Molecular Docking Studies of 3-thioindoles as Potent Antiviral and Antibacterial Agents

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***Abstract*—Viral and bacterial infections are still prevalent worldwide. Infection with the Human Immunodeficiency Virus (HIV) causes Acquired Immunodeficiency Syndrome (AIDS), and in 2023, around 39.9 million people across the globe were living with HIV/AIDS. On the other hand, bacterial infections account for 15% of global deaths, despite the discovery of antibiotics, due to bacterial drug resistance. Developing new, selective, and safe inhibitors for the treatment of these diseases remains a high priority for medical research. Many indole derivatives have specifically been used in pharmaceutical applications. By targeting HIV-1 glycoprotein 120 (gp 120) and FabH (β-ketoacyl-acyl carrier protein (ACP) synthase III) enzymes, 3-sulfenylated indoles have shown promise in HIV-1 gp 120 and FabH inhibition, respectively. In this study, previously synthesized indole derivatives, using a newly developed protocol involving sulfa-Michael addition and intramolecular reductive N-heteroannulation, are assessed for their antiviral and antibacterial activity. Toxicity analysis and molecular docking is performed on twenty-seven indole derivatives to assess their affinity against the targets. Among the 27 compounds, compound 5b, phenyl(3-(p-tolylthio)-1*H*-indol-2-yl)methanone, with a binding score of -8.91 kJ, was found to be the most potent HIV-1 gp 120 inhibitor, while compound 5a, phenyl(3-(phenylthio)-1*H*-indol-2-yl)methanone, with a binding score of -7.96 kJ, showed the most promise against FabH inhibition. Interaction diagrams indicate that the affinity of the studied indole-based derivatives is governed by hydrogen bonding (H-bond), which occurs either with a nitrogen or oxygen atom of the indole group, as well as hydrophobic and pi interactions within the binding pocket. Upon comparison with compounds previously assessed for their HIV-1 gp 120 and FabH inhibition abilities, respectively (r1-r4), it is found that the new compounds compare favorably with the reference compounds in terms of binding score. Analysis of results show that the indole-based analogs can be a starting point for the synthesis of drugs designed as both antiviral and antibacterial agents.**

***Keywords*—FabH, HIV-1, indole derivative, molecular docking, toxicity analysis**

I. Introduction

Infection with Human Immunodeficiency Virus (HIV) causes the Acquired Immunodeficiency Syndrome (AIDS) which is observed worldwide. In 2023, there were approximately 39.9 million people across the globe with HIV/AIDS [1]. HIV infections are caused by one of two retroviruses, HIV-1 or HIV-2 with the former being the more common cause of infections worldwide [2]. At present, there is no known cure for this disease, however, several drugs designed to lower the level of HIV within the body are being administered [3]. Developing new, selective, and safe inhibitors for HIV treatment, remains a high priority for medical research [4]. Many inhibitors are being designed for HIV inhibition to find safe and effective drugs. Indole derivatives, specifically, have been considered as one class of promising HIV-1 inhibitors [5]. In a recent study, molecular docking was done on indole-based analogs as HIV-1 attachment inhibitors. The study showed that indole derivatives exhibit good potential for inhibition by specifically targeting the HIV-1 glycoprotein 120 (gp 120) which is one of the key targets for treatment of the disease [6].

Infections caused by bacteria are still a global cause of illness and death. The World Health Organization lists bacterial infections as the cause of approximately 15% deaths globally [7]. Though the discovery of antibiotics has helped combat this disease, the antibacterial effect has reduced significantly due to the rise of bacterial drug resistance. A study estimates that around 1.2 million people died from antibiotic-resistant bacterial infections in 2019, highlighting the urgent need for new antibiotics [8]. Identifying new antibacterial agents, including those targeting bacterial fatty acid biosynthesis has been a focus of several studies. Fatty acid biosynthesis is a vital metabolic pathway in bacteria. FabH (β-ketoacyl-acyl carrier protein (ACP) synthase III) enzyme, in particular, which catalyzes the first step in fatty acid biosynthesis, has been identified as a promising target for antibacterial agents [9–11]. Indoles have been evaluated as potential inhibitors of the FabH enzyme. Several studies have reported the inhibitory activity of indole-based derivatives against FabH in various bacterial strains, including *E. coli*, S. aureus, and M. tuberculosis [12].

Since indole derivatives show potential as HIV and FabH inhibitors, this study focuses on conducting molecular docking studies on synthesized thioindoles to be used as HIV and FabH attachment inhibitors. This investigation can pave way for the design of drugs which specifically target HIV and FabH.

Indoles are heteroaromatic compounds consisting of a fused benzene and pyrrole ring [13]. They are usually referred to as the "king of heterocycles" owing it to their presence in pharmaceuticals, agrochemicals, organic electronics, along with the vast of natural products alkaloids containing indole moieties [14]. Many indole derivatives have specifically been synthesized and used in pharmaceutical applications. 3-sulfenylated indoles specifically reveal a variety of biological properties, such as anti-HIV, anticancer, antiallergy, anti-obesity, anti-inflammatory, and cardiovascular activities as well as applications as building blocks in the syntheses of natural products and potent drugs. Synthesis of 3-sulfenylated indoles is typically done via transition-metal- or metal-

catalyzed sulfenylation of indoles in the presence of Ru, Pd, V, Cu, Co, Ni, or Mg employing sulfenylating reagents, such as sulfonyl chlorides, disulfides, thiols, and thiophthalimide. Transition-metal-free synthetic approaches such as iodine- or organophosphorus-catalyzed, base-promoted, and electro-catalytic sulfenylation of indoles with sulfenylating reagents have also been achieved. Recently, domino C-S/C-N bond formation using well-defined copper-phosphine complex catalyst demonstrated by preparing 2-benzoyl-3-sulfenylated indoles from commercially available 2-nitrochalcones and thiols which exhibited good chemoselectivity and broad substrate scope was done [15].

