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A quantum chemistry background of sickle cell anemia and gaps in antisickling drug development

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ABSTRACT

Sickle cell anemia disease has been a great challenge for the world in the present situation. It occurs only due to the polymerization of sickle hemoglobin (HbS) having Pro-Val-Glu (PVG) typed mutation, while the polymerization does not occur in normal hemoglobin (HbA) having Pro-Glu-Glu (PGG) residues. According to data from the literature, Val-beta6 of Pro-Val-Glu is hydrophobic in nature, which appears to fit into a hydrophobic pocket in the adjacent HbS. After the insertion of Pro-Val-Glu into a hydrophobic pocket on the adjacent HbS, the polymerization is started. This is a questionable point on how the replacement of glutamic acid with valine in HbS makes it more reactive to fit into a hydrophobic pocket on adjacent HbS for polymerization. No data from the literature on the reactivity of HbS for polymerization was found yet. This is the first time that the theoretical calculation was done in both HbA and HbS where they were structurally different. After that, a comparative study between PVG and PGG was done at quantum level for the evaluation of the reactivity to fit into a hydrophobic pocket on adjacent HbS. At a quantum level, it was found that the HOMO-LUMO gap of Pro-Val-Glu was lower than that of Pro-Glu-Glu. According to the data from the literature, the lesser HOMO-LUMO gap promotes the initiation of the polymerization reaction. On the basis of the results, it was also shown how the mutation point (Pro-Val-Glu) in HbS becomes more reactive to polymerization, whereas Pro-Glu-Glu in HbA does not. The computational method developed for the first time will be very helpful not only for molecular biologists but also for computational and medicinal chemists. Additionally, the required modifications based on gaps in anti-sickling drug development are also suggested in the presented article.

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1. Introduction

Of course, sickle cell anemia disease (SCAD) has been a very old one. Therefore, many studies have been done to evaluate this disease, but Pauling [1] noted the main point for the first time when he found that sickle cell anemia is a molecular disease. Subsequently, Ingram [2] studied the structures of sickle hemoglobin (HbS) and normal hemoglobin (HbA) to confirm the exact change at the gene mutation point. He reported [2] that one of the glutamic acid residues of Pro-Glu-Glu (PGG) present in normal hemoglobin (HbA), is replaced by the valine residue, that is, Pro-Val-Glu (PVG) in sickle cell, also called the E6V mutation. Although sickle hemoglobin (HbS) has normal oxygen binding affinity, its polymerized form does not [3]. The low affinity of a hemoglobin mutant is a characteristic of sickle cell anemia disease that was confirmed in detail more recently [4]. The production of abnormal red blood cells (RBCs) is also associated with the polymerization that occurs under hypoxic conditions [3,5], that is, a low concentration of oxygen. Gill *et al.* [6] showed a sharp decrease in oxygen affinity just after partial deoxygenation. It proved that the deoxygenated form of HbS (dHbS) takes part in the polymerization reaction rapidly while the oxygenated form (OHbS) does not. Therefore, abnormal RBC production is caused by the polymerization of deoxygenated sickle hemoglobin under hypoxic conditions. Different studies were done to understand the effect of pH, carbon monoxide, and other factors on polymerization. For example, the findings regarding the pH effect showed that lowered pH over the physiological range promotes the polymerization of HbS, and it causes sickling of red cells [7], but it was shown that none of these factors could be responsible for the different behavior of sickle cell disease under hypoxic conditions [8]. Hence, the truth behind the actual reason for the HbS polymerization is yet to come.

The normal Pro-Glu-Glu link in HbA is hydrophilic in nature, while Pro-Val-Glu is a hydrophobic link in HbS due to the presence of a hydrophobic valine residue [7-9]. Therefore, researchers [7-9] have suggested that the hydrophobic R-group of Val-beta6 (E6V) appears to fit into a hydrophobic pocket constituted by β88 leucine (Leu-beta88), β85phenylalanine (Phe-beta85), and β73 aspartic acid (Asp-beta73) residues on adjacent deoxygenated sickle hemoglobin molecules (dHbS-M).

