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Computational Studies of 5-methoxypsolaren as Potential Deoxyhemoglobin S Polymerization Inhibitor

Ikechukwu K. Ijoma [1]*, Chinyere E. Okafor [2], Vincent I.E. Ajiwe [1]

[1] *Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria.*
[2] *Department of Pure and Industrial Chemistry, Chukwuemeka Odumegwu Ojukwu University, Uli, Nigeria*

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ABSTRACT

Ficus thonningii is a native Southeast Nigerian tree. The leaves are medicinal, and it is reportedly used in sickle cell disease (SCD) management by ethnic people of Ebonyi State, Southeast, Nigeria. Previously we characterized the *in vitro* antisickling activity of its crude leaf methanol extract and observed that it functioned via the sickle polymerization inhibition pathway and 5-methoxypsolaren (5-MPS) labelled FTH1 was isolated as one of its constituents. Therefore, this research aim and objectives are to comprehend in silico the mechanism of the observed *in vitro* sickle deoxyhemoglobin (DeOxyHbS) polymerization inhibitory activity of 5-MPS. **T**he structure of the target protein (2HBS) was chosen based on advanced BLAST analysis. Molecular docking and molecular dynamics simulation studies were carried out using blind docking and distant-dependent dielectric assays, respectively whereas ADMET was performed using SwissADME and protox-II webserver. The ability of 5-MPS to interfere with the processes that leads to DeOxyHbs polymerization was evident in the binding affinity of -6.4 Kcal/mol. The MD simulation analysis of the binding site amino acid residue confirmed its antisickling potentials due to observed variation in perturbation between the bound (DeOxyHbS-5-MPS) and unbound (DeOxyHbS) simulation studies whereas the ADMET showed that 5-MPS is a potential CYP1A2 and CYP2D6 inhibitor. The results suggest that 5-MPS is a potential antisickling drug candidate.

***Keywords*:** Sickle Cell Disease, Root Mean Square Deviation, Radius of Gyration, Antisickling, Potential Energy

Introduction

Low antioxidant level and related oxidative stress is the result of SCD is caused by erythrocyte distortion, which results in serious clinical implications such as microvascular occlusion, anemia, excruciating pain, renal failure, infections, ischemia, priapism, strokes, and pulmonary hypertension [1, 2]. The condition was named for the structural change of red blood cells (RBCs) from a biconcave disc to rigid elongated crescents. A point mutation in sickle cell hemoglobin (HbS) causes the sixth amino acid of the β chains to convert from hydrophilic glutamic acid to hydrophobic valine [3-5]. This structural alteration leads DeOxyHbS to aggregate into long straight rods, which deforms the RBC [1]. In the oxy form, HbS functions like regular hemoglobin, however at low oxygen tension i.e. in the deoxy form, the resultant Val6 on one hemoglobin complementary site binds hydrophobically with Phe85 and Leu88 from an adjacent hemoglobin molecule [1]. This action can be described by a lock and key binding mechanism in which valine on the surface of the hemoglobin structure connects to a complementary location on another hemoglobin molecule, thereby initiating the polymerization process. Perutz's extensive research into hemoglobin conformational changes reveals that oxy and deoxy hemoglobin have different quaternary structures [6].

*Corresponding author. E mail: ikechukwuijoma@gmail.com
Tel: +2348144250562

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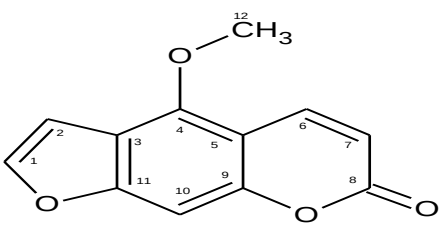
According to research, any harmless chemical that can bind to the region implicated in sickling is likely to significantly modify the binding site and prevent sickling [7]. Although sickling is not caused solely by a single complimentary site, additional molecular binding sites play an important role, therefore, interfering with any of these contact points may avert sickling. Researchers have demonstrated that a biomolecule's ability to prevent in vitro polymerization is dependent on its efficiency and likelihood to interact with DeoxyHbS monomer's complimentary contact region, alteration of amino acid residues that play a key role in HbS contact region and other critical sites' three-dimensional structures, and stabilization of the HbS molecule's R (relaxed) state [8-10]. Thus, based on molecular standpoint, protein aggregation is dependent on a delicate equilibrium of hydrophobic and electrostatic (i.e. primarily non-contact) interactions brought about by osmolytes and water [11, 12], which regulate protein activity. As a result, knowing the molecular mechanisms and thermodynamics of protein aggregation is crucial for developing treatment techniques and designing inhibitors. Therefore, the specific aim of this research is to comprehend in silico the antisickling mechanism of 5-MPS (Figure 1) derived from *Ficus thonningii*, a renowned antisickling plant, which crude leaf methanol extract function via the sickle hemoglobin polymerization inhibition route [13].