The synthesized indole products (1a–5e) by Tamargo, Kim & Lee require further investigation to determine its potential applications. Among these, compounds 5a–5d in particular have been synthesized for the first time and much is still unknown regarding its potential applications.

The study aims to conduct molecular docking studies of 3-thioindoles. Moreover, this study specifically aims to: assess the toxicity of the synthesized indole derivative products; perform molecular docking studies on the synthesized indole derivative products to assess its affinity for HIV-1 gp 120 inhibition; and perform molecular docking studies on the synthesized indole derivative product to assess its affinity for FabH inhibition.

II. Literature Review

A. Human Immunodeficiency Virus: Prevalence, Types and Treatment

The Human Immunodeficiency Virus (HIV) targets immune systems which weakens the body's defense against infection and some types of cancer. Immunodeficiency increases susceptibility to a wide range of infections, cancers, and diseases which normally healthy immune systems fight off. Infection with the virus causes the Acquired Immunodeficiency Syndrome (AIDS). AIDS is the most advanced stage of HIV infection and is defined by the development of certain cancers, infections, or other severe long term clinical manifestations. Having claimed about 42.3 million lives to date, HIV continues to be a major global public health issue [16]. By the end of 2023, there were approximately 39.9 million people across the globe infected with about 630,000 people who died from AIDS-related illnesses [1, 16].

HIV infections are caused by one of two retroviruses, HIV-1 or HIV-2. Most HIV infections worldwide are caused by HIV-1, however, several HIV infections in West Africa have been caused by HIV-2 [2]. HIV-1 and HIV-2 are genetically distinct viruses which can cause AIDS. They show significant sequence variation with only 55% nucleotide sequence identity in the viral genome [17]. Although there is no known cure at this time, advancements have been made in treatments which made HIV a manageable disease. HIV treatment is often referred to as antiretroviral therapy (ART), highly active antiretroviral therapy (HAART) or Antiretrovirals (ARVs) [18]. Antiretroviral therapy manages the infection by controlling viral load and disease evolution. HAART is also known as the 'AIDS cocktail therapy' because it involves a combination of two, three or more drugs in a treatment regimen, which typically includes Reverse Transcriptase Inhibitors (RTIs) [19]. However, some risk factors for diseases such as atherosclerotic Cardiovascular disease (CV) are associated with HIV therapy [20]. Drug treatment failures can also result because of the emergence of drug resistant virus strains [21]. Among the FDA-approved New Chemical Entities (NCEs) which target the various stages of HIV-1 replication, HIV-1 RTIs is predominant, demonstrating their importance. Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs) are the two categories of HIV-1 RTIs [22].

Developing new, selective, and safe inhibitors for HIV treatment, however, remains a high priority for medical research. The discovery of new drugs with improved potency, selectivity, and pharmacokinetic properties, reduced side effects, and new therapeutic targets is still necessary in combating the problems associated with the current treatments.

B. Antibacterial Infection: Prevalence and Treatment

Infections caused by bacteria are still a global cause of human illness and death. The World Health Organization lists bacterial infections as the cause of approximately 15% deaths globally [7]. Bacterial infections are illnesses which are caused by bacterial growth or toxins. These infections can affect any part of the body, such as the skin, gastrointestinal tract, lungs, heart, brain, and blood. They can be caused by a variety of bacteria, including streptococcus, staphylococcus, *E. coli*, and salmonella. Some symptoms of bacterial infections include fever, chills, fatigue, and headache. In severe cases, bacterial infections can lead to sepsis, a life-threatening condition where the body's immune system overreacts to the infection [23].

Bacterial infections can be treated with antibiotics. Despite the discovery of antibiotics to help combat the disease, the rise of bacterial drug resistance has significantly reduced the antibacterial effect. A study estimates that based on data from 132 countries, around 1.2 million people died from antibiotic-resistant bacterial infections in 2019, highlighting the urgent need for new antibiotics and more effective infection prevention and control measures [8].

Identifying new antibacterial agents, including those targeting bacterial fatty acid biosynthesis has been a focus of several studies. Fatty acid biosynthesis is a vital metabolic pathway in bacteria. Targeting this pathway has been shown to be an effective approach in inhibiting bacterial growth. FabH (β-ketoacyl-acyl carrier protein (ACP) synthase III) enzyme, in particular, which catalyzes the first step in fatty acid biosynthesis, has been identified as a promising target for antibacterial agents. FabH is an essential enzyme found in pathogens which acts as a homodimer and is responsible for initiating the fatty acid synthesis pathway, which controls the entire carbon chain and is crucial for bacterial survival [9–11]. The FabH enzyme is highly conserved for both sequence and structure across Gram-positive and Gram-negative bacteria. It has a distinct structure from human fatty acid synthesis. As a result, FabH has emerged as a promising target for antibacterial agents [12].