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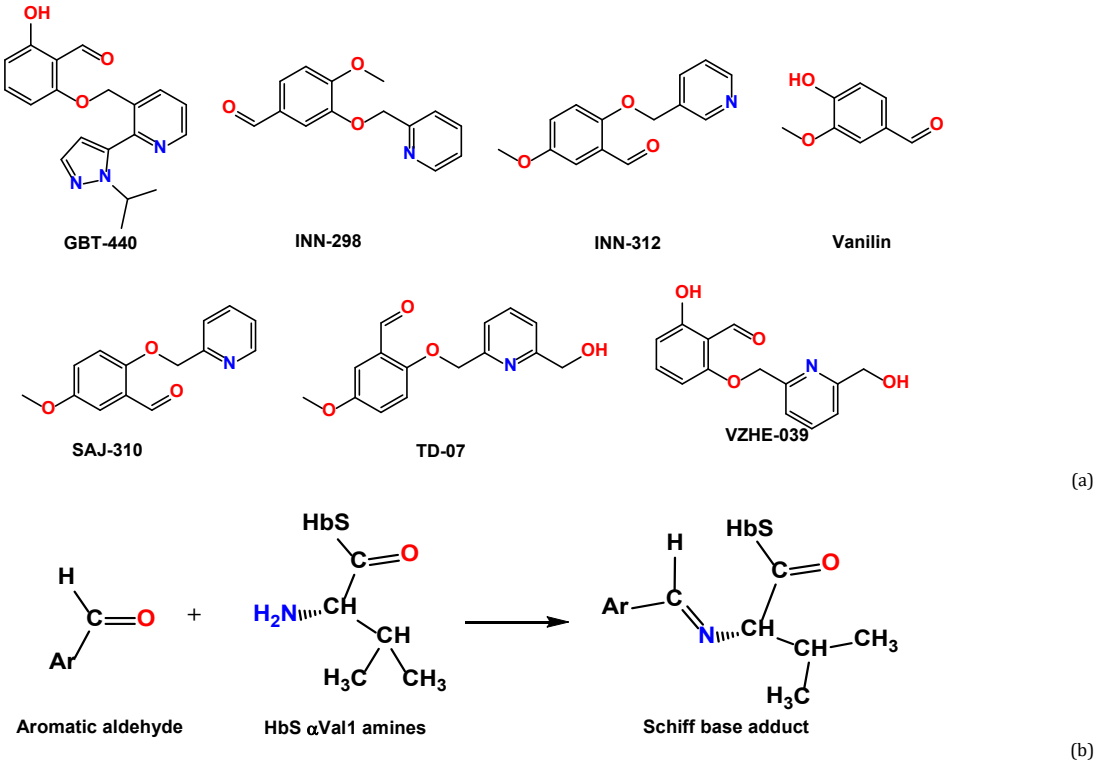

Figure 1. (a) The structure of anti-sickling drugs [13], and (b) their literature data-based mechanism [13-19].

Due to this, the hydrophobic β valine (Valbeta6) generates a 'sticky patch' on the β-globin chain of dHbS-M [10,11]. The hydrophobic interaction is stereospecific to the Leu-beta88 side chain in the acceptor pocket regions on adjacent dHbS-M [7]. Besides, the contact position on dHbS-M is also favourable for the introduction of the R-group of Val-beta6 in this hydrophobic pocket [7,10,11]. On the other hand, the hydrophilic R-group of β glutamic acid (Glubeta6) does not easily fit in the hydrophobic pockets, which is why polymerization does not occur in deoxyHbA [12]. Here, the question of why Pro-Val-Glu is so reactive that it attaches to the adjacent hydrophobic pocket, while PGG does not arise and remains constant. Consequently, no research explained the reactiveness of β-valine (Valbeta6) of (Pro-Val-Glu) to fit into hydrophobic pockets on adjacent dHbS-M.

