East Asian Journal of Multidisciplinary Research (EAJMR)
Vol. 1, No. 9, 2022 : 1793-1800

New Inhibitors of Glucose-6-Phosphate Dehydrogenase Discovered by Molecular Docking

Haitham Al-Madhagi[1]*, Hisham Al-Ward[2]
[1]Biochemical Technology Program, Faculty of Applied Sciences, Thamar University
[2]Shanghai Institute of Stem Cell Research and Clinical Translation, School of Medicine, Tongji University
Corresponding Author: Haitham Al-Madhagi bio.haitham@gmail.com

A R T I C L E I N F O

Keywords: Virtual Screening, Molecular Docking, G6PD, 6-Aminonicotinamide, ADME

Received : 06 October
Revised : 16 October
Accepted: 26 October

A B S T R A C T

The aim of this study is to *in silico* screen new glucose-6-phosphate dehydrogenase (G6PD) inhibitors. glucose-6-phosphate dehydrogenase is the first and regulator of pentose phosphate pathway providing NADPH and ribose-5-phosphate required for various syntheses from fatty acids to DNA. G6PD is linked to oxidative stress and hence, to inflammation as well. Therefore, G6PD inhibition is a useful target against inflammation, cancer and some infections. Virtual screening of 15 ligands in the NADP-binding site in comparison with the standard inhibitor 6-aminonicotinamide using iGEMDOCK. Besides, ADME properties of the selected compounds were performed via SWISSADME webserver. All the tested ligands were better than reference inhibitor in terms of binding energy as well as pharmacokinetic and ADME parameters. Moreover, of all tested compounds, ligand 15 showed best docking fitness (-115 Kcal/mole total energy). Novel compounds were screened to be lead inhibitors of G6PD enzyme and ligand 15 ranked first.

DOI: https://10.55927/eajmr.v1i9.1191
ISSN-E: 2828-1519
https://journal.formosapublisher.org/index.php/eajmr/index

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD; EC: 1.1.1.49) is the first catalyzing and rate-limiting enzyme in pentose phosphate pathway (PPP). It promotes the oxidation of glucose 6 phosphate (G6P) to 6 phosphogluconolacto ne, concomitant with the reduction of nicotinamide adenine dinucleotide phosphate ($NADP^+$) into NADPH (Fig 1). The formed NADPH is utilized for various biosynthetic pathways including fatty acid, cholesterol and nucleotides. Furthermore, NADPH is an indispensable component for the antioxidants defense system of cells especially for red blood cells (RBC) and white blood cells (WBC) where it acts as a coenzyme of glutathione reductase, needed for the regeneration of active glutathione and thus protecting cells from accumulating free radicals (Stanton, 2012; Tian et al., 1998).

Besides, if the reactions of the first phase (the oxidation phase of PPP) is moving on, ribose-5-phosphate is produced in the second (non-oxidative phase of PPP). The latter is necessary as a building blocks for the synthesis of nucleotides and thus even more important processes such as transcription and replication (Hutchings et al., 2005; Li et al., 2020).

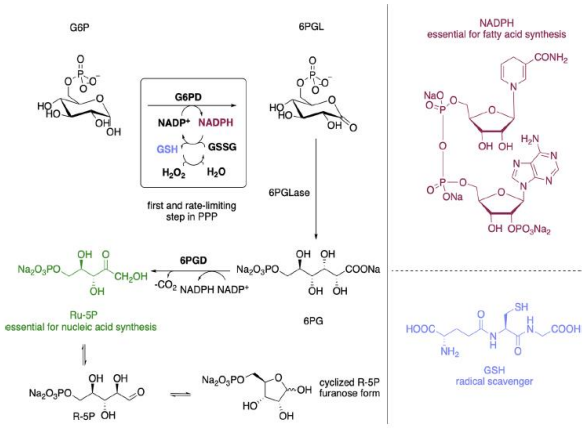

Figure 1. Pentose Phosphate Pathway with Role of G6PD Elucidated

Also, the structure of NADPH and glutathione (GSH, radial scavenger) are depicted. Reprinted with permission from (Koperniku et al., 2022). Copyright 2022, American Chemical Society. Structurally, G6PD is a homodimer consisting of 515 amino acid residues each with a molecular weight of 59 kDa. Human G6PD is similar to *Leuconostoc mesentroides* G6PD, with a 'Rossmann-fold' coenzyme-binding domain and a β+α domain which forms the dimer interface (Fig 2) (Kotaka et al., 2005). In each subunit, there is an active site and another coenzyme-binding site away from the active site. G6PD deficiency is the most common type of enzymophathies, accounting for 300 million afflicted people annually. The mutation-to-structure impact is due to destabilization of dimer interface, reducing the activity of the enzymatic activity (Au et al., 2000).

