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Design and evaluation of novel glutaminase inhibitors

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ABSTRACT

A novel set of GAC (kidney glutaminase isoform C) inhibitors able to inhibit the enzymatic activity of GAC and the growth of the triple negative MDA-MB-231 breast cancer cells with low nanomolar potency is described. Compounds in this series have a reduced number of rotatable bonds, improved *C*log*P*s, microsomal stability and ligand efficiency when compared to the leading GAC inhibitors BPTES and CB-839. Property improvements were achieved by the replacement of the flexible *n*-diethylthio or the *n*-butyl moiety present in the leading inhibitors by heteroatom substituted heterocycloalkanes.

1. Introduction

In 1955, Eagle, in his paper 'Nutrition needs of mammalian cultured cells' reported that glutamine, a non-essential amino acid, is quite essential for the growth of tumor cells in culture media.[1] Since that report, research in tumor cell metabolism has revealed that high glutamine utilization and dependence, a property termed 'glutamine addiction', is a key attribute for a number of tumor cell lines.[2–6]

The key step in glutamine processing in mitochondria is the hydrolysis of the glutamine amide group by the enzyme glutaminase. There are two main glutaminase isoforms, the kidney isoform (KGA or GLS1) and the liver isoform (LGA or GLS2). Starting with the 1969 paper of Knox et al., where it was shown that kidney glutaminase activity is proportional to the growth rate of tumors in rats, the evidence accumulated over the years point to the fact that KGA and its splice variant kidney glutaminase isoform C (GAC) particularly, is a target of interest for cancer therapy.[7–9] GAC upregulation is present in multiple cancer cell lines, correlates with increased proliferative rates, and it is linked to the dysregulation of a number of pathways, including the dysregulation/amplification of the Myc oncogene.[10–13]

Inhibition of GAC through antisense, siRNA and/or synthetic molecules like BPTES, CB-839 and compound 968 leads to reduction of tumor cell proliferation in vitro and in vivo and suggests that glutaminase is a valid anticancer target.[14–18]

Among the selective small molecule GAC inhibitors, BPTES and CB-839 are the most similar, having as a key feature the presence of a lipophilic connecting chain (diethylthio in BPTES and *n*-butyl in CB-839) between two heterocyclic aromatic moieties (Fig. 1). Being straight and lipophilic these chains contribute to the relatively high *C*log*P*s and the high number of rotatable bonds (NRB) of these compounds (BPTES: *C*log*P* = 4.15, NRB = 12; CB-839: *C*log*P* = 4.74, NRB = 13). Herein we describe a novel and potent set of inhibitors with NRB values within the generally accepted drug-like range (⩽10),[19] improved ligand efficiency (LE),[20] lipophilic efficiency (LiPE/LLE)[21] and/or *C*log*P*s when compared to the leading inhibitors.

2. Results and discussion

2.1. Design principles for new compounds

Catalytically active GAC units are tetrameric and recent evidence suggests that in cells GAC may in fact operate as an oligomer of tetramers.[22]

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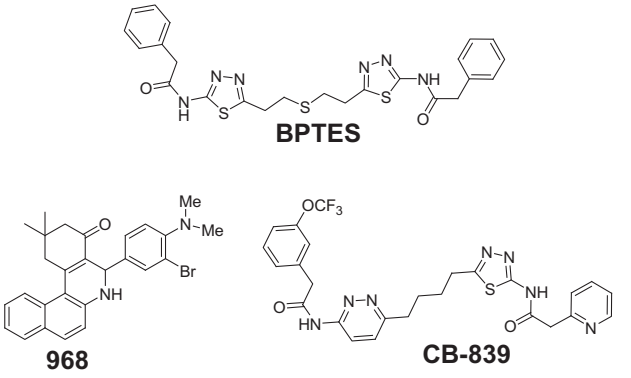

Figure 1. GAC inhibitors.

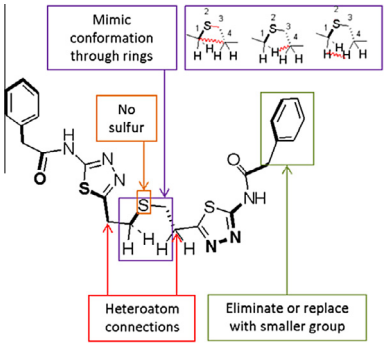

Figure 3. Design principles for new GAC inhibitors.

With respect to structural information there are three crystal structures of human GAC in complex with BPTES in the Protein Data Bank (PDB), namely structures 3UO9, 3VOZ and 3VP1.[23,24] These structures show that BPTES binds in a stoichiometry of 2 molecules of inhibitor per GAC tetramer and at an allosteric pocket, that is, formed at the interface between GAC dimers (Fig. 2).

