Discovery of Potential Sorbitol Dehydrogenase Inhibitors from Virtual Screening

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Abstract: Sorbitol dehydrogenase (SDH) is the second enzyme in the polyol pathway of glucose metabolism and is a possible target for the treatment of the complications of diabetes. In this study the molecular modelling program DOCK was used to analyse 249,071 compounds from the National Cancer Institute Database and predict those with high affinity for SDH. From a total of 21 tested the 7 compounds including flavin adenine dinucleotide disodium hydrate, (+)-Amethopterin, 3-hydroxy-2-napthoic(2-hydroxybenzylidene) hydrazide, folic acid, N-2,4-dinitrophenyl-L-cysteic acid, Vanillin azine and 1H-indole-2,3-dione,5-bromo-6-nitro-1-(2,3,4-tri-O-acetyl-α-L-arabinopyranosyl)-(9Cl), were shown to inhibit SDH and displayed IC_{50} values of 0.192 μM, 1.1 μM, 1.2 μM, 4.5 μM, 5.3 μM, 7 μM and 28 μM, respectively. These compounds may aid the design of pharmaceutical agents for the treatment of diabetes complications.

INTRODUCTION

Sorbitol dehydrogenase (SDH) is expressed in all mammalian tissues, including the brain, lens, erythrocytes and liver [1,2]. The enzyme has attracted considerable interest owing to its implication in the development of diabetic complications such as cataracts, neuropathy, retinopathy and nephropathy [3]. The structure of human SDH with and without the bound inhibitor 2-hydroxymethyl-4-(4′-*N*,*N*-dimethylaminosulfonyl-1-piperazino) pyrimidine (SDI-158) has been recently published [4]. The overall structure was found to be similar to those of rat SDH [5], NADPH-dependant whitefly ketose reductase [6], and human liver ADH [7]. The catalytic zinc atom was coordinated by His69, Cys44, Glu70 and a water molecule (tetra-coordination) in both the apo- and holoenzyme structures. In the ternary structure, penta-coordination of the zinc occurred with simultaneous interactions with the N1 nitrogen and O30 oxygen of SDI-158 and dissociation of Glu70.

Prior to the year 2000, only one known *in vivo* prototype SDH inhibitor (SDI), SDI-158, was reported in the literature. Since then the synthesis of pyridine derivatives, which had a 4′-*N*,*N*-dimethylaminosulfonyl-1-piperazino group at the 4-position and various functionalities, including hydrogen, CH_2OH, CHO, $CONH_2$ and CN, at the 2-position were reported [8]. The inhibitory activities of these derivatives were investigated to determine their potential as novel SDIs. The pyridine group was selected as a template because of its similarity to the pyrimidine group in SDI-158 and the various functionalities at 2-position of the pyridine ring were then used to establish structure-activity relationships. However, when tested against SDH, these compounds were shown to be less effective inhibitors than SDI-158, hence it was concluded that the pyrimidine moiety is important for the active site interactions. New SDH inhibitors were synthesised by replacing the dimethylaminosulfonyl group in SDI-158 with a variety of heterocycles and found to inhibit SDH with the most potent IC_{50} value equal to 10 nM [9]. Based on these findings, the dimethylaminosulfonyl group was targeted and an SDI with an IC_{50} value of 4 nM that has very good drug-like properties, including a long plasma half-life, was recently reported [10].

The program DOCK (version 4.0) [11] is used to orient small molecules into potential binding sites of proteins by matching the receptor pocket to the ligand atoms or surfaces. An energy score is given based on the complementarity between the ligand and the receptor, i.e. geometry as well as overall chemical complementarity and steric fit. The docking methodology used in this study has been described in detail in the literature [12,13]. In this case, the crystal structure of the human SDH holoenzyme [4] together with the DOCK program were used to search the NCI database [14] for possible compounds that may inhibit SDH or serve as new templates for further inhibitor development.