($C_{12}H_8O_4$)

Figure 1: Structure of 5-MPS isolated from *F. thonningii* leaves as reported by Ijoma and Ajiwe [14]

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Materials and Methods

The molecular docking used AutoDock tools via Vina script. Molecular dynamic simulation was carried out using Nanoscale molecular dynamic (NAMD) software. Both methods were based on a procedure described by Ijoma and Ajiwe [7]. PDB file was used for both receptor and ligand. The structure of 5-MPS was drawn using Chemsketch then optimization and energy minimization of ligand was carried out using Avogadro software. Autodock tool GUI (Graphical User Interface) was used to create a PDBQT file for 2HBS. Ligand bonds were set to rotatable. The docking procedure was based on the Lamarckian Genetic Algorithm (LGA) method. The docking was performed using the blind docking procedure. The docking conformation with the lowest binding energy was chosen for further analysis using BIOVIA Discovery Studio and PyMol [7].

Molecular dynamics (MD) simulation study employed the generalized CHARMM27 all-force field parameters. Energy minimization was done through the steepest descent algorithm under the NVT ensemble followed by the NPT ensemble. On completion of the simulation, five hundred structures at intervals of 1 ps each were considered for further analysis using Visual molecular dynamics (VMD) and Microsoft Excel [7]. Parameters such as root mean square deviation (RMSD), radius of gyration (RoG), solvent accessible surface area (SASA), potential energy (PE), electrostatic internal energy (EIE) and Van der Waal (VDW) energy were calculated for both apo and holo simulations.

ADME prediction was evaluated using the SwissADME webserver (http://swissadme.ch). The toxicity assay was profiled using the Protox-II webserver (https://tox.charite.de). The crystal structure of Deoxyhemoglobin S (DeOxyHbS) used was PDB file 2HBS obtained from the protein data bank repository (https://rcsb.org) based on advanced BLAST analysis.

Results and Discussion

Molecular docking analysis

Figure 2 shows the 2-dimensional DeOxyHbS-5-MPS binding interactions. Research suggest that VDW interactions are involved in the uptake of oxygen by deoxyhemoglobin [6, 15]. 5-MPS made significant VDW interaction with βCys93 which is a vital target in the design of DeOxyHbS polymerization inhibitors [16]. 5-MPS interaction with βcys93 stabilizes the relaxed (R) state and distorts the salt-bridge interaction between β-His146 and β-Asp94 hence, destabilizing the tense (T) state. Similar mechanism was reported by Nakagawa *et al.* [17] for triazole disulfide compounds. Compounds that modifies βcys93 has been shown to possess antisickling potentials [17] since hemoglobin's allosteric properties, antisickling, and oxidative modulations is mediated significantly by βCys93 [18-20]. Thus, βCys93 occupies a significant and critical location at the β/α terminal and it's critically participates in the hemoglobin R → T transition. Therefore, compounds that modifies the allosteric equilibrium through βCys93 interactions are known to possess both antisickling and antioxidant properties [16].

Figure 3 depicts the Interaction of DeOxyHbS-5-MPS with binding site amino acids and the corresponding bond length of these interactions. The bond length for 5-MPS-HIS97 interaction was 2.02Å while 5-MPS-LYS40 had bond length of 2.06Å and both were within typical hydrogen bond interaction. 5-MPS-LYS40 bond had bond length of 4.62Å, 5-MPS-LYS40 had bond length of 4.80Å, 5-MPS-LEU48 had bond length of 3.62Å, 5-MPS-LEU48 bond length of 3.79Å, whereas 5-MPS-THR41 was 3.19Å (figure 3).

Previous research showed that hydrophobic group such as Leu48 are known to affects the solubility of hemoglobin [21]. Lys40 has been reported as part of the critical residue in the formation of deoxyhemoglobin tetrameric interaction with each other and with all the four heme groups [22] as such, interactions with Lys40 has the potentials of distorting or delaying the polymerization of DeOxyHbS. Similarly, Lalezari *et al.*, [23] reported Lys, Leu, Pro, Phe, Thr, Arg, Glu and Asp as some of the potential binding site residues for allosteric effector of hemoglobin capable of modifying human deoxyhemoglobin solubility.

A Study found that the FG corner residue β2His97, situated between α1Pro44 and α1Thr41 in the T structure's "switch region" adjust to locate α1Thr41 and α1Thr38 in the R structure, where β2His97 initiates hydrogen-bond contact with α1Thr38 [15]. Therefore, the interaction of 5-MPS must have to destabilize the interdimer salt-bridge and/or hydrogen bond formed by β2His97, α1Pro44, and α1Thr41 and thus will promote T→R transition because deoxyhemoglobin is constrained by salt bridges [24] subsequently, this will distort/delay polymer formation.