Looking at the available BPTES/GAC crystal structures and particularly the bent conformation assumed by the thiadiazole-connecting diethylthio chain, it became apparent to us that this flexible connector could be replaced by small to medium size ring systems (Fig. 3). Morphing this diethylthio chain connector into a cyclic structure would be highly beneficial as it would result in inhibitors with reduced number of rotatable bonds, a property inversely related to the probability of good absorption.[19] An added benefit of this decrease in rotatable bonds would be a reduction in the entropic energy penalty for binding, that is, inherently higher in molecules with a high number of rotatable bonds, and as such it could lead to greater potency inhibitors.[25]

As a means of maintaining the log*P* as low as possible, we envisioned the use of saturated ring systems that contained other-than-sulfur heteroatoms as surrogates for the conformation assumed by the BPTES flexible chain. Ease of synthesis considerations and our desire to have more than one heteroatom present on the small to medium size ring systems that would not clash with the walls of the binding pocket suggested to us that heteroatom substituents on prospective saturated ring systems should serve as connectors between the BPTES thiadiazoles and/or their isosters, and not as stand-alone substituents. In that regard, non-sulfur-containing ring systems such as 4-hyrdoxypiperidine, 4-aminopiperidine, 3-amino azetidine, etc. appeared as very suitable heteroatom containing rigid surrogates for the flexible connector chains of BPTES/CB-839.

B-factors in the 3UO9 X-ray structure suggest that one of the BPTES phenyls is particularly flexible/mobile (Fig. 2c).[23] This suggests that this phenyl moiety most likely does not contribute significantly to binding. As such, this phenyl group and possibly the whole phenylacetic acid moiety in that part of the molecule, could

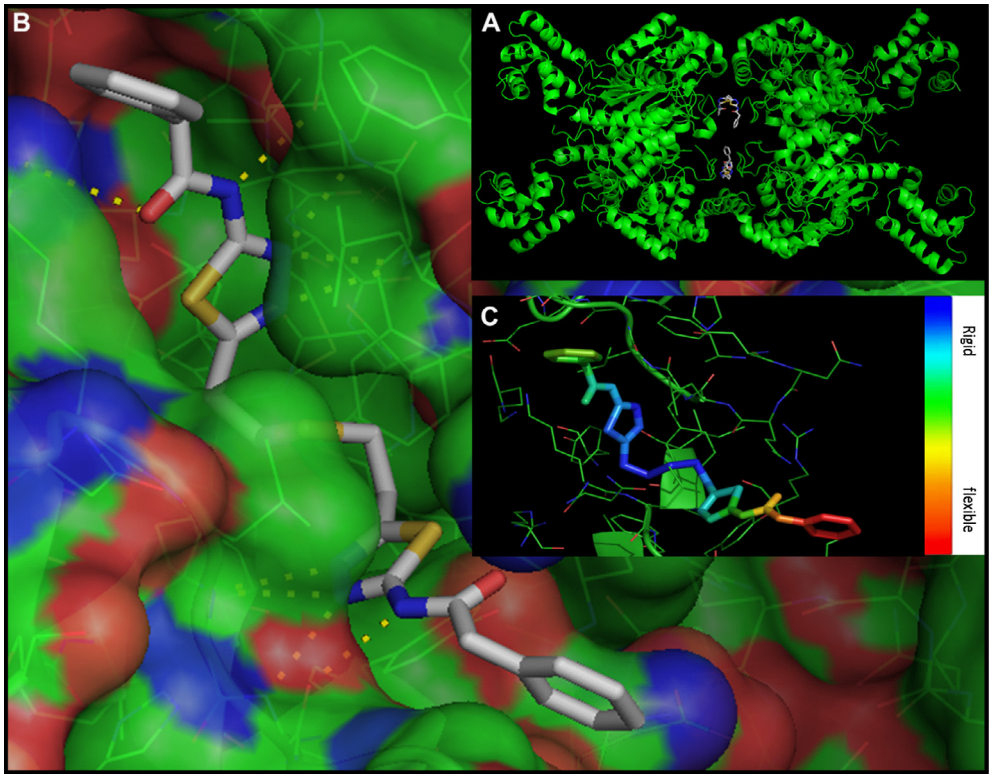

Figure 2. (A and B) Binding of BPTES to glutaminase as appears in the 3UO9 X-ray structure. (C) Heat map of B-factors for the BPTES atoms in the 3UO9 structure.