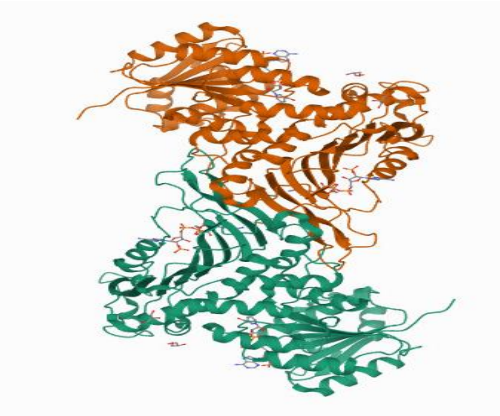

Figure 2. 3D Structure of Human G6PD Complexed with G6P in its Active Site and NADP+ as well as some Glycerol Molecules (PDB ID: 2BH9)

Given its pivotal role in providing both (i) NADPH required for redox homeostasis of RBC, oxidative burst in WBC, reducing equivalents for major biosntheses and (ii) ribose-5-phosphate, necessary for the synthesis of purines thereby DNA synthesis and cell division, G6PD is an interesting target as antimicrobial (Berneburg et al., 2022), anti-inflammatory (Yang et al., 2016), anti-tumor activities (Koperniku et al., 2022; Stanton, 2012). Recently, its role was expanded to be also implicated in cardiovascular pathology (Chhabra et al., 2018) and neurodegenerative diseases (Tang, 2019).

The current standard G6PD inhibitor of the NADP-binding site is 6-aminonicotinamide (6-AN) (Chen et al., 2018; Köhler et al., 1970). However, it exhibits weak androgenic activity and serves as a native substrate for other enzymes *in vivo* (Koperniku et al., 2022). So, there is a need for developing new inhibitors of G6PD that are more potent with no or, at least, milder side effects and this is the goal of this *in silico* study.

THEORETICAL REVIEW

The crystal structure of human G6PD enzyme was retrieved from PDB (PDB ID: 2BH9). The crystal structure showed a substrate binding site occupied by G6P and another coenzyme-binding site occupied by the $NADP^+$. The site chosen for docking investigation was the coenzyme-binding motif (NAP) with a binding site radius of 8 Å. A list of 15 natural and synthetic compounds (Table 1) were selected from PubChem dataset according to their similarity index to the established inhibitor 6-AN. The selected ligands were retrieved from PubChem database as SDF files and then converted to MOL2 using Open Babel software (http://openbabel.org) (O'Boyle et al., 2011) to be inputted into the iGEMDOCK software.

Table 1. List of Screened Compounds in the Current Study along with Their Pubchem ID

NO	PubChem ID	Ligand Name
1	132279092	3,5'-Dimethoxy-resveratrol
2	72344	Nobiletin
3	96118	4',5,6,7-Tetramethoxyflavone
4	96539	Gardenin B
5	96892	6-(diethylamino)pyridine-3-carboxamide
6	97332	Quercetin pentamethyl ether
7	136417	5-Hydroxy-3,6,7,8,3',4'-hexamethoxyflavone
8	145659	Sinensetin
9	261592	6-(Pyrrolidin-1-yl)pyridine-3-carboxamide
10	629965	Zapotin
11	3010100	4'-Hydroxy-5,6,7,8-tetramethoxyflavone
12	3031415	6-(Cyclohexylamino)pyridine-3-carboxamide
13	5315263	Casticin
14	5352005	Retusin
15	132228215	N-(1-(1-(L-alanyl)piperidin-4-yl)ethyl)-6-aminonicotinamide

