
5		CH_2	—H	15.0
6		$H_2C—CH_2$	—H	1.9
7		$H_2C—CH_2$	—H	7.2
8		$H_2C—CH_2$	—H	22.0
9		$H_2C—CH_2$	—H	4.5
10		$H_2C—CH_2$	—H	2.6
11		$H_2C—CH_2$	—H	12.0
21		$H_2C—CH_2$		1.8
22		$H_2C—CH_2$		0.21
23		$H_2C—CH_2$		0.38
24		$H_2C—CH_2$		3.0
25		$H_2C—CH_2$		0.12
26		$H_2C—CH_2$		0.17
27		$H_2C—CH_2$		0.07
28		$H_2C—CH_2$		0.07
29		$H_2C—CH_2$		0.54
30		$H_2C—CH_2$		13.0
31		$H_2C—CH_2$		0.45
32		$H_2C—CH_2$		0.07
33		$H_2C—CH_2$		0.11
34		$H_2C—CH_2$		2.3
35		$H_2C—CH_2$		1.2