The US FDA has approved GBT-440 (voxelotor) as an antisickling agent [13]. In addition to GBT-440, many drugs [13-19] have also been synthesized (Figure 1a) whose aldehyde moieties form the Schiff base adduct with αVal1 N-terminal amines (Figure 1b). The main purpose for the synthesis of these drugs was just to increase the HbS protein affinity for oxygen. No description was found about that drug, which may attach with the Pro-Val-Glu mutation point so that the changes observed due to the replacement of glutamic acid with valine could be nullified. The presented article suggests that it could be possible in the future if we notice the gap found in the development of antisickling agents.

2. Experimental

2.1. Software and tools

All software and tools used for the current study are the Gaussian 03 program [20] and GaussView 6.0 [21], Discovery Studio Visualizer 2019 (v19.1.0.18287) [22], MarvinSketch (16.9.12 version), ChemDraw Ultra (12.0.2 version) [23], and Protein Data Bank (https://www.rcsb.org/).

2.2. Simulation method

Of course, the simulation study has played an important role in the reaction mechanism [24], chiral separation [25-28], isolation of the most active gradient from the plant [29], and biological chemistry [30-34], but this is the first time the computational method has been applied to know the quantum background of the molecular disease. For the study at the quantum level, the pdb file of deoxygenated hemoglobin S (βGlu6 → Val) with PDB Code 2hbs [35] was obtained from the protein data bank. All amino acids were removed except the mutation point (PVG) using Discovery Studio (Figure 2). The obtained file was saved in mol file format. For the preparation of the mol file of PGG, the mol formatted file of PVG was opened again, and valine was replaced with glutamic acid with the help of a Marvin sketch and saved in the same format, that is, the mol file format (Figure 2). After that, both files were used for theoretical calculation in the Gaussian(R) 03 program [20]. Three basis sets ONIOM(RHF/6-31G) [36], HF/3-21+G* [37], and B3LYP/3-21+G* [38,39] were used in the oniom method, Hatree-Fock method and DFT method, respectively. The results obtained were analyzed by GaussView 6.0 [21]. The DFT method with the basis set B3LYP/3-21+G* was used to reduce the approximation in the results of the other two methods. In the presented study, the HOMO-LUMO energy gap in both structures was studied in depth. The interpretation of the results removed the curtain from the unsolved question regarding the more reactiveness of the mutation point PVG in HbS as compared to PGG in HbA. Due to its more reactivity to fit into a hydrophobic residue on adjacent dHbS-M, PVG generates a 'sticky patch' on the β-globin chains of dHbS-M [10,11]. However, the hydrophobic nature of both links (PGG and PVG) was also valued so that the hydrophobicity-based difference between PGG and PVG could be evaluated. For this purpose, all parameters related to hydrophobicity were studied computationally. For instance, the hydrophobicity-related Log P (Partition coefficient), CMR (Calculated molar refractivity), MR

Table 1. E_{gap} variation in PGG and PVG.

Method	Basis set	Link	HOMO (eV)	LUMO (eV)	E_{gap} (eV)	Reactivity result
DFT	B3LYP/3-21+G*	PGG	-6.79300	-1.61036	5.18264	PVG > PGG
		PVG	-6.62323	-1.64192	4.98131	
Hartree Fock	HF/3-21+G*	PGG	-10.6388	1.19185	11.83065	
		PVG	-10.3748	1.23321	11.60801	
Hartree Fock	ONIOM (RHF/6-31G)	PGG	-10.9637	2.71024	13.67394	
		PVG	-10.18872	2.88222	13.07094	

