Journal of Molecular Graphics and Modelling 76 (2017) 364–378

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

Investigation of novel pharmacological chaperones for Gaucher Disease

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ARTICLE INFO

Article history:
Received 28 March 2017
Received in revised form 16 July 2017
Accepted 17 July 2017
Available online 20 July 2017

Keywords:
GBA
Gaucher disease
Molecular docking
Substructure search
Molecular dynamics
Blood brain barrier
Pharmacological chaperones

ABSTRACT

Beta-Glucocerebrosidase (GBA) is a lysosomal protein that is responsible for the hydrolysis of glycosylceramide into glucose and ceramide. Mutations in GBA lead to the accumulation of glycosylceramide in the lysosome causing an enlargement of the spleen and the liver and skeletal deformations. This disease is called Gaucher Disease. Enzyme replacement therapies and substrate reduction methods that are used to treat Gaucher Disease fail when the disease is neuropathic because they fail to pass the blood brain barrier. In this work, QSAR, virtual screening, docking and molecular dynamics simulations were performed to obtain a set of compounds that might be pharmacological chaperones for GBA. ZINC Database was screened using ligand-based and structure-based pharmacophore hypotheses. After docking of these molecules and filtration based on druglikeness, top ranking ligands were identified and their binding stabilities were examined using MD simulations. As a result, seven new compounds that can potentially cross the blood brain barrier were proposed as GBA inhibitors. Three of the seven compounds have a tricyclic pyrido-thieno-pyrimidine scaffold and one has the dioxino quinolone scaffold. Derivatives of these scaffolds have been reported as antiallergic agents, antibiotic and anticancer compounds. These results offer a new approach for the development of new drugs against neuropathic Gaucher Disease Type 2 and Type 3.

1. Introduction

Beta-glucocerebrosidase (GBA) protein catalyzes the breakdown of sphingolipid glycosylceramide into ceramide and glucose [1]. Mutations in GBA protein lead to accumulation of glycosylceramide in the lysosome, and consequently to Gaucher Disease (GD) resulting in an increase in spleen and liver volumes and skeletal deformations [2]. Out of 250 GD associated mutations of GBA, 203 of them are missense mutations, which influence the correct folding of GBA protein [3]. N370S is the most prevalent mutation in type 1 GD, and L444P, G202R, 84GG, F213I, R496H, V15M, S364R and G377S are other common mutations. L444P is a severe mutation that leads to neuropathic GD, which is very difficult to treat. GBA works with the cofactor Saposin C (SapC) in the degradation of glycosylceramide [4] and functional impairment of SapC is also linked with a rare variant form of GD [5]. Prevalence of GBA mutant forms implicated in GD makes GBA a key target in the drug design and discovery studies against GD.

To date, there are two therapeutic options for GD, both of which aim to prevent the accumulation of glycosylceramide; Enzyme Replacement Therapy (ERT) and Substrate Reduction Therapy (SRT). ERT is an intravenous therapy, which is based on increasing the GBA level in the Gaucher patient by administering recombinant GBA, imiglucerase, which breaks down glycosylceramide. However, the method is ineffective in neurological GD because the recombinant protein is too large to cross the blood brain barrier (BBB) [1], it is expensive and requires bi-weekly infusion of recombinant GBA. SRT aims to reduce the level of glycosylceramide by inhibiting glucosylceramide synthase, which catalyzes the synthesis of glycosylceramide. Treatment with iminosugars, such as Miglustat (*N*-(*n*-butyl) deoxynojirimycin, NB-DNJ) can reduce mean liver and spleen volumes by 12% and 19%, respectively, but patients suffer from low selectivity and off target effects such as gastrointestinal problems [6]. Unfortunately, ERT and SRT are effective only for nonneuropathic GD, and their biodistribution is very unfavorable leading to many side effects [2].