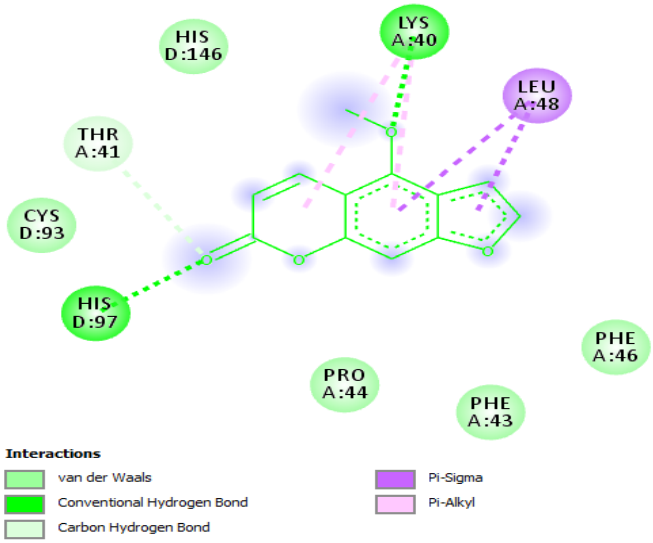

Figure 2: DeOxyHbS-5-MPS binding interactions

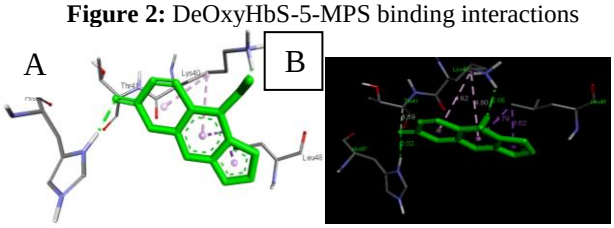

Figure 3: Interaction of DeOxyHbS-5-MPS showing binding site amino acids (A) and their corresponding bond length (B)

The Classical studies suggest that structural variation in histidine interactions leads to changes in the solubility of deoxyhemoglobin [21, 25]. Bond formation by ligands can destabilize deoxyhemoglobin [26]. But since an allosteric effector can bind to the same site and produce an opposite allosteric effect it implies that the direction in the shift in allosteric equilibrium caused by the binding of an allosteric effector do not solely depends on the molecule's binding location but also on how its interactions with the hemoglobin dimer–dimer interface favors the stabilization or destabilization of that allosteric state [15] hence, binding does not connote positive activity/effect. Reports have shown that interaction with histidine opens up deoxyhemoglobin for oxygen uptake [25] thus, 5-MPS may possess oxygen regulatory functions as an effector ligand because drugs have been shown to act on the allosteric equilibrium of a receptor in the same path as the natural effector even though they differ chemically because protein may probably offer various binding sites not used in nature [27].

Figure 4 illustrates the pose view of 5-MPS in the DeOxyHbS allosteric core. The binding affinity of 5-MPS in 2HBS is –6.4 kcal/mol (Figure 4). Ross *et al.* [28] and Ross *et al.* [29] reported the binding affinity for the DeOxyHbS polymerization process as -3.0kcal/mol at 37ºC hence, the molecular docking results show a better interaction for 5-MPS and thus suggest that it can distort the polymerization process because, smaller binding affinity signifies better interaction and better receptor activity *in vitro* [30, 31]. Votano and Rich, [32] showed a strong correlation between DeOxyHbS solubility and dissociation constant this suggests that the interactions of 5-MPS may play a key role in DeOxyHbS solubility

Over time, research has established that ligand binding to receptors' stereochemistry is defined by accessible VDW space and, within that space, by arrays of polarity interactions. However, the detailed stereochemistry is geared towards maximizing the sum of the energy of electrostatic interactions, by aligning effectors relative to the receptor to maximize mutual polarizabilities [25]. Ideally, the structure of the

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ligand-receptor complex affects the specificity and efficiency of the target (protein) action with high binding affinity marked by lower energy values, connoting better interaction between ligand and receptor hence, presumably better activity [30, 31].

Figure 5 indicates the binding interaction of DeOxyHbS-5-MPS in the presence of surrounding amino acid residues. Bonding and non-bonding interactions are within 4.80Å. Summarily, the sum of binding interactions of 5-MPS indicates the presence of VDW interactions as a contributing factor to deoxyhemoglobin polymerization (Figure 2 and Figure 5) and VDW interactions play a vital role in sickle hemoglobin polymerization [33]

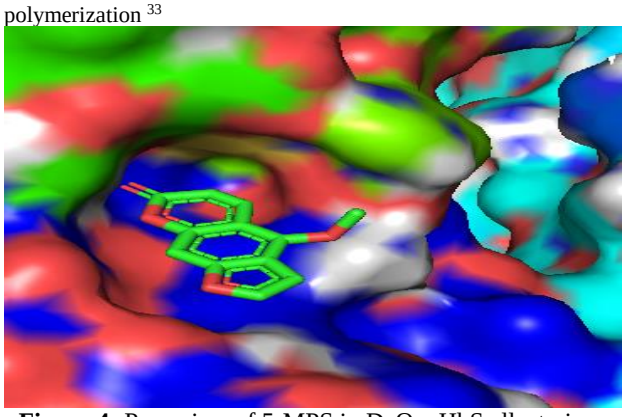

Figure 4: Pose view of 5-MPS in DeOxyHbS allosteric core

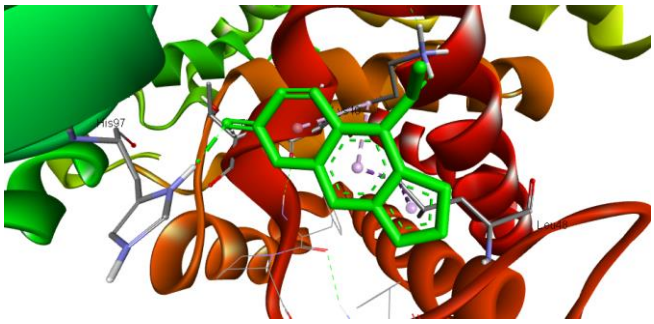

Figure 5: Binding interaction of DeOxyHbS-5-MPS in the presence of surrounding amino acid residues.