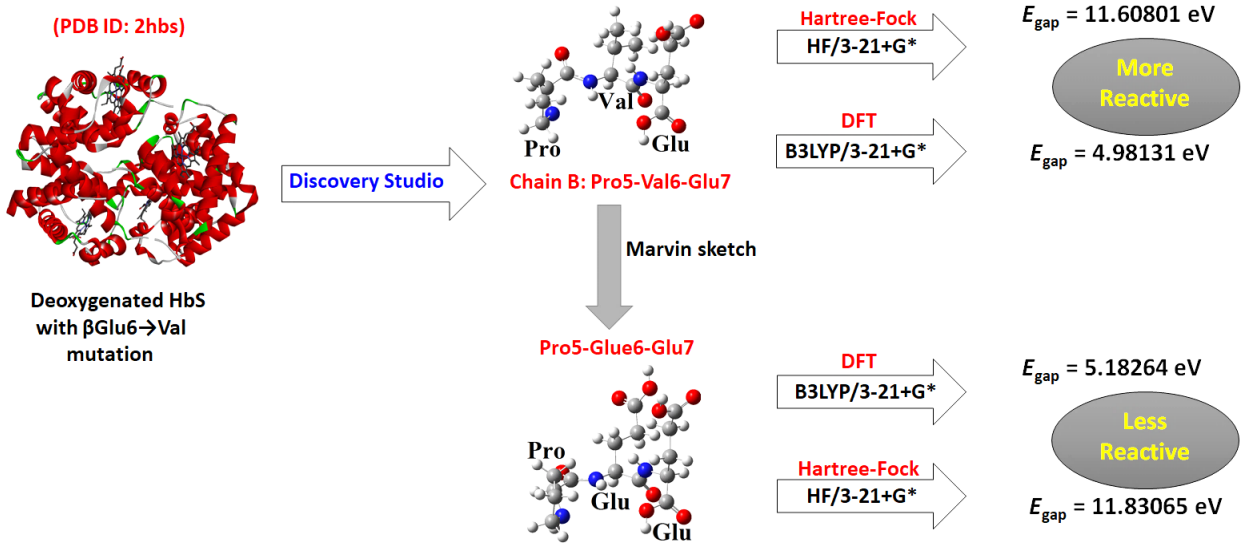

Figure 2. The protocol for the quantum level study (E_{gap} = HOMO-LUMO Energy Gap).

(Remanent magnetization) and tPSA (topological Polar Surface Area) of PGG and PVG were observed using Chemdraw. The logP is a negative value for hydrophilic compounds (higher affinity for the aqueous phase), and a positive value for hydrophilic compounds [40]; CMR is a calculated molar refractivity of one mole of a substance [41]; MR is the magnetization that remains after removal a sufficiently large field to reach the saturation magnetization [42] and tPSA is the surface sum over all polar atoms or molecules, primarily oxygen and nitrogen, also including their attached hydrogen atoms [43]. In addition to these things, the values of Henry's law, heat of form, dipole moment, CLogP, and other important parameters were also renowned.

3. Results and discussion

After Hartree-Fock, DFT, and ONIOM calculations, both structures were found to be different in their HOMO-LUMO energy gaps (E_{gap}). Three basis sets used for the quantum calculation gave the same results, *i.e.,* E_{gap} of PGG was found to be higher than that of PVG (Table 1). Figure 3 shows a 3D pose of HOMO-LUMO of both structures, *i.e.,* PGG of HbA and PVG of HbS. According to data from the literature [44-47], a molecule with a higher frontier orbital gap (HOMO-LUMO energy gap) has low chemical reactivity and high kinetic stability because it is energetically unfavorable to add an electron to the high-lying LUMO in order to remove electrons from the low-lying HOMO. If E_{gap} is higher, it becomes energetically unfavorable for an electron to jump from HOMO to LUMO [24,48], which is why conditions become very difficult for a molecule to take part in a chemical reaction. On the other hand, it becomes very easy for an electron to jump from HOMO to LUMO, if E_{gap} is smaller; therefore, the conditions become very easy for a molecule to participate in a chemical reaction [24]. Based on these facts, Pro-Glu-Glu in HbA must be less reactive to fit into a hydrophobic pocket constituted by β88 leucine (Leu-beta88), β85phenylalanine (Phe-beta85), and β73 aspartic acid (Asp-beta73) residues on adjacent dHbS-M. It is because its HOMO-LUMO energy gap is greater compared to Pro-Val-Glu (Table 1). On the other hand, the HOMO-LUMO energy gap of Pro-Val-Glu is lesser than that of Pro-Glu-Glu (Table 1). Therefore, Pro-Val-Glu in HbS must be more reactive to fit into a hydrophobic pocket constituted by β88 leucine (Leu-beta88), β85phenylalanine (Phe-beta85), and β73 aspartic acid (Asp-beta73) residues on adjacent dHbS-M. Moreover, the HOMO-LUMO gap of the hydrophobic compound should be lower for the initiation of the polymerization reaction [49]. Therefore, the reactivity of Pro-Val-Glu to fit into the hydrophobic pockets is more. Now, the reactiveness regarding the point between the Pro-Glu-Glu of HbA and the Pro-Val-Glu of HbS to fit into hydrophobic points for polymerization is resolved.