NCI DATABASE SEARCH

The coordinates of the human SDH/NAD^+ complex (PDB code: 1PL8) and the NCI database (249,071 compounds) were used to search for potential inhibitors or drug templates of SDH. The DOCK program [11] read 234,244 compounds, of which 230,747 compounds were docked successfully. A total number of 3,497 compounds were skipped due to the chemical and physical filters utilised by the program. This includes ignoring compounds that contain heavy atoms such as Zn, Al, Au or Ag or compounds that had too many or incorrectly assigned rotatable bonds. 15,756 compounds (6.3% of the database) were found to be chemically toxic (i.e. containing atoms such as Pt, Hg, Pu, Cr or Ir) and were also excluded from the database. Hydrogen atoms, partial

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1573-4064/06 $50.00+.00

charges, atomic potentials and bond orders for the complexes were assigned using the automatic procedures within the InsightII 2.1 package (Biosym Technologies Inc., San Diego, CA). Arginine, lysine, aspartate and glutamate amino acids were charged while the histidines were uncharged, with hydrogen atoms fixed at the Nε2. The Zn atom present in the active site of SDH was charged (2+). Using the program Concord the compounds were downloaded from the NCI database without altering their ionization states. Based on the SDH/SDI structure, a box was used to define the active site and included the Zn atom and Zn binding ligands. The docked compounds were restricted to the specified box. The top-ranked 3,000 compounds were checked for commercial availability using the NCI database website (www.cactus.nci.nih.gov) and only those compounds that were available were analysed thoroughly for their interactions with the active-site residues and Zn atom using the molecular modelling program InsightII (Biosym Technologies, San Diego, CA, USA). Compounds that had good interactions were then chosen for IC_{50} measurements as described below.

SDH EXPRESSION AND PURIFICATION

The coding region of human SDH (SORD) was isolated from the liver cDNA library, inserted into a prokaryotic expression vector (pET23 (+) Novagen, Madison, WI, USA) and transformed into *E. coli* BL21 (DE3) (Novagen) [15]. Briefly, SDH was purified from the supernatant by ammonium sulfate precipitation, anion exchange chromatography and affinity chromatography following established procedures [16]. The concentration, purity and enzymatic activity of SDH were examined at each step.

INHIBITORY ASSAY FOR SDH

Enzyme activity was determined using a Shimadzu UV-vis spectrophotometer (model UV160A) by following the increase in absorbance of NADH at 340 nm. SDH was tested for compound inhibition using compound stocks made up in 50% DMSO. The reaction was carried out with a 1 ml assay sample containing 42 mM glycine buffer pH 9.9, 9.9 mM D-sorbitol and 0.5 mM β-NAD^+ and different concentrations of compounds. Initially, the buffer and water were equilibrated to a constant temperature of 25° C in a hot water bath and a final concentration of DMSO in the assay not exceeding 2% was used. The reaction was commenced by addition of the substrate. One milli-unit (mU) of activity was defined as the amount of enzyme needed to oxidise 1 milli-mole of substrate per minute under initial velocity conditions at room temperature (20° C).