Molecular Dynamics

Figure 6 illustrates the plotting of the RMSD change as a function of simulation time in Pico seconds (ps). After the least-squares best fit, the RMSD of DeOxyHBS and DeOxyHbS-5-MPS from the beginning to the end of the 500 ps production run was computed in order to assess the overall behavior of the examined systems as a measure of stability. Throughout the simulation trajectory, the overall RMSD of the DeOxyHbS-5-MPS model was greater than that of DeOxyHbS. This is anticipated to line up with the structural modification brought about by 5-MPS docking. Based on the plotted RMSD fluctuations, significant conformational changes were observed in the trajectories which signify changes in the structure of DeOxyHbS. The mean RMSD for DeOxyHbS and DeOxyHbS-5-MPS was 2.541Å and 3.678Å respectively showing a deviation of 1.137Å. The RMSD variations of the two systems on 500 ps time scales MD simulation indicate that the complex atomic coordinates in DeOxyHbS-5-MPS and the initial starting structure differ hence, indicating structural perturbation due to 5-MPS docking

Figure 7 shows the RoG values of DeOxyHbS and DeOxyHbS-5-MPS simulations, the RoG shows the degree of protein structure compactness [34]. The RoG value of DeOxyHbS-5-MPS was 13.922 Å, which was significantly higher than that of DeOxyHbS (8.075 Å), this change was attributed to the binding of 5-MPS to DeOxyHbS structure. Galamba and Pipolo [33] attributed the sickle hemoglobin aggregation to residue-residue interaction, residue-residue repulsion, PE, electrostatic interactions, and VDW interactions; in their simulation, they observed that close compact dimer-dimer interactions were responsible for sickle hemoglobin aggregation, this observations were supported by earlier work of Prabhakaran and Johnson [35]. Prabhakaran and Johnson [35] at 50 ps MD simulation observed that HbS structure is more compact when compared to HbA, they also attributed sickle hemoglobin polymerization *in vitro* to their observation.

From Figure 7, RoG for DeOxyHbS-5-MPS increased from the minimized starting structure value of 5.061 Å during the simulation to a mean value of about 20.749Å. It was observed that DeOxyHbS exhibited significant contraction when compared to DeOxyHbS-5-MPS. However, a graphical examination of the trajectory showed that both DeOxyHbS and DeOxyHbS-5-MPS simulations did not maintain their general structural shapes during the simulation run indicating possible changes in structural conformations.

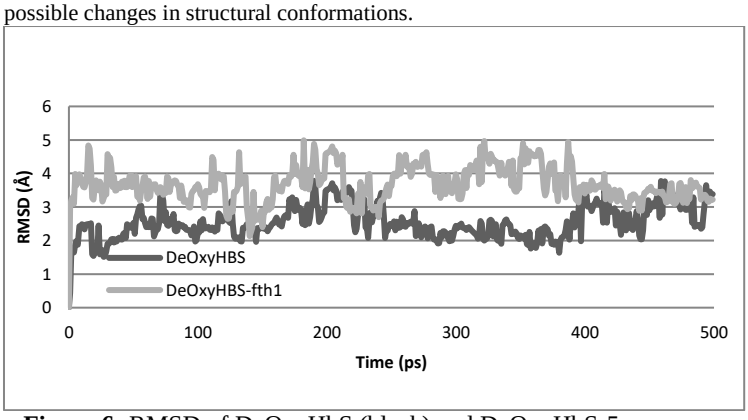

Figure 6: RMSD of DeOxyHbS (black) and DeOxyHbS-5-MPS (gray) simulations

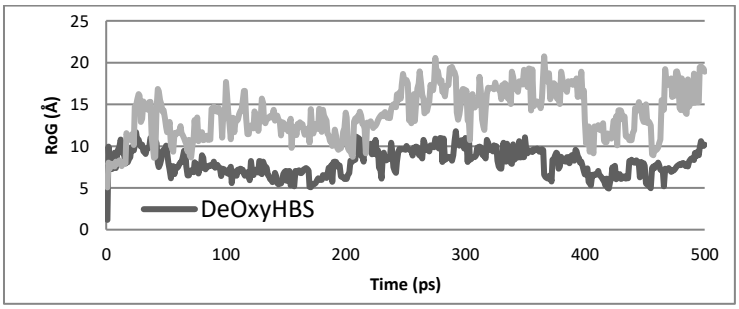

Figure 7: RoG of DeOxyHbS (black) and DeOxyHbS-5-MPS (gray) simulations

Figure 8 displays the computed SASA values along the MD simulation trajectories for DeOxyHbS and DeOxyHbS-5-MPS. Surface tension close to the protein–solvent interface as well as the hydrophobic and hydrophilic interactions of the amino acids with water molecules determine the values of SASA [36]. An increase or decrease in SASA denotes an alteration in the protein's tertiary structure and surface exposure of the amino acid residues [34]. DeOxyHbS-5-MPS have larger SASA compared to DeOxyHbS indicating a possible change in the tertiary structure of DeOxyHbS on binding 5-MPS. The computed average SASA for the DeOxyHbS-5-MPS simulation was 197.7926Å^2 while that of the DeOxyHbS simulation was calculated as 146.267Å^2