Another reason for the same thing is the hydrophobic nature of the mutant link, *i.e.* Pro-Val-Glu. The computationally evaluated hydrophobicity of PGG and PVG showed that the hydrophobic nature of PVG is greater than that of PGG. In the case of PGG, the values of LogP and CLogP were less than those of PVG, which clearly showed that PVG is more hydrophobic than PGG. On the other hand, the values of tPSA, MR, and CMR were lower in the case of PVG than those of PGG, and this also showed that the hydrophobic nature of PVG is greater than that of PGG. Besides these hydrophobicity-based things, the values of Henry's law, the heat of formation, CLogP, dipole moment, and other belongings are also mentioned in Table 2. The dipole moment value (Table 2) of PVG and PGG clearly shows that PGG is more polar compared to PVG. It is just because of the presence of proline and valine residues which are nonpolar or hydrophobic groups [50] in PVG, while glutamic acid is hydrophilic in nature [50]. On the other hand, there are two hydrophilic groups (Glutamic acid) and only one hydrophobic group (Proline) in the Pro-Glu-Glu link. Therefore, the presence of two hydrophobic amino acids in the structure of Pro-Val-Glu makes it hydrophobic.

Table 2. Parameters computed to distinguish the nature of PGG and PVG.

Software	Parameters	PGG	PVG
ChemDraw	Gibbs energy, kJ/mol	-955.09	-613.62
	Henry's law	31.12	25.87
	Heat of form, kJ/mol	-1540.43	-1195.44
	tPSA	182.13	144.83
	Log P	-2.71	-1.32
	CLogP	-4.4154	-2.5994
	MR, cm³/mol	83.69	83.24
	CMR	8.7009	8.5121
Gaussian 03	Electronic energy, a.u.	-1344.39	-1195.92
	Dipole moment, Debye	9.5386	8.2611