C. Indoles as HIV-1 gp 120 Inhibitors

Indole derivatives, specifically, have been considered as one class of promising HIV-1 inhibitors. Delavirdine, an indole derivative, was approved for HIV treatment by FDA

in 1997. Indolyl aryl sulfones used as potent class of NNRTIs were well reviewed in 2005 [4]. Several indole compounds have been identified with the potential to inhibit HIV-1 [5]. Various non-nucleoside reverse transcriptase inhibitors from indole-based α-amino acids were synthesized and determined to be efficient NNRTIs [24]. Patel *et al.*, in 2016, synthesized indole-based allosteric inhibitors for HIV-1 integrase which had good activity [25]. Indole derivatives as a promising HIV inhibitor was investigated by Chen *et al.* in 2022 [26]. Their analysis found that the indole derivates can hinder HIV reverse transcriptase activity by binding to specific pocket enzymes. Although the study concluded that indoles have a potential to become an HIV drug, more research is required to optimize the effectiveness and minimize toxicity. A recent study conducted molecular docking on indole-based analogs as HIV-1 attachment inhibitors. This study showed that indole derivatives exhibit good potential for inhibition by specifically targeting the HIV-1 glycoprotein 120 (gp 120) which is one of the key targets for treatment of the disease [6].

D. Indoles as FabH Inhibitors

Indoles have been examined for their ability to inhibit the FabH enzyme, which is a prospective target for antibacterial drugs. Studies have reported the inhibitory activity of indole-based derivatives against FabH in various bacterial strains, such as *E. coli*, *S. aureus*, and *M. tuberculosis* [12]. Several 3-(4-chlorophenyl)-1-(1H-indol-3-yl)prop- 2-en-1-ones compounds have been synthesized and biologically evaluated. The synthesized compounds were assessed for their anti-inflammatory activity using a carrageenan-induced rat paw edema model. Among the compounds, one showed the most potent anti-inflammatory activity due to its high reducing activity and very strong antibacterial activity against *E. faecalis* [27]. A study by He *et al.* [28] determined the ability of indole substituted compounds as potential inhibitors of the FabH enzyme. Some compounds were found to have highly potent inhibitory activity. The study concluded that indole-based compounds could serve as a starting point for the development of novel FabH inhibitors with potential antibacterial activity. In 2019, Jia *et al.* [29] synthesized a series of indole diketopiperazine alkaloids (indole DKPs) and determined the antimicrobial activity and Structure-Activity Relationship (SAR) of 24 indole DKPs. The in-silico study revealed some compounds showed significant binding affinity to the FabH protein from *E. coli*, which has been identified as the key target enzyme of Fatty Acid Synthesis (FAS) in bacteria. The indole compounds are not only promising as new antibacterial agents but also potential FabH inhibitors.

E. Indoles

1) Occurrence and conventional synthesis

Indoles are heteroaromatic compounds consisting of a fused benzene and pyrrole ring specifically the benzo[b]pyrrole. Since indoles are analogues of naphthalene, their basic reactivity patterns can be understood because of the fusion of an electron-rich protein pyrrole ring with a benzene ring. Indole reactions include electrophilic aromatic substitution (e.g., halogenation, nitration, C-acylation, and alkylation), N-alkylation, arylation, lithiation and subsequent transformations, and oxidation [13]. Indoles are usually referred to as the "king of heterocycles" owing it to their presence in pharmaceuticals, agrochemicals, organic electronics, along with the vast of natural products alkaloids containing indole moieties. Among these, alkaloids like gramine, strychnine and lysergic acid, amino acids and other bioactive compounds like tryptophan, tryptamine and serotonin and pharmaceuticals like vincristine, delavirdine and yohimbine are just some examples of compounds employing the indole [14]. Indoles comprise of heterocyclic ring systems which are widely studied for their broad range of applications in pathophysiological conditions such as cancer, microbial and viral infections, inflammation, depression, migraine, emesis, hypertension, etc. The molecular architecture of indoles is very interesting which makes them suitable candidates for drug development [30].

Many indole derivatives have specifically been synthesized and used in pharmaceutical applications. Some of the more important indole derivatives applied in the pharmaceutical industry are the nonsteroidal anti-inflammatory agent, indomethacin, and the β-adrenergic blocker, pindolol [13]. 3-sulfenylated indoles reveal a variety of biological properties, such as anti-HIV, anticancer, antiallergy, anti-obesity, anti-inflammatory, and cardiovascular activities as well as applications as building blocks in the syntheses of natural products and potent drugs [15]. Furthermore, the wide-ranging biological activities of indoles extends to its antiviral activity against some pathogenic virus such as Yellow Fever Virus (YFV) and Bovine Viral Diarrhea Virus (BVDV), Herpes Simplex Virus type-1 (HSV-1) and Human Cytomegalovirus (HCMV); antitumor activities against L1210, A549 and HCT8 cell lines, and to P388 leukemia cells; tubulin polymerization inhibitors; antimicrobial activities; 5-HT6 receptor antagonists; Severe Acute Respiratory Syndrome (SARS)-CoV 3CLpro inhibitors; and antituberculosis activity [4]. Synthesis of indoles typically start with an aromatic compound (either monosubstituted or ortho-disubstituted). Examples of indole synthesis processes include the Fischer indole synthesis from arylhydrazones and related sigmatropic syntheses, reductive cyclizations of nitrocompounds, the Madelung synthesis from anilides and related base catalyzed condensations, and transition-metal catalyzed cyclizations [13].