Figure 9 depicts the VDW and EI energies of DeOxyHbS-5-MPS and DeOxyHbS for apo and holo simulations while Figure 10 shows the PE plot for DeOxyHbS-5-MPS and DeOxyHbS simulations. From Figure 9, the VDW (upper graph) and EI energy (lower graph) were calculated using the Charmm force field generated using Charmm GUI, and analysis of the generated trajectory was performed using the NAMD energy tool in VMD [37, 38]. From the observed variation in the simulated trajectory, there is an increase in the VDW energy and a subsequent decrease in the EI energy in the bound region for the DeOxyHbS-5-MPS simulation. The variation in VDW energy has been attributed to the distortion of the hemoglobin dimer-dimer interaction [33] therefore, although the VDW interactions are much weaker when compared to electrostatic interactions however, their sum significantly influences the bound regions PE (Figure 10). The decrease in the EI energy indicates

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that 5-MPS was able to reduce the PE of the residues in the bound region through direct molecular electrostatic interaction and indirect association (VDW) therefore the docking of 5-MPS to DeOxyHbS reduced the EI energy of these residues and thus confirms the antisickling characterization of 5-MPS *in vitro*. Similarly, it is important to highlight that L-Glutamine, licensed for the treatment of SCD is not predicated on an antisickling mechanism of this kind, which may upset the electrostatic balance [39-41]. The calculated PE for DeOxyHbS and DeOxyHbS-5-MPS simulations were -435.725kJ/mol and -597.509kJ/mol respectively. For the DeOxyHbS-5-MPS simulation, the computed EI energy was -535.629kJ/mol while VDW was -61.8798kJ/mol. Similarly, for the DeOxyHbS simulation the computed EI energy was -334.755 while VDW was -100.97kJ/mol

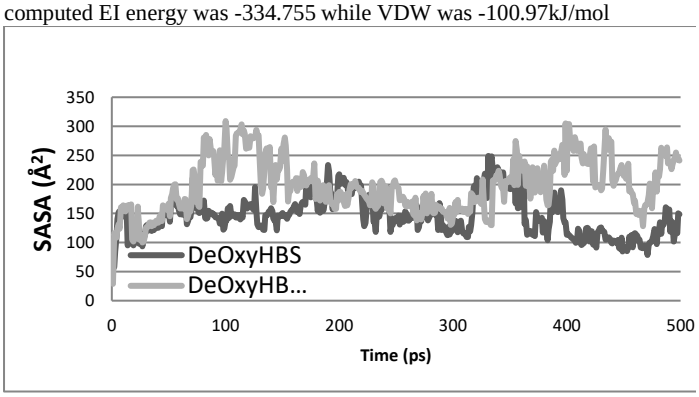

Figure 8: SASA of DeOxyHbS (black) and DeOxyHbS-5-MPS (gray) simulations

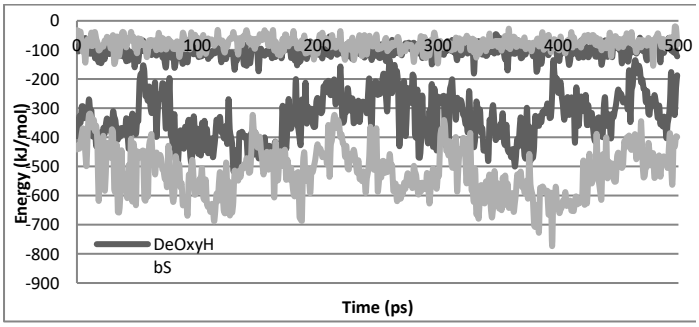

Figure 9: VDW and EI energies of DeOxyHbS-5-MPS (gray) and DeOxyHbS (black)

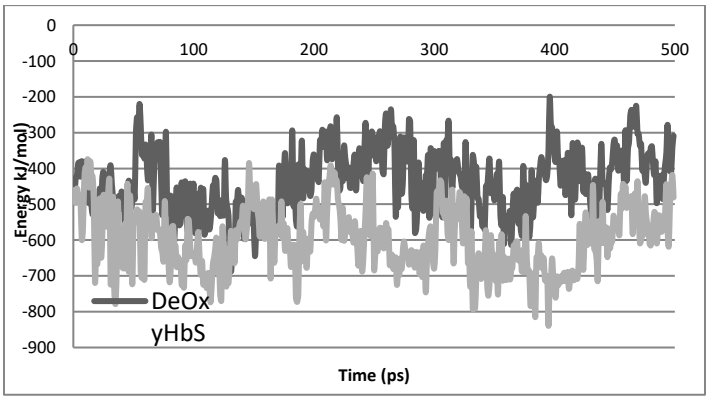

Figure 10: PE plot for DeOxyHbS-5-MPS (gray) and DeOxyHbS (black)

Admet analysis

Table 1 depicts the physicochemical, Pharmacokinetics, drug-likeness, lead-likeness, and medicinal chemistry properties of the assayed compound. 5-MPS showed no violation for the Lipinski, Ghose, Veber, Egan, and Muegge rules indicating its drug-like characteristics however, the lead-likeness filter had one violation owing to molecular weight being < 250. Also, it showed one violation of BRENK alert because of the presence of coumarin hence, 5-MPS may probably be chemically and metabolically unstable [42]as well as supposedly toxic [43] however, this may not be of serious concern because the structural moiety producing the alert i.e. coumarin however, this should be factored into prioritization of 5-MPS for drug-likeness attributes. 5-MPS showed a bioavailability score of 55% indicating its chances to reach the systemic circulation.