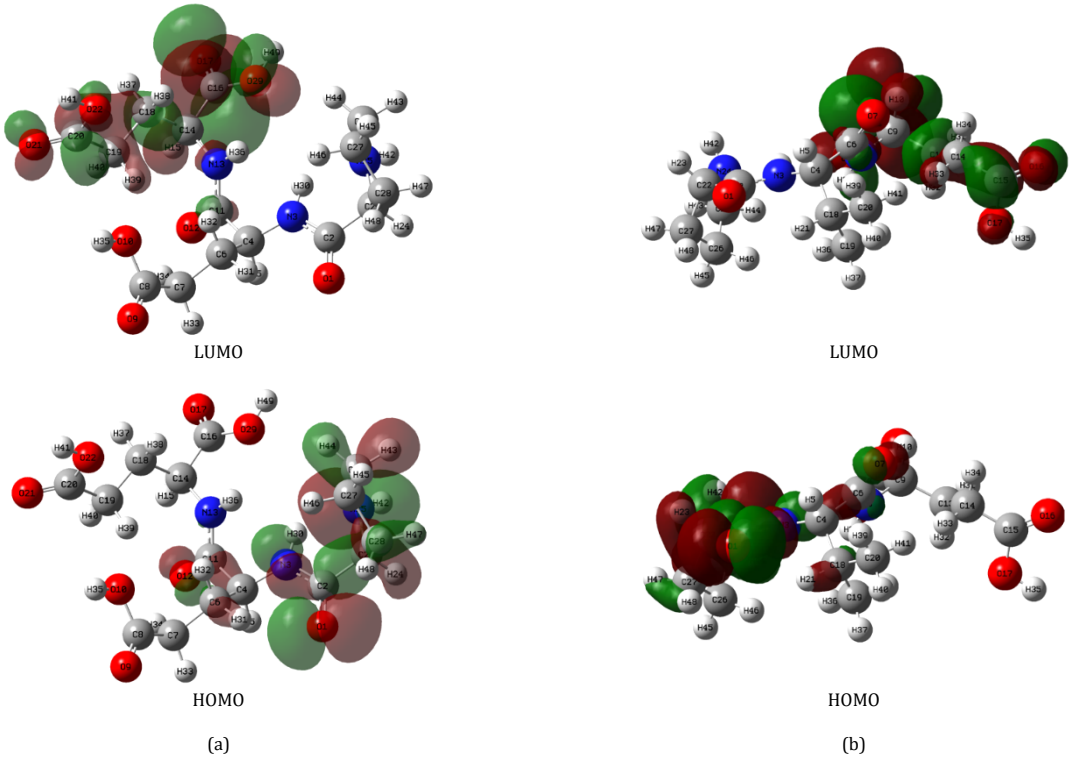

Figure 3. HOMO-LUMO of (a) PGG (HbA) and (b) PVG (HbS) after DFT and Hartree Fock calculation.

On the other hand, the binding sites *i.e.* β88 leucine (Leu-beta88), β85phenylalanine (Phe-beta85), and β73 aspartic acid (Asp-beta73) residues on the adjacent dHbS-M are also hydrophobic [7,10,11]. Hence, the attachment of hydrophobic Pro-Val-Glu of HbS with the hydrophobic residues of the adjacent dHbS-M is stronger compared to that of hydrophilic Pro-Glu-Glu of HbA. It is because the hydrophilic compound does not have a greater affinity towards the hydrophobic complex, but the hydrophobic compound does according to the universal truth. Hence, there are two factors associated with Pro-Val-Glu before polymerization under hypoxia conditions: (i) The decreased E_{gap} value of Pro-Val-Glu for its quick participation in the reaction, and (ii) its hydrophobic nature. These two factors are responsible for the generation of a 'sticky patch' on the β-globin chains of dHbS-M [7,10,11].

4. Gaps in the development of anti-sickling agents

After a literature survey, it was found that the development of antisickling agents has a large gap to be more potent. Here are some important gaps observed during the literature survey, as follows:

i. According to data from the literature, polymerization occurs under hypoxia conditions [3,5], so most scientists synthesize only those drugs or their derivatives that could stabilize oxy HbS [13-18], so that polymerization could be stopped. For example, vanillin stabilizes oxy HbS and stops the polymerization of HbS to some extent, but not 100%. Although other drugs having structural differences with vanillin were also suggested [51], those drugs were also used just to increase the stability of oxy HbS. No one focused on the synthesis of such a drug that could work under hypoxia conditions.

ii. Antisickling drugs synthesized [13-18] or available on the market (Figure 1a) attack only the alpha chain of hemoglobin (Figure 1b), while the mutation-causing residue is present on the beta chain of hemoglobin. Hence, no such drug has been found / synthesized yet that could show affinity to attach to the β-chain of hemoglobin.

iii. Antisickling drugs synthesized [13-18] or available on the market (Figure 1a) react with only Hb αVal1 amines to form Schiff base adducts (Figure 1b), but what about β valine (Valbeta6) whose presence causes polymerization? Therefore, no such drug has yet been found / synthesized that could attack β valine (Valbeta6) of Pro-Val-Glu to make the behavior of HbS like HbA.