Synthesis of 3-sulfenylated indoles, specifically, is typically done via transition-metal- or metal-catalyzed sulfenylation of indoles in the presence of Ru, Pd, V, Cu, Co, Ni, or Mg employing sulfenylating reagents, such as sulfonyl chlorides, disulfides, thiols, and thiophthalimide. Transition-metal-free synthetic approaches such as iodine- or organophosphorus-catalyzed, base-promoted, and electro-catalytic sulfenylation of indoles with sulfenylating reagents have also been achieved [15]. Shi et.al. synthesized indoles through a DABCO (1,4- diazabicyclo[2.2.2]octane)-promoted decarboxylative acylation of cinnamic acids toward formation of cinnamides [31]. Despite this, there is still a high need for a more efficient and straightforward protocol. Finding the optimum reaction conditions for the two-step synthesis of novel amide-containing 3-thioindoles is essential.

2) Synthetic methodologies to access 2-Amido-3-thioindoles

Recently, domino C-S/C-N bond formation using well-defined copper-phosphine complex catalyst demonstrated by

preparing 2-benzoyl-3-sulfenylated indoles from commercially available 2-nitrochalcones and thiols which exhibited good chemoselectivity and broad substrate scope was done [15]. A schematic diagram of the protocol is shown in Fig. 1.

Fig. 1. Schematic Diagram of the protocol by Tamargo *et al.* [15].

F. Toxicity Analysis

Conducting an ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) investigation of compounds is one of the crucial steps in drug development. Toxicities of compounds are often investigated by doing animal experiments however, this is time-consuming and takes animal lives. A faster and cheaper alternative is the use of in silico toxicity predictions. In silico toxicity predictions rely on known toxicity date to develop a model which predicts the toxicity of new compounds. One compound can be active for multiple toxicity endpoints. When a chemical interacts with a protein target, it can interact with multiple proteins in varying affinities resulting in the activation of different functional pathways. The disturbed pathways have interconnected relationships which can lead to synergistic or mitigating effects on the system which could extend across organs, tissues, and cellular levels of connectivity, ultimately leading to severe and pronounced toxic profiles [32].

One such server used in toxicity analysis is ProTox-II which enables uncomplicated predictions of different levels of toxicity. It classifies the compounds according to the globally harmonized system of classification of labeling chemicals (GHS). The classification is as follows:

Class I: fatal if swallowed (LD50 ≤ 5)

Class II: fatal if swallowed (5 < LD50 ≤ 50)

Class III: toxic if swallowed (50 < LD50 ≤ 300)

Class IV: harmful if swallowed (300 < LD50 ≤ 2000)

Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000)

Class VI: non-toxic (LD50 > 5000)

where LD50 is the median lethal dose or the dose at which 50% of test subjects die upon exposure to a compound often given in mg/kg body weight.

The server also includes methods for prediction of four toxicological endpoints such as cytotoxicity, mutagenicity, carcinogenicity and immunotoxicity. Mutagenicity refers to the adverse effects of chemicals that change the genetic material, usually DNA, of an organism. Carcinogenicity refers to the adverse effects of chemicals that can cause cells to become cancerous by altering their genetic structure so that they multiply continuously and become malignant. While immunotoxicity refers to the adverse effects of chemicals that alters the functioning of the immune system upon exposure. All of the models are based on machine learning methods which predict the results with a confidence score [33].

G. Molecular Docking Studies

Molecular docking, widely used in drug discovery, is a bioinformatic modeling dealing with the interaction of two or more molecules (receptor, R and ligand, L) which results in a stable adduct. The 3D structure of the Receptor-Ligand (R-L) complex is predicted depending on their binding properties. Docking is done to attain the optimized receptor-ligand complex conformation with lesser binding free energy [34]. Drug binding is often expressed in terms of the dissociation constant, K_d, wherein

$$K_d = \frac{[R][L]}{[R-L]} \quad (1)$$

The inhibition constant, K_i, is also used to describe the binding affinity of molecule or macromolecule has for an enzyme or receptor. While K_d is a more general, all-encompassing term, K_i which also represents a dissociation constant more narrowly describes the binding of an inhibitor to an enzyme [35]. Smaller constant values are preferred. K_d/K_i values within the range of 1–100 nanomolar are considered good inhibitors while values within the range of 1–100 micromolar are generally good initial "hit" compounds. Consequently, binding free energy is given in terms of K_d using the equation:

$$\Delta G = -RT \log K_d \quad (2)$$

Often used for study of protein-ligand interactions and for drug discovery and development, computational docking starts with a target of known structure. Then, docking is used to predict the bound conformation and binding free energy of small molecules to the target. Docking software generate, rank and group together several possible adduct structures using a scoring function. AutoDock is a suite of free open–source software used for computational docking and virtual screening of small molecules to macromolecular receptors [36].