METHODOLOGY

To calculate the binding affinity of the selected ligands for G6PD enzyme, iGEMDOCK software (Hsu et al., 2011) was used. 15 virtual screenings of the selected ligands against the standard inhibitor of the coenzyme-binding site 6-aminonicotinamide. The docking parameters were set as stable docking with 300 population size, 80 generations, and 10 solutions. Upon finishing the docking process, post docking analysis was performed to figure out the best docking pose and its corresponding energy values. The empirical scoring function of iGemdock software was estimated using the formula:

Energy = vdW + Hbond + Elec

2D Visualization of docking results was done using Discovery Studio Client 21. Pharmacokinetics as well as Lipinski's rule of five of the all ligands were calculated by the webserver SWISSADME (Daina et al., 2017). SWISSADME is a quick, accurate, simple interface, and freely available online tool for the prediction of pharmacokinetic parameters ADME (absorption, distribution, metabolism, and excretion) as well as the leadlikeness of the ligand molecules. This step can eliminate unnecessary testing in the wet lab setting.

RESULTS AND DISCUSSIONS

G6PD is an essential metabolic enzyme regulate key metabolic pathway (PPP) which is required for many tissues and cells. This makes the enzyme an important step to be targeted for treating cancer, parasite infections and inflammation (Koperniku et al., 2022). So, this study aimed to evaluate the potential inhibitors of G6PD. A set of 15 ligands were tested in the current study using iGEMDOCK software.

Based on the obtaining results from iGEMDOCK, all of the tested ligands showed higher binding affinities (Table 2) than the standard inhibitor 6-AN. The total energy ranged from -72 to -115 Kcal/mole, the best of which was ligand 15 with a total energy of -115 Kcal/mole, (-88.7 VDW and -27 H-bonds with no electrostatic attractions). Therefore, the presented ligands are lead candidates to be potent inhibitors of G6PD and thus drugs of cancer or inflammation. Fig 3 shows the NADP-binding site residues interaction with the ligand 15 (the best docked ligand) in 3D as well as in 2D diagram along with the type of interactions. Ser 40, Arg 72, Tyr 112, Ala 141, Tyr 147, and Lys 171 form H-bonds with the ligand whereas Leu 43, Leu 142 and Pro 143 pose Van der Waal's interaction with the heterocyclic rings of the ligand.

The interactions found between reference inhibitor 6-AN and protein showed three amino acid residues (Ser 40, Ala 141 H-bonded and Leu 142 via hydrophobic interactions) in common with ligand 15 (Fig 3). This indicates the fact that docked ligands fit well within the NADP-binding pocket of the enzyme. Still, bonding amino acids are more in the ligand 15 compared to 6-AN due to the larger conformation of the ligand, hence, occupies larger area within the binding pocket.

Table 2. Molecular Docking Result of all Ligands at NADP-Binding Site of 2BH9 Protein

Ligand	Total Energy	VDW	HBond	Elec
1	-93.7302	-68.8719	-24.8583	0
2	-97.0042	-70.3835	-26.6207	0
3	-88.1441	-79.4254	-8.71866	0
4	-94.0464	-67.1559	-26.8905	0
5	-72.1477	-52.9871	-19.1605	0
6	-96.5266	-81.6642	-14.8624	0
7	-99.9191	-82.6126	-17.3066	0
8	-97.0619	-86.4126	-10.6492	0
9	-72.2487	-53.3623	-18.8863	0
10	-87.7474	-65.4561	-22.2913	0
11	-91.3706	-63.3928	-27.9779	0
12	-84.2082	-63.4956	-20.7125	0
13	-99.7863	-80.2791	-19.5073	0
14	-91.159	-72.3642	-18.7948	0
15	-115.787	-88.7524	-27.0349	0
6-AN	-64.2528	-34.6957	-29.557	0