iv. All antisickling agents are aromatic aldehydes [13-19] that react with Hb αVal1 amines to form the Schiff base adduct, but what about the effect of pH on it? As Schiff base adduct formation is a pH-dependent condensation reaction [52] that occurs between carbonyl compounds (aldehydes/ketones) and ammonia derivatives (amine),

no pH effect was studied in the formation of Schiff base adducts. In the future, if it is done, it will be very helpful in increasing the potency of the available antisickling agent.

v. Of course, benzenoid aromatic aldehydes and their derivatives [13-18] are found promising drugs in the treatment of sickle cell anemia, but what about non-benzenoid aromatic aldehydes *e.g.* 5-HMF [19]? Therefore, non-benzenoids, aromatic aldehydes, and their derivatives should also be taken under trial. It will also be very helpful in the development of a more potent antisickling agent.

5. The tactic for the selection of a more potent anti-sickling drug before synthesis

For the selection of the anti-sickling drug before its synthesis, first, the binding affinity or docking study of different compounds with the β-valine residue of Pro-Val-Glu must be done computationally. Among the studied compounds, only that compound must be selected which will show the highest binding affinity with the β-valine residue of Pro-Val-Glu. After this, the binding affinity study of the selected compound with β-valine residue should be done in a polypeptide chain of HbS. If the drug shows the same result in a polypeptide chain, then we also have to go for its synthesis and then for its derivatization to increase its potency. In the future, if the drug attached to β valine (Valbeta6) of Pro-Val-Glu is synthesized, there would be two main things associated with its mechanism of action which are as follows:

i. It will not form Schiff base adducts with β valine (Valbeta6) of Pro-Val-Glu as other available antisickling agents do. It is because β valine (Valbeta6) amine group is used in the formation of a peptide bond. Hence, there will be no chance to build a Schiff base adduct. Therefore, the mechanism of the β valine (Valbeta6) attacking the drug would be different from the mechanism of action of the available / known antisickling drug.

ii. It will increase the E_{gap} of Pro-Val-Glu of HbS to make it less or equally reactive to HbA, so that HbS could behave as HbA. For this purpose, the drug having a greater affinity for β valine (Valbeta6) in a polypeptide chain would first be synthesized as described above. Before its derivatization, the binding site variation of β valine (Valbeta6) with respect to the introduction of different groups in the synthesized drug would be computationally studied. Only those binding sites of β valine (Valbeta6) would be considered better where the attachment of the synthesized drug would cause an increase in the E_{gap} of Pro-Val-Glu of HbS.

6. Conclusion

The above sections have left a large amount of work for scientists working in the fields of computational chemistry, molecular biology, and medicinal chemistry. The presented paper also describes how antisickling drug development can be improved. Of course, many computationally evaluated anti-sickling drugs were found during the literature survey, but the gaps found in the development of such drugs cannot be ignored. Keeping gaps in the development of antisickling drugs into consideration, not only the potency of anti-sickling agents can be increased but also new/different drugs with more potency can be synthesized.

Supporting information

The supporting data of the presented work are publicly available on Mohd Suhail. (2023). Output files of the theoretical calculations done for Pro-Glu-Glu and Pro-Val-Glu (Gaussian(R) 03 program and GaussView 6.0) [Data set]. Zenodo. https://doi.org/10.5281/zenodo.7729828.

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CRediT authorship contribution statement

Conceptualization: Mohammad Suhail; Methodology: Safwana Usmani; Software: Mohammad Suhail; Validation: Mehmood Ahmad; Formal Analysis: Safwana Usmani; Investigation: Mohammad Suhail; Resources: Mohammad Suhail; Data Curation: Mohammad Suhail; Writing – Original Draft: Mohammad Suhail; Writing - Review and Editing: Mohammad Suhail; Visualization: Mehmood Ahmad; Funding acquisition: Mohammad Suhail; Supervision: Mohammad Suhail; Project Administration: Mohammad Suhail.

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