HIV-1 gp 120 is one of the key targets in inhibiting the virus. The envelope protein of HIV-1 gp120 is a crucial component in the multi-tiered viral entry process [37]. Various studies such as those by Tamamura *et al.* [38], Woo *et al.* [39], and Kagiampakis *et al.* [40] have been conducted to inhibit HIV by studying the inhibitor's binding to gp120. On the other hand, FabH enzymes is a promising target for antibacterial agents. Studies such as those by Pathak *et al.* [27], He *et al.* [28], and Jia *et al.* [29] have been conducted to inhibit FabH from E. faecalis and *E. coli*.

The synthesized indoles by the protocol of Tamargo, Kim & Lee will be docked on HIV-1 gp 120 and FabH from *E. coli* to assess its affinity for inhibition.

To evaluate the inhibitory potential of the synthesized indoles, their interactions with HIV-1 gp120 and FabH will be analyzed, focusing on key molecular interactions. Aromatic (π-π stacking), hydrogen bonding and hydrophobic interactions are the most important interactions between HIV-1 glycoprotein 120 and indole-based inhibitors which allow for virus inhibition [6]. Similarly, aromatic (π-π stacking), hydrogen bonding and hydrophobic interactions are the most important interactions between FabH and indole-based inhibitors that showed the most promising inhibitory activity [41].

III. Materials and Methods

A. Data and Materials Gathering

The procedure used for synthesizing the indoles in this study was adapted from the developed protocol by Tamargo

et al. [15]. This method involves a complex process of obtaining diverse 3-thioindoles through a sulfa-Michael addition and an intramolecular reductive N-heteroannulation, using a well-defined catalyst called chloro-tris(triphenylphosphine)-copper(I). The procedure shows a high level of tolerance for various substituted aryl, alkyl, heteroaryl, bulky secondary, and tertiary thiol systems, as well as different 2-nitrocinnamaldehydes, which results in good to excellent yields [15].

Twenty-seven of the synthesized indole-based analogues are assessed for both their HIV-1 gp 120 and FabH inhibitory activity. The molecular editor and visualizer software Avogadro is used to generate the synthesized indole derivative (1a-5e) structures.

In order to conduct the docking analysis, the three-Dimensional (3D) structure of the ligands are generated using Avogadro and saved as PDB files which is the format required by Autodock. The 3D structures are shown in Fig. 2.

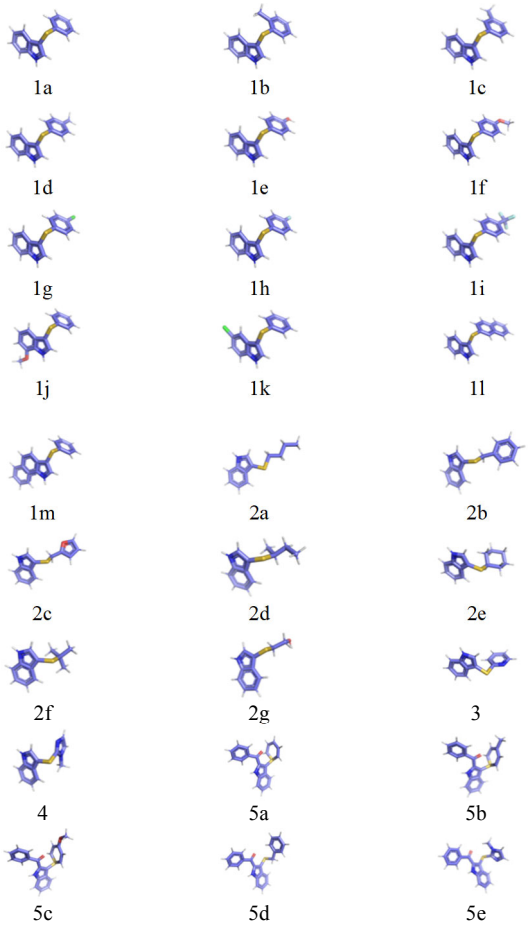
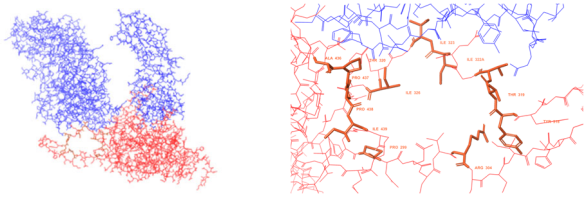


Fig. 2. Three-dimensional (3D) structures of the indole-based derivatives generated using Avogadro and PyMOL.

The PDB files of the target HIV-1 gp 120 (PDB ID: 2B4C) and FabH receptors (PDB ID: 1HNJ) are imported from Maestro's built-in Protein Data Bank. For the case of HIV-1 gp 120 as receptor, Chain G, which contains the 12 residues surrounding the binding pocket: PRO299, ARG304, TYR318, THR319, THR320, ILE322A, ILE323, ILE326, ALA436, PRO437, PRO438 and ILE439, is specifically isolated during docking to simplify the process [6]. The structures are shown in Fig. 3.



a b

Fig. 3. (a) Glycoprotein 120 structure highlighting chain G (red) and (b) the twelve residues surrounding the binding pocket (orange).

On the other hand, for the case of FabH as receptor, the active site containing the catalytic triad tunnel consisting of CYS112, HIS244, and ASN274 is highlighted and shown in Fig. 4 [21].