One novel therapeutic alternative to ERT and SRT is the use of pharmacological chaperones (PCs) that may facilitate folding of GBA. A PC is a small molecule that binds to target protein in the endoplasmic reticulum and stabilizes the correct fold of the protein and allows the protein to traffic through the pathway to lysosome [7,8]. Most PCs proposed for lysosomal storage disease

Abbreviations: GBA, Glucocerebrosidase; MD, Molecular Dynamics; GD, Gaucher Disease; PC, Pharmacological Chaperone; SP, Standard Precision; XP, Extra Precision.

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http://dx.doi.org/10.1016/j.jmgm.2017.07.014
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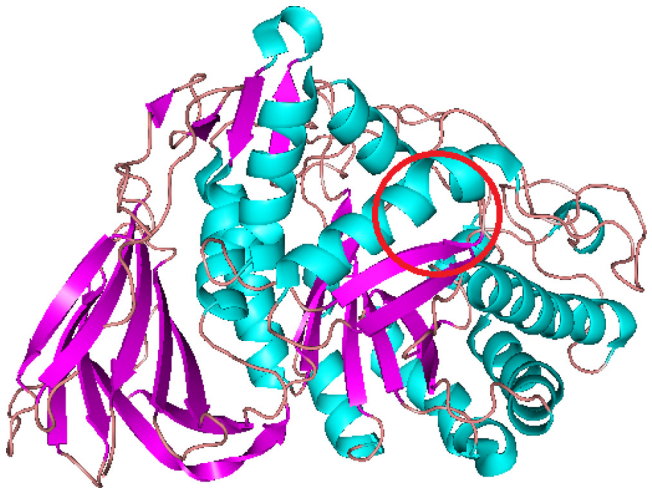

Fig. 1. The structure of GBA, the active site is shown with red circle (Structure figures are prepared using Pymol). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

treatment are usually natural ligand mimetics that bind to the active site in the ER and then are released in the low acidic environment of the lysosome [7,9–11]. Because of their similarity to the natural ligand, carbohydrate mimetics (iminosugars, carbasugars, azasugars etc.) and non-carbohydrate compounds (Ambroxol, Diltiazem) have been proposed as PCs [2]. Miglustat (NB-DNJ), which is an inhibitor of glucosylceramide synthase, also functions as a pharmacological chaperone for GBA. The activity of wild type GBA and the S364R, N370S, V15M and M123T mutants was found to increase upon incubation with 10 μM Miglustat. Another effective iminosugar derived PC is *N*-(*n*-nonyl) deoxynojirimycin (NN-DNJ). Treatment of N370S Gacuher fibroblasts with 10 μM of NNDNJ resulted in 2-fold increase in GBA activity [12] and treatment of N188S Gaucher fibroblasts with 5 μM NNDNJ fibroblasts resulted in 20% increase in GBA activity [13] while the L444P mutant was not affected by treatment with NNDNJ [12]. Crystallographic studies on the interaction between GBA and NB-DNJ and NN-DNJ (Fig. 1) showed that both inhibitors bind to the active site of GBA by hydrogen bonding via the iminosugar moiety as well as by hydrophobic interactions with the alkyl chain of iminosugars [14]. The azosugar mimetic, isofagomine also formed hydrogen bonds with GBA active site and increased the stability of protein in wild-type cells as well as in pre-treated N370S/N370S and F213/L444P patient fibroblasts [15,16] while incubation of 30 μM *N*-Octyl-Beta-Valienamine, a carbomimetic carbasugar, enhanced the activity of F213I/F213I mutant of GBA 6-fold [17]. ε-Glucoimidazole derivatives were more effective than iminosugar in enhancing the activity of N370S mutants by 2.4 fold [18]. C-2 derivatives of glucoimidazole enhanced the activity of N370S mutant, and provided 2- to 30 fold better inhibition of imiglucerase (recombinant, wild type GBA) than NN-DNJ [19]. Aminocyclitols mimicking carbohydrate were highly active as chaperones in the treatment of Gaucher patients with the G202R mutation [13] and in enhancing GBA activity of the L444P variant at nanomolar or subnanomolar concentrations [20]. Docking studies with aminocyclitols derivatives showed that the cyclitol hydroxyls interacted with the active site residues Asp127, Trp179, Glu235, Trp381, and Asn396 [21]. Fluorous iminoalditol forms ionic bonds with carboxyl groups in the GBA active site and the oligofluoro moiety increased the hydrophobicity and activity or selectivity of GBA more than simple lipophilic *N*-alkyl derivatives [22]. A computational study [3] showed that binding of Ambroxol stabilized residues 243–249, 310–312, and 386–400 near the GBA active site. Treatment with 10 μM of Ambroxol enhanced both GBA activity and protein level of the F213I/L444P mutant respectively, but it was not effective for L444P/L444P mutation [3] and Ambroxol failed in clinical trials. Pyrrolidine-based iminosugars were studied both experimentally and computationally [23]. DAB with octyl chain inhibited GBA, and as the alkyl chain length increased, the activity of GBA increased [23]. Nitrogen and the hydroxyl groups of α-1-C-tridecyl-DAB formed hydrogen bonding with the substrate binding residues, Glu235, Glu340, Asp127 and Trp179 [23].