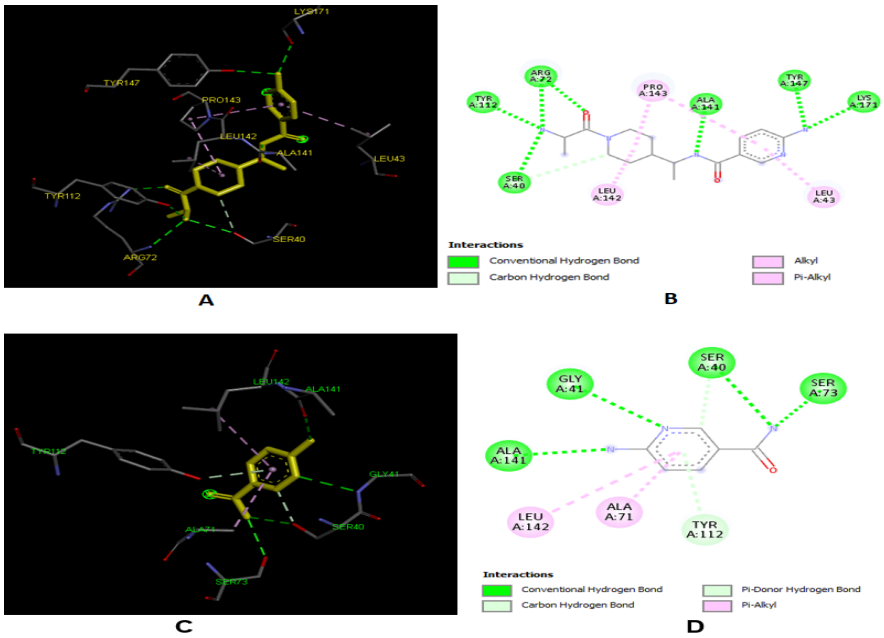

Figure 3. G6PD-Ligand 15 and Reference Inhibitor 6-AN Interactions in 3D Representation (A,C) and 2D Diagram (B,D) Depicted using Discovery Studio

ADME prediction assists in the assessment of druglikeness of the lead candidates. All the of the tested compounds were found to satisfy Lipinski's rule of five and ADME properties with no violation. ADME results are summarized in Table 3. In addition, the gastrointestinal absorption of all tested ligands is high and thus can be prepared as pharmaceuticals given orally.

Table 3. SWISSADME Results Showing ADME Parameters of Compounds (1-15) along with the Standard Inhibitor 6-AN

Ligand	MW (<500)	RB (≤10)	HA (<10)	HD (<5)	MR (40-130)	TPSA (≤140)	LogP (<5)	GI absorption (>30% high)
1	290.31	4	5	3	79.49	79.15	2.41	High
2	402.39	7	8	0	106.87	85.59	3.51	High
3	342.34	5	6	0	93.89	67.13	3.49	High
4	358.34	5	7	1	95.91	87.36	3.2	High
5	193.25	4	2	1	56.15	59.22	1.03	High
6	372.37	6	7	0	100.38	76.36	3.5	High
7	418.39	7	9	1	108.9	105.82	3.22	High
8	372.37	6	7	0	100.38	76.36	3.5	High
9	191.23	2	2	1	56.97	59.22	0.4	High
10	342.34	5	6	0	93.89	67.13	3.49	High
11	358.34	5	7	1	95.91	87.36	3.2	High
12	219.28	3	2	2	63.56	68.01	1.73	High
13	374.34	5	8	2	97.93	107.59	2.91	High
14	358.34	5	7	1	95.91	87.36	3.2	High
15	319.4	6	4	3	92.5	114.34	0	High

6-AN	137.14	1	2	2	36.74	82	-0.23	High

MW: molecular weight; RB: rotatable bonds; HA: hydrogen acceptor; HD: hydrogen donor; MR: molar refractivity; TPSA: topological polar surface area.

CONCLUSIONS AND RECOMMENDATIONS

Here, we have carried out a molecular docking of potential inhibitors of human G6PD that can be utilized as possible drugs mainly against inflammation and cancer. All of the screened ligands showed more favorable binding energy as well as pharmacokinetics and ADME properties than the reference drug inhibitor 6-AN. The best docked candidate was ligand 15 with total free energy -115 Kcal/mole. On applying Lipinski's rule of five, all tested compounds fit well with no violation. Hence, the present study proposes ligand 15 for further evaluation as G6PD inhibitor *in vitro* and *in vivo*.

FURTHER STUDY

Therefore, G6PD inhibition is a useful target against inflammation, cancer and some infections. Virtual screening of 15 ligands in the NADP-binding site in comparison with the standard inhibitor 6-aminonicotinamide using iGEMDOCK. Besides, ADME properties of the selected compounds were performed via SWISSADME webserver. All the tested ligands were better than reference inhibitor in terms of binding energy as well as pharmacokinetic and ADME parameters. Moreover, of all tested compounds, ligand 15 showed best docking fitness (-115 Kcal/mole total energy). Novel compounds were screened to be lead inhibitors of G6PD enzyme and ligand 15 ranked first.