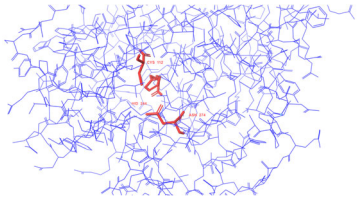


Fig. 4. FabH structure highlighting the catalytic triad tunnel (red) of the active site.

B. Toxicity Analysis

Before conducting the docking, a toxicity analysis of the indole-based analogues is conducted using ProTox-II. The structures of the compounds are drawn in the designated input of the server and the results of the toxicity analysis is generated. The indole derivatives synthesized by Hdoufane *et al.* (r1–r3) [6] and Jia *et al.* [29] (r4) which are found to have the highest affinity for HIV-1 gp 120 and FabH inhibition respectively used as reference compounds also underwent toxicity analysis for comparison.

C. Molecular Docking Studies

Following the toxicity analysis is the molecular docking analysis. The ligands are docked onto the target receptor using Autodock to determine their best binding score, inhibition constant value, and the location of the ligand in the lowest-energy pose. One hundred runs were employed during the docking in Autodock to maximize the search and ensure that the docking occurred within the binding pocket. The Genetic Algorithm (GA) method is selected. The reference compounds were also docked for comparison.

The results of docking experiments are analyzed to evaluate the potential of indole derivatives as HIV-1 gp 120 and FabH inhibitors. The binding interactions between the ligand and receptor are identified, and the results are further analyzed using various open-access software tools, such as Maestro, Protein Plus, Protein Ligand Interaction Profiler (PLIP), and Ligplot+. These tools provided a range of features for molecular modeling, simulation, and analysis, including energy minimization, molecular dynamics simulations, and docking studies. Analysis from the tools helped further visualize and assess the potential of each derivative to inhibit its target receptor. These tools are commonly used in academic research, drug development, and other areas of molecular biology and biophysics.

IV. Result and Discussion

A. Toxicity Analysis

The results of the toxicity analysis done using ProTox-II is shown in Table 1. It highlights the toxicity class as well as the active toxicological endpoints of the newly synthesized indole-based analogues as well as the reference structures.

The 27 indole-based derivatives fall in either toxicity Class 3 or Class 4. The toxicity of the indole-based derivatives compares with the reference compounds in terms of classification level.

B. Indoles as HIV-1 gp 120 Inhibitors

A summary of the lowest binding score poses with their corresponding inhibition constants and ligand-receptor interactions is shown in the Table 2.

The binding score value is a common metric used to measure the strength of interactions between molecules or between a molecule and a surface. A higher negative binding score value usually indicates a stronger interaction [19]. The molecular docking analysis evaluated the potential of the 27 synthesized compounds as HIV-1 gp 120 inhibitors. Results revealed that all of the compounds are able to form stable complexes within the protein, with their binding energies ranging from −8.91 kJ to −6.01 kJ. Among the compounds, 5b exhibited the most negative binding score and is found to be the most potent inhibitor. This compound falls under toxicity Class 4. This compound is also observed to form hydrogen bonds and hydrophobic interactions with key binding residues of HIV-1 gp 120 including Phe210.

Table 1. Toxicity analysis results using ProTox-II

Compound	Toxicity Class	Toxicity endpoints active
1a	3	hepatotoxicity, mutagenicity
1b	3	hepatotoxicity, mutagenicity
1c	3	hepatotoxicity, mutagenicity
1d	3	hepatotoxicity, mutagenicity
1e	3	hepatotoxicity, mutagenicity
1f	3	hepatotoxicity, mutagenicity
1g	3	hepatotoxicity, mutagenicity
1h	3	hepatotoxicity, mutagenicity
1i	3	hepatotoxicity, mutagenicity
1j	3	hepatotoxicity, mutagenicity, immunotoxicity
1k	3	hepatotoxicity, mutagenicity
1l	3	hepatotoxicity, mutagenicity
1m	3	hepatotoxicity, mutagenicity
2a	4	hepatotoxicity
2b	4	hepatotoxicity, mutagenicity
2c	4	carcinogenicity
2d	3	hepatotoxicity
2e	4	mutagenicity
2f	4	-
2g	3	-
3	3	hepatotoxicity, mutagenicity
4	3	hepatotoxicity, carcinogenicity
5a	4	hepatotoxicity
5b	4	hepatotoxicity
5c	3	hepatotoxicity, mutagenicity, carcinogenicity
5d	4	hepatotoxicity
5e	4	hepatotoxicity, carcinogenicity
r1	4	-
r2	4	carcinogenicity, mutagenicity
r3	4	mutagenicity
r4	4	immunotoxicity

Table 2. Summary of molecular docking results of indole-based derivatives on HIV-1 gp 120 receptor