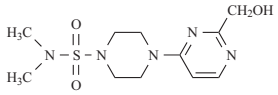
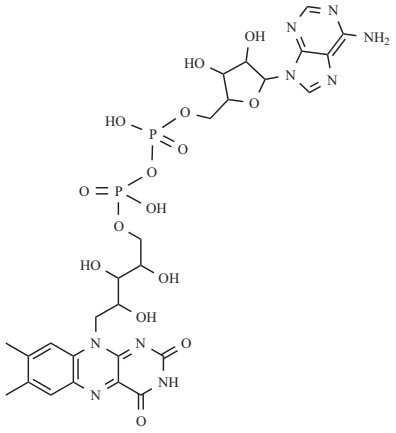
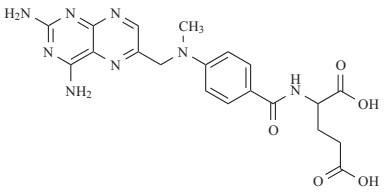
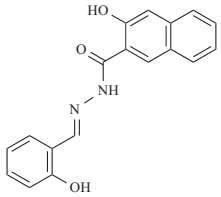
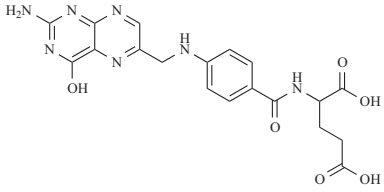
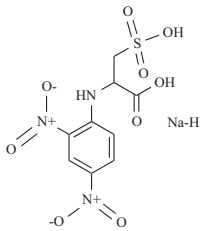
The DOCK program results suggested 21 compounds that may potentially inhibit SDH. These compounds were purchased and after the initial testings 7 compounds were found to be active. The active compounds included flavin adenine dinucleotide disodium hydrate, (+)-Amethopterin, 3-hydroxy-2-napthoic(2-hydroxybenzylidene) hydrazide, folic acid, N-2,4-dinitrophenyl-L-cysteic acid, Vanillin azine and 1H-indole-2,3-dione,5-bromo-6-nitro-1-(2,3,4-tri-O-acetyl-α-L-arabinopyranosyl)-(9Cl). The corresponding IC_{50} values were 0.192 μM, 1.1 M, 1.2 μM, 4.5 μM, 5.3 μM, 7 μM and 28 μM, respectively. The molecular formulae and chemical structures of the 7 active compounds are shown in Table **1**. For comparison and validation of the inhibitory assay the IC_{50} value of SDI-158 was also determined and was found to be in agreement with the published value [8]. In addition, SDI-158 was docked into the active site of SDH and the resulting orientation was found to be similar to that observed in the crystal structure of the ternary complex [4]. The most potent compound, with an IC_{50} value of 0.192 μM and 5.2-fold greater potency than SDI-158, is flavin adenine dinucleotide disodium hydrate. 1H-indole-2,3-dione,5-bromo-6-nitro-1-(2,3,4-tri-O-acetyl-α-L-arabinopyranosyl)-(9Cl) was the least potent with a 28-fold less potency compared to SDI-158.

While previous studies on SDIs were focused on the design and synthesis of analogues for SDI-158 [9-12], our study revealed novel classes of potential inhibitors with *in vitro* potencies in the micro molar range. The new compounds include a ring system, which seems to be a pre-requisite for SDH inhibitors and bind to a hydrophobic portion of the active site. Additionally, hydroxyls, sulfonic or nitro functional groups interacted with the polar residues and the catalytic zinc atom. Vanillin azine and 3-hydroxy-2-napthoic(2-hydroxybenzylidene) hydrazide have comparable structures including hydroxyl groups and benzyl rings, while (+)-Amethopterin is an analogue of folic acid. The proposed interactions between the most active compound (flavin adenine dinucleotide disodium hydrate) and the active site residues of SDH are shown in Fig. **1**.

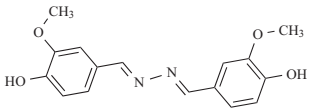
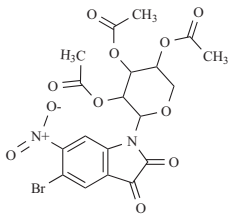
Similar to SDI-158 [4], the potencies of the compounds *in vitro* are likely due to their interactions with the catalytic zinc. Additional interactions may include the stacking against the nicotinamide ring of NAD^+, the numerous van der Waals contacts and a small number of H-bonds between the compounds and the protein. The proposed models suggest that flavin adenine dinucleotide is coordinated to the zinc atom by oxygen and nitrogen atoms (1.8 Å and 1.9 Å, respectively) from the flavin ring which π-stacks against the nicotinamide ring of NAD^+ (Fig. **1**). (+)-Amethopterin is H-bonded to His49 (3.0 Å) and Thr121 (2.9 Å). The zinc atom is likely coordinated by nitrogen atoms of the diamino-pteridin ring (3.2 Å and 3.9 Å). In case of the 3-hydroxy-2-napthoic(2-hydroxybenzylidene) hydrazide the zinc atom may be coordinated by a nitrogen atom from the 2-hydroxybenzylidene moiety (2.7 Å). Folic acid is H-bonded to Ser46 (3.7 Å), Thr121 (3.1 Å), Glu155 (2.8 Å) and Ser276 (2.3 Å), and may coordinate to the zinc atom through two nitrogens from its 4-pteridinol ring (3.2 Å and 3.8 Å). N-2,4-Dinitrophenyl-L-cysteic acid is likely coordinated through its sulfomethyl oxygen to the zinc atom (2.4 Å). Vanillin azine forms an H-bond with Thr121 (3.0 Å) and may coordinate to the zinc atom through an imine nitrogen (2.4 Å). In the case of 1H-indole-2,3-dione,5-bromo-6-nitro-1-(2,3,4-tri-O-acetyl-α-L-arabinopyranosyl)-(9Cl), the zinc atom is likely coordinated by an ester oxygen (2.3 Å).