Table 2 reveals the predicted toxicity of 5-MPS. 5-MPS was inactive on hepatotoxicity, immunotoxicity, and cytotoxicity parameters while active on carcinogenicity and mutagenicity (Table 2). Evidence from our in silico results corroborates the classification of 5-MPS as a mutagenic and carcinogenic agent [43]. Similarly, *in vitro*, 5-MPS has been implicated as a potent cytochrome P450 inhibitor [44] as evidenced by Table 1. Also, the reduced function of CYP2D6 has been implicated as a contributory factor in painful episodes in SCD [45]. Based on oral toxicity 5-MPS was classified as not toxic with LD50 of 8000mg/kg (Table 3).

Table 1: ADME analysis of 5-MPS showing the physicochemical, Pharmacokinetics, drug-likeness, lead-likeness and medicinal chemistry properties of 5-MPS

Physicochemical properties	Lipophilicity	Water solubility	Pharmacokinetics	Drug-likeness	Medicinal chemistry
Formula:	**LogPo/w (iLOGP):**	Moderately soluble	**GI absorption:**	**Lipinski:**	**PAINS:**
$C_{12}H_8O_4$	2.29		High	Yes; 0 violation	0 alert
Molecular weight:	**LogPo/w (XLOGP3):**		**BBB permeant:**	**Ghose:**	**Brenk:**
216.19g/mol	1.93		Yes	Yes; 0 violation	1 alert; coumarin
No. of heavy atoms:	**LogPo/w (WLOGP):**		**P-gp substrate:**	**Veber:**	**Leadlikeness:**
16	2.55		No	Yes; 0 violation	No; 1 violation: MW < 250
No. of aromatic heavy atoms:	**LogPo/w (MLOGP):**		**CYP1A2 inhibitor:**	**Egan:**	**Synthetic accessibility:**
13	1.18		Yes	Yes; 0 violation	2.90
No. of rotatable bond:	**LogPo/w (SILICOS-IT):**		**CYP2C19 inhibitor:**	**Muegge:**	
1	2.88		No	Yes; 0 violation	

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No. H-bond acceptors:	**Consensus LogPo/w:**	**CYP2C9 inhibitor:**	**Bioavailability**
4	2.16	No	**score:**
			0.55
No. of H-bond donors:		**CYP2D6 inhibitor:**	
0		Yes	
Molar refractivity:		**CYP3A4 inhibitor:**	
58.75		No	
TPSA:		**Log Kp:**	
52.58Å^2		-6.25cm/s	

TPSA: Topological polar surface area; GI: gastrointestinal; BBB: Blood brain barrier; P-gp: P-glycoprotein; CYP: cytochrome P450; PAINS: pan assay interference structures; MW: molecular weight;

Table 2: Protox-II predicted toxicity of 5-MPS

Classification	Target	Prediction	Probability
Organ toxicity	Hepatotoxicity	Inactive	0.79
Toxicity end points	Carcinogenicity	Active	0.84
	Immunotoxicity	Inactive	0.83
	Mutagenicity	Active	0.75
	Cytotoxicity	Inactive	0.85
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon receptor	Active	1.0
	Androgen receptor	Inactive	0.99
	Androgen receptor ligand binding domain	Inactive	1.0
	Aromatase	Inactive	0.99
	Estrogen receptor alpha	Inactive	0.90
	Estrogen receptor ligand binding domain	Inactive	0.99
	Peroxisome proliferator activated receptor gamma	Inactive	0.99
Tox-21 stress response pathway	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf/ARE)	Inactive	0.99
	Heat shock factor response element (HSE)	Inactive	0.99
	Mitochondria membrane potential (MMP)	Inactive	0.92
	Phosphoprotein (tumor suppressor) p53	Inactive	0.98
	ATPase family AAA domain-containing protein 5 (ATAD5)	Inactive	0.90

Table 3: Protox-II predicted oral toxicity of 5-MPS

Predicted LD_{50}	Predicted toxicity class	Average similarity	Prediction accuracy
8000mg/kg	6	100%	100%