Even though several different classes of compounds have shown potential as PCs, their inability to pass the BBB prohibits their use in the neuropathic type 2 and type 3 GD. In this work, our aim was to identify novel ligands with the potential of crossing the BBB using the methodology developed previously by our group for drug discovery [24]. Ligand-based and structure-based pharmacophore modeling methods were used to obtain pharmacophore hypotheses. Then ZINC database was screened using these hypotheses in order to obtain a set of putative druglike compounds. After strain energy correction and GScore filtration, surviving molecules were clustered and the common scaffolds were used for substructure search to rescreen the ZINC database. The identified compounds were pooled together and docked to the protein. After post-docking analyses, the surviving molecules were redocked flexibly to the GBA active site. Then, promising molecules were examined by molecular dynamics simulations in order to assess the dynamic interactions and the stability of protein-ligand complex. The seven compounds identified through this detailed drug discovery campaign can serve as leads both due to their high affinity, as predicted by free energy calculations, and due to their potential to cross BBB.

2. Methods

Schrödinger Suite [25] was utilized to perform docking and molecular dynamics simulations. The flowchart of the present study for the discovery of new pharmacological chaperones is shown in Fig. 2.

2.1. Pharmacophore models

Structure-based and ligand-based pharmacophore modeling methods were used.

2.1.1. Ligand based pharmacophore modeling

Experimentally known 182 different inhibitors of GBA were retrieved from the CHEMBL database [26]. These compounds belonged to families such as iminosugars, isofagomine and had IC50 values lower than 500 mM [26]. These molecules were clustered based on their structural similarity via Hierarchical Clustering tool in Chemmine [27]. 30 Pyrrolidine-based compounds were used to generate pharmacophore hypotheses of three to five sites via Phase [28–30]. LigPrep was utilized to generate 3D structures of ligands from 2D structures [31]. Ionization states of ligands were determined at pH = 7 and maximum 32 stereoisomers of each ligand were generated. Compounds were energy minimized and maximum 100 different conformations of each ligand were selected using ConfGen [32,33].

2.1.2. Structure-based pharmacophore modeling

Crystal structure of GBA bound to NN-DNJ (PDB Code: 2V3E) [14] was used to prepare the protein structure with Protein Preparation tool of Maestro [34,35]. Missing side chains and missing loops (Chain A residues: 31, 498, 499, 500, 501, 502 and 503) were built. All water molecules except the molecules near Glu235 (seven water molecules) were deleted because water molecules near this residue are important for inhibitor binding [14]. The protein was optimized and minimized by allowing the RMSD of heavy atoms to be 0.30 Å using the OPLS 2005 Force field. Grid Generation was performed with Glide [36–38], and the center of the grid was set