This was the first study on the discovery of potential SDH inhibitors from virtual screening and resulted in a 33% success rate. From a total of 21 compounds tested 7 compounds were found to inhibit SDH with IC_{50} values in the micro molar range. Similar studies performed on the much more investigated aldose reductase, the first enzyme of the polyol pathway, have resulted in a similar success rate [17].

Table 1. IC_{50} Values, Molecular Formulae and Chemical Structures of SDI-158 and the Seven SDH Inhibitors Identified from the NCI Database

Compound	Molecular Formula	IC_{50} (μM)	Structure
SDI-158	$C_{11}H_{17}N_5O_3$	1	
Flavin adenine dinucleotide disodium hydrate	$C_{27}H_{33}N_9O_{15}P_2$	0.19	
(+)-Amethopterin	$C_{20}H_{22}N_8O_5$	1.1	
3-Hydroxy-2-napthoic(2-hydroxybenzylidene) hydrazide	$C_{18}H_{14}N_2O_3$	1.2	
Folic acid	$C_{19}H_{19}N_7O_6$	4.5	
N-2,4-Dinitrophenyl-L-cysteic acid	$C_9H_{10}N_3NaO_9S$	5.3	

(Table 1. Contd....)

Compound	Molecular Formula	IC_{50} (μM)	Structure
Vanillin azine	$C_{16}H_{16}N_2O_4$	7	
1H-Indole-2,3-dione,5-bromo-6-nitro-1-(2,3,4-tri-O-acetyl-α-L-arabinopyranosyl)-(9Cl)	$C_{19}H_{17}BrN_2O_{11}$	28	

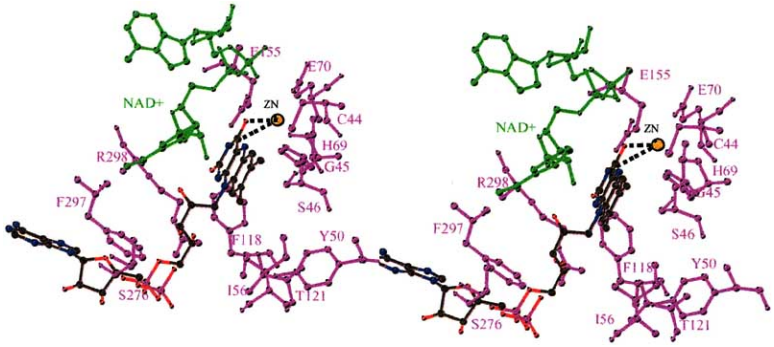


Fig. (1). Stereoview of the most active compound (flavin adenine dinucleotide disodium hydrate) identified from the NCI database and its proposed interactions with the SDH active site. Residues within 4 Å of the compound, H-bonds and the proposed coordination between the zinc atom and the compound (dashed lines) are shown.

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Received: 03 October, 2005 Revised: 16 December, 2005 Accepted: 17 December, 2005