Conclusion

The antisickling mechanism of 5-MPS was studied in silico. It was reviewed that it binds to DeOxyHbS and interacts with βcys93. Also, compounds that modify βcys93 are viable antisickling agents, thus implying that it may possess the ability to potentially modify βcys93. Similarly, molecular dynamic simulations studies showed an overall increase in the RMSD, SASA and RoG of DeOxyHbS-5-MPS relative to DeOxyHbS which suggest that 5-MPS perturbs DeOxyHbS structure, modifies its tertiary structure and makes it less compact. Furthermore, the EI energy, VDW, and PE for DeOxyHbS-5-MPS were also, lower than DeOxyHbS. This depicts 5-MPS's ability to reduce the PE of the amino acids at the bound region of DeOxyHbS including βcys93. ADMET analysis suggests that 5-MPS is potentially mutagenic and carcinogenic. Summarily, the mechanism of 5-MPS is through modifying βcys93 by increasing the RoG of DeOxyHbS resulting in the modification of its tertiary structure, this generally results in the overall decrease in the potential energy at the bound region of 5-MPS resulting in sickle polymerization inhibition/reversal. Our observations suggest that 5-MPS may be a useful drug candidate for the inhibition of sickle hemoglobin polymerization, improve erythrocyte solubility, or act as a potential deoxyhemoglobin S allosteric effector however, the ADMET properties should be factored in while considering its drug-like uses. Future studies should carry out in vivo, ex vivo, and clinical trials on the assayed compound and its synthetic analogue.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Phanus-umporn C, Shoombuatong W, Prachayasittikul V, Anuwongcharoen N, Nantasenamat C. Privileged substructures for antisickling activity via cheminformatic analysis. RSC Adv. 2018; 8: 5920–5935
2. Abere TA, Okoye CJ, Agoreyo FO, Eze GI, Jesuorobo RI, Egharevba CO, Pauline O, Aimator PO. Antisickling and toxicological evaluation of the leaves of *Scoparia dulcis* Linn (Scrophulariaceae). BMC Compl Altern Med. 2015; 15:414
3. Eaton WA. Hemoglobin S polymerization and sickle cell disease: A retrospective on the occasion of the 70th anniversary of Pauling's science paper. Am J. Hematol. 2020; 95: 205-211
4. Madigan C, Malik P. Pathophysiology and therapy for haemoglobinopathies. Part I: Sickle cell disease. Expert Rev Mol Med. 2006; 8, 1–23
5. Safo MK, Abdulmalik O, Danso-Danquah R, Burnett JC, Nokuri S, Joshi GS, Musayev FN, Asakura T, Abraham DJ. Structural basis for the potent antisickling effect of a novel class of five-membered heterocyclic aldehydic compounds. J. Med Chem. 2004; 47(19): 4665–4676
6. Perutz MF. Mechanisms regulating the reactions of human hemoglobin with oxygen and carbon monoxide. Annu Rev Physiol. 1990; 52:1-25
7. Ijoma KI, Ajiwe VIE. Methyl ferulate induced conformational changes of DeoxyHbS: Implication on sickle erythrocyte polymerization. Mediterr J. Chem. 2022; 12(1):100-111
8. Oyewole O, Malomo SO, Adebayo, JO. Comparative studies on antisickling properties of thiocyanate, tellurite and hydroxyurea. Pak J. Med Sci. 2008; 24: 18-22.
9. Ibraheem NK, Ahmed JH, Hassan MK. The effect of fixed oil and water extracts of *Nigella sativa* on sickle cells: an *in vitro* study. Singapore Med J. 2010; 51(3):230–234
10. Chikezie CP. Sodium metabisulfite–induced polymerization of sickle cell hemoglobin incubated in the extracts of three medicinal plants (*Anacardium occidentale, Psidium guajava*, and *Terminalia catappa*). Pharmacogn Mag. 2011; 7(26):126–132.
11. Ball P. Water Is an Active Matrix of Life for Cell and Molecular Biology. Proc Natl Acad Sci. 2017; 201703781.
12. Bellissent-Funel MC, Hassanali A, Havenith M, Henchman R, Pohl P, Sterpone F, Van der Spoel D, Xu Y, Garcia AE. Water Determines the Structure and Dynamics of Proteins. Chem. Rev. 2016; 116: 7673–7697.
13. Ijoma KI, Ajiwe VIE, Ndubuisi JO. Evidenced based preferential *in vitro* antisickling mechanism of three Nigerian herbs used in the management of sickle cell disease. Malays J. Biochem Mol Biol. 2023; 3, 9-17
14. Ijoma KI, Ajiwe VIE. Antibacterial activity of *Ficus thonningii* leaves extracts against some selected pathogenic bacterial prevalent in sickle cell anemia patient. Jordan J. Pharm Sci. 2023; 16(2): 345-354
15. Safo MK, Ahmed MH, Ghatge MS, Boyiri T. Hemoglobin-Ligand binding: Understanding Hb Function and Allostery on atomic level. Biochim Biophy Acta 2011; 1814: 797 –809
16. Kassa T, Wood F, Strader MB, Alayash AI. Antisickling Drugs Targeting βCys93 Reduce Iron Oxidation and Oxidative Changes in Sickle Cell Hemoglobin. Front Physiol. 2019; 10:931
17. Nakagawa A, Ferrari M, Schleifer G, Cooper MK, Liu C, Yu B, Berra L, Klings ES, Safo RS, Chen Q, Musayev FN. A triazole disulfide compound increases the affinity of hemoglobin for oxygen and reduces the sickling of human sickle cells. Mol Pharm. 2018;15, 1954–1963
18. Jana S, Strader MB, Meng F, Hicks W, Kassa T, Tarandovskiy I, De Paoli S., Simak J, Heaven MR, Belcher JD, Vercellotti GM, Alayash AI. Hemoglobin oxidation-dependent reactions promote interactions with band 3 and oxidative changes in sickle cell-derived microparticles. JCI Insight 2018; 3:120451
19. Jia Y, Buehler PW, Boykins RA, Venable RM, Alayash AI. Structural basis of peroxide-mediated changes in human hemoglobin: a novel oxidative pathway. J. Biol Chem. 2007; 282: 4894–4907
20. Omar AM, Mahran MA, Ghatge MS, Chowdhury N, Bamane FH, El-Araby, ME, Abdulmalik, O, Safo M. Identification of a novel class of covalent modifiers of hemoglobin as potential antisickling agents. Org Biomol Chem. 2016; 13, 6353–6370
21. Adachi K, Konitzer P, Kim J, Welch N, Surrey S. Effect of beta 6 aromatic amino acids on polymerization and solubility of recombinant Hemoglobins made in yeast. J. Biol. Chem. 1993; 268 (29): 21650-21656
22. Garret, R.H., and Grisham, CM. (2016). Biochemistry 6th edition. Cengage Learning, Pp1280
23. Lalezari I, Lalezari P, Poyart C, Marden M, Kister J, Bohn B, Fermi, G, Perutz MF. New effectors of Human Hemoglobin: Structure and Function. Biochem. 1990; 29: 1515-1523
24. Perutz MF. Stereochemistry of Cooperative Effects in Haemoglobin: Haem–Haem Interaction and the Problem of Allostery. Nat. 1970; 228, 726–734.
25. Perutz MF. Mechanisms regulating the reactions of human hemoglobin with oxygen and carbon monoxide Annu Rev Physiol. 1990; 52:1-25
26. Fermi G, Perutz MF, (1981). Atlas of Molecular Structures in Biology: Haemoglobin & Myoglobin. Oxford: Clarendon. pp 104
27. Perutz MF, Fermi G, Abraham DJ, Poyart C, Bursaux E. Hemoglobin as a Receptor of Drugs and Peptides: X-Ray Studies of the Stereochemistry of Binding. J. Am. Chem. Soc. 1986; 108, 1064–1078
28. Ross PD, Hofrichter J, Eaton WA. Calorimetric and optical characterization of sickle cell hemoglobin. J. Mol. Biol. 1975; 96(2): 239-256.
29. Ross PD, Hofrichter J, Eaton WA. Thermodynamics of gelation of sickle cell hemoglobin. J. Mol. Biol. 1977; 115(2): 111-134
30. Abdulfatai U, Uzairu A, Uba S. Molecular docking and quantitative structure-activity relationship study of anticonvulsant activity of aminobenzothiazole derivatives. Beni-Suef Univ J. Basic and Appl. Sci. 2018; 7: 204-214
31. Vijesh AV, Isloor AM, Telkar S, Arulmoli T, Hoong-kun F. Molecular docking studies of some new imidazole derivatives for antimicrobial properties. Arab J. Chem. 2013; 6: 197-204
32. Votano JR, Rich A. Inhibition of Deoxyhemoglobin S Polymerization by Biaromatic Peptides Found to Associate with the Hemoglobin Molecule at a Preferred Site. Biochem. 1985; 24: 1966-1970
33. Galamba N, Pipolo S. On the Binding Free Energy and Molecular Origin of Sickle Cell Hemoglobin Aggregation, J. Phys Chem B 2018; 2-30
34. Gupta A, Agarwal R, Singh A, Bhatnagar S. Calcium-induced conformational changes of Thrombospondin-1 signature domain: implications for vascular disease. J. Recept Signal Transduct Res. 2017; 1-12
35. Prabhakaran M, Johnson ME. Molecular Dynamics of Sickle and Normal Hemoglobins. Biopolymers 1993; 33: 735-742
36. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ. GROMACS: fast, flexible, and free. J. Comput Chem. 2005; 26:1701–1718