REFERENCES

Au, S.W., Gover, S., Lam, V.M., Adams, M.J., 2000. Human glucose-6-phosphate dehydrogenase: the crystal structure reveals a structural NADP+ molecule and provides insights into enzyme deficiency. Structure 8, 293–303.

Berneburg, I., Peddibhotla, S., Heimsch, K.C., Haeussler, K., Maloney, P., Gosalia, P., Preuss, J., Rahbari, M., Skorokhod, O., Valente, E., 2022. An Optimized Dihydrodibenzothiazepine Lead Compound (SBI-0797750) as a Potent and Selective Inhibitor of Plasmodium falciparum and P. vivax Glucose 6-Phosphate Dehydrogenase 6-Phosphogluconolactonase. Antimicrobial Agents and Chemotherapy e02109–21.

Chen, X., Xu, Z., Zhu, Z., Chen, A., Fu, G., Wang, Y., Pan, H., Jin, B., 2018. Modulation of G6PD affects bladder cancer via ROS accumulation and the AKT pathway in vitro. International journal of oncology 53, 1703–1712.

Chhabra, A., Mishra, S., Kumar, G., Gupta, A., Keshri, G.K., Bharti, B., Meena, R.N., Prabhakar, A.K., Singh, D.K., Bhargava, K., 2018. Glucose-6-phosphate dehydrogenase is critical for suppression of cardiac hypertrophy by H2S. Cell death discovery 4, 1–16.

Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports 7, 1–13.

Hsu, K.-C., Chen, Y.-F., Lin, S.-R., Yang, J.-M., 2011. iGEMDOCK: a graphical environment of enhancing GEMDOCK using pharmacological interactions and post-screening analysis. BMC bioinformatics 12, 1–11.

Hutchings, D., Rawsthorne, S., Emes, M.J., 2005. Fatty acid synthesis and the oxidative pentose phosphate pathway in developing embryos of oilseed rape (Brassica napus L.). Journal of Experimental Botany 56, 577–585.

Köhler, E., Barrach, H.-J., Neubert, D., 1970. Inhibition of NADP dependent oxidoreductases by the 6-aminonicotinamide analogue of NADP. FEBS letters 6, 225–228.

Koperniku, A., Garcia, A.A., Mochly-Rosen, D., 2022. Boosting the Discovery of Small Molecule Inhibitors of Glucose-6-Phosphate Dehydrogenase for the Treatment of Cancer, Infectious Diseases, and Inflammation. Journal of Medicinal Chemistry 65, 4403–4423.

Kotaka, M., Gover, S., Vandeputte-Rutten, L., Au, S.W.N., Lam, V.M.S., Adams, M.J., 2005. Structural studies of glucose-6-phosphate and NADP + binding to human glucose-6-phosphate dehydrogenase. Acta Crystallogr D Biol Crystallogr 61, 495–504. https://doi.org/10.1107/S0907444905002350

Li, R., Wang, W., Yang, Y., Gu, C., 2020. Exploring the role of glucose-6 phosphate dehydrogenase in cancer. Oncology Reports 44, 2325–2336.

O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., Hutchison, G.R., 2011. Open Babel: An open chemical toolbox. Journal of cheminformatics 3, 1–14.

Stanton, R.C., 2012. Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. IUBMB life 64, 362–369.

Tang, B.L., 2019. Neuroprotection by glucose-6-phosphate dehydrogenase and the pentose phosphate pathway. Journal of Cellular Biochemistry 120, 14285–14295.

Tian, W.-N., Braunstein, L.D., Pang, J., Stuhlmeier, K.M., Xi, Q.-C., Tian, X., Stanton, R.C., 1998. Importance of glucose-6-phosphate dehydrogenase activity for cell growth. Journal of Biological Chemistry 273, 10609–10617.

Yang, H.-C., Wu, Y.-H., Liu, H.-Y., Stern, A., Chiu, D.T.-Y., 2016. What has passed is prolog: new cellular and physiological roles of G6PD. Free radical research 50, 1047–1064.