Compound	Binding score (kJ)	Inhibition Constant (μM)	H Bonds Present	Pi-pi Interactions Present	Drug Score
1a	−7.32	4.33	ASN425G	-	0.81
1b	−6.91	8.54	TYR384G	-	0.81
1c	−7.06	6.72	TYR384G	PHE382G (perpendicular)	0.81
1d	−7.43	3.58	VAL255G	PHE382G (perpendicular)	0.81
1e	−6.64	13.58	GLU370G, SER375G, PHE383G	PHE382G (perpendicular)	0.81
1f	−6.5	17.14	ASN377G, GLY379G	-	0.81
1g	−8.18	1.02	VAL255G	PHE382G (perpendicular)	0.81
1h	−7	7.4	VAL255G	PHE382G (perpendicular)	0.81
1i	−6.63	13.86	VAL255G	PHE382G (perpendicular)	0.81
1j	−7.25	4.88	ASN425G	-	0.81
1k	−6.13	32.17	SER264G, GLN287G, LYS485G	TYR484G (perpendicular)	0.81
1l	−7.24	4.96	ARG444G	-	0.81
1m	−8.62	0.47687	ASN425G	-	0.81
2a	−6.86	9.3	ASN377G	PHE382G (perpendicular)	0.81
2b	−7.5	3.18	VAL255G	PHE382G (perpendicular)	0.81
2c	−6.84	9.73	SER446G	-	0.81
2d	−6.65	13.4	ASN425G	-	0.81
2e	−8.28	0.85657	GLY473G	-	0.81
2f	−6.58	15.04	ASN425G	-	0.81
2g	−6.01	39.21	PHE376G, PHE383G, ASN425G, TRP427G	TRP427G (perpendicular)	0.81
3	−7.02	7.15	VAL255G	PHE382G (perpendicular)	0.81
4	−6.42	19.79	GLU370G, ASN425G	TRP427G (perpendicular)	0.81
5a	−7.97	1.43	PHE210G	-	0.81
5b	−8.91	0.2924	PHE210G	-	0.81
5c	−8.47	0.61816	PHE210G, SER446G	-	0.81
5d	−8.3	0.82239	PHE210G	-	0.81
5e	−6.31	23.6	ILE326G	ARG298G	0.81

Also shown in the table is the drug score which was calculated by inputting the results from Autodock in Protein Plus, taking into account several factors such as druglikeness, molecular weight, and toxicity risks to evaluate the overall potential of a compound to qualify as a drug. The drug score is a useful tool for evaluating the suitability of the compounds for further development as drug candidates.

The ligand-protein interaction diagrams generated using Maestro software provided a visual representation of the binding interactions between the inhibitors and the protein. These diagrams revealed that the inhibitors formed several hydrogen bonds and hydrophobic interactions with the protein residues. Specifically, the interaction diagram for compound 5b from Fig. 5 showed the formation of hydrogen bonds and hydrophobic interactions with the protein receptor.

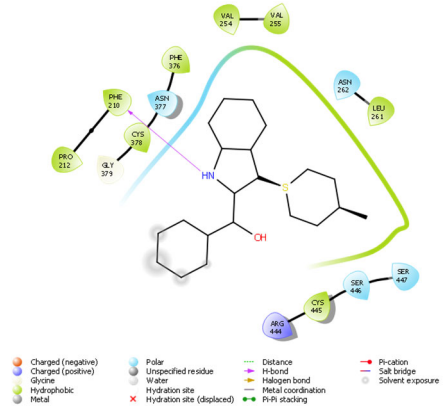


Fig. 5. Ligand interaction diagram of compound 5b and HIV-1 gp 120.

The indole derivatives synthesized by Hdoufane *et al.* [6] (r1–r3) which are found to have the highest affinity for HIV-1 gp 120 inhibition are also docked for comparison as shown in Table 3. The binding score of the reference indole compounds range from −9.25 kJ to −8.67 kJ. Results show that the data gathered from the 27 indole-based derivatives compare to the reference structures. Though the reference structures form a more stable complex with the receptor, due to forming more hydrogen bonds, the 27 newly synthesized indole derivatives still compare and exhibit potential to inhibit HIV-1 gp 120.

Table 3. Summary of molecular docking results of reference compounds on HIV-1 gp 120 receptor

Compound	Binding score (kJ)	Inhibition Constant (μM)	Hydrogen Bonds Present	Pi-pi Interactions Present	Drug Score
r1	–9.25	0.16531	SER209G, ARG440G, SER446G	-	0.81
r2	–9.2	0.18144	SER446G, SER447G	-	0.81
r3	–8.67	0.44399	ARG252G, SER446G	-	0.81

Overall, the results of the molecular docking analysis suggest that the synthesized indole compounds have potential as HIV-1 gp 120 inhibitors, with compound 5b showing the highest binding affinity and most favorable interactions with the protein receptor. These findings can provide a basis for further optimization and development of the compounds as potential therapeutic drugs for HIV.

C. Indoles as FabH Inhibitors

A summary of the lowest binding score poses with their corresponding inhibition constants and ligand-receptor interactions is shown in the Table 4.

Table 4. Summary of molecular docking results of indole-based derivatives on FabH receptor

Compound	Binding score (kJ)	Inhibition Constant (μM)	Hydrogen Bonds Present	Pi-pi Interactions Present	Drug Score
1a	−6.61	14.3	GLY209A	-	0.84
1b	−6.93	8.4	GLY209A	-	0.84
1c	−7	7.35	PHE304A	-	0.84
1d	−7.04	6.96	ALA246A, ASN247A	-	0.84
1e	−6.94	8.25	HIS244A, ALA246A, ASN274A, PHE304A	-	0.84
1f	−7.33	4.23	HIS244A, ASN247A, PHE304A	-	0.84
1g	−7.14	5.89	GLY209A	-	0.84
1h	−6.56	15.62	GLY209A	-	0.84
1i	−6.72	11.82	GLY209A, PHE213A	-	0.84
1j	−7.24	4.91	GLY209A	-	0.84
1k	−6.61	14.39	LEU189A, LEU191A	-	0.84
1l	−8.62	0.47662	VAL212A	-	0.84
1m	−7.86	1.74	GLY209A	-	0.84
2a	−5.8	55.6	GLY209A	-	0.84
2b	−6.7	12.34	PHE304A	-	0.84
2c	−6.64	13.63	ASN274A	-	0.84
2d	−5.68	68.09	GLY209A	-	0.84
2e	−7.12	6.06	PHE304A	-	0.84
2f	−6.55	15.84	ALA246A, ASN247A	-	0.84
2g	−5.31	127.52	HIS244A, ALA246A, ASN247A	-	0.84
3	−6.49	17.58	HIS244A, PHE304A	-	0.84
4	−5.84	52.72	GLY209A, ASN247A	-	0.84
5a	−7.96	1.47	LEU189A, LEU191A	-	0.84
5b	−7.76	2.03	LEU189A, LEU191A	-	0.84
5c	−7.83	1.81	LEU189A, LEU191A, ASN193A	-	0.84
5d	−7.41	3.71	LEU189A, LEU191A	-	0.84
5e	−7.36	4.05	THR81A, LEU191A	-	0.84

The potential of the 27 synthesized indole-based compounds as FabH inhibitors is evaluated from the docking results. The results revealed that all of the compounds are able to form stable complexes with the protein, and their binding energies range from −8.62 kJ to −5.8 kJ. Among the compounds, 1l exhibited the most negative binding score and is found to be the most potent inhibitor. However, since this compound falls under toxicity Class 3, compound 5a with the next most negative binding score of −7.96 kJ is selected as the best compound among the 27 derivatives most suited for

FabH inhibition. Compound 5a is observed to form hydrogen bonds with LEU189 and LEU191 and hydrophobic interactions with key the binding residues.

The ligand-protein interaction diagrams generated using Maestro software also provided a visual representation of the binding interactions between the inhibitors and the FabH protein. The diagrams revealed that the inhibitors formed several hydrogen bonds and hydrophobic interactions with the protein residues. Specifically, the interaction diagram for compound 5a which showed the formation of hydrogen bonds and hydrophobic interactions with the receptor protein is shown in Fig. 6.

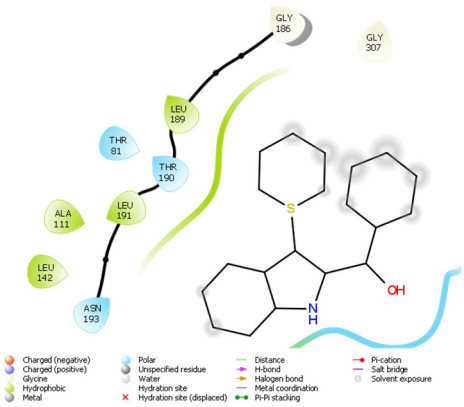


Fig. 6. Ligand interaction diagram of compound 5a and FabH.

The indole derivative synthesized by Jia *et al.* [29] (r4) which is found to have the highest affinity for FabH Inhibition is docked for comparison and results are shown in Table 5. The reference compound with a binding score of −8.74 kJ forms a more stable complex with the FabH receptor dur to the formation of more hydrogen bonds. Still the data gathered from the 27 indole-based derivatives indicate the ability to compare with reference structure, signaling the potential to be used as antibacterial agents.

Table 5. Summary of molecular docking results of reference compound on FabH receptor

Compound	Binding score (kJ)	Inhibition Constant (μM)	Hydrogen Bonds Present	Pi-pi Interactions Present	Drug Score
r4	−8.74	0.39041	THR81A, LEU189A, LEU191A, ASN193A		0.82

The results of the molecular docking analysis indicate that the synthesized compounds have potential as inhibitors of FabH. Compound 5a is found to be the best compound with a suitable toxicity class and high binding affinity as well as favorable interactions with the FabH protein. These findings can also serve as a starting point for optimizing and developing the indole compounds as antibacterial agents.

V. Conclusion

A more negative free binding score is needed for binding to be favorable. The K_i values of the ligands all fall within 1–100 micromolar for the case of both targets. This range is a common range for good initial "hit" compounds. In the case of indoles for HIV-1 gp 120 inhibition, the results show that compound 5b, phenyl(3-(p-tolylthio)-1*H*-indol-2-yl)methanone, has the lowest binding score while for FabH inhibition, compound 1l, 3-(Naphthalen-2-ylthio)-1*H*-indole, generates the lowest binding score. Considering the toxicity of the compounds is also an important step in drug development. Compound 5b, phenyl(3-(p-tolylthio)-1*H*-indol-2-yl)methanone, and compound 5a, phenyl(3-(phenylthio)-1H-indol-2-yl)methanone, which both fall under Class 4 are found to be the best candidates for HIV-1 gp 120 and FabH inhibition, respectively, based on the results of toxicity analysis and molecular docking studies. Protein