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37. Kukol, A. (2016). NAMD/VMD tutorial (uses VMD 1.9.2, NAMD 2.10_Win64-multicore) Molecular dynamics simulation of 'protein folding. University of Hertfordshire School of Life and Medical Sciences
38. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J. Mol Graph. 1996; 14:33–38.
39. Niihara Y, Zerez CR, Akiyama DS, Tanaka KR. Increased Red Cell Glutamine Availability in Sickle Cell Anemia: Demonstration of Increased Active Transport, Affinity, and Increased Glutamate Level in Intact Red Cells. J. Lab Clin Med. 1997; 130, 83–90
40. Niihara Y, Zerez CR, Akiyama DS, Tanaka KR. Oral L-Glutamine Therapy for Sickle Cell Anemia: I. Subjective Clinical Improvement and Favorable Change in Red Cell NAD Redox Potential. Am J. Hematol. 1998; 58: 117–121
41. Ortiz de Montellano PRA (2017). New Step in the Treatment of Sickle Cell Disease: Published as Part of the *Biochemistry* Series "Biochemistry to Bedside." Biochem. (Mosc.) 2017; 57: 470–471
42. Ononamadu CJ, Ibrahim A. Molecular docking and prediction of ADME/drug-likeness properties of potentially active antidiabetic compounds isolated from aquoues-methanol extracts of Gymnema sylvestre and Combretum micranthum. BioTechnologia, 2021 102(1):85-89
43. *WHO-IARC. Monograph on the evaluation of carcinogenic risk of chemicals to humans. P. S7 66. https://monographs.iarc.who.int/agents-classified-by-the-iarc/ Retrieved January, 2023*
44. Aldred E. (2009). Haschek and Rousseaux's Handbook of Toxicologic pathology (third edition). Churchill livingstone
45. Brousseau DC, Panepinto JA. Sickle cell pain crisis: the effect of CYP2D6 polymorphism. Blood 2005; 106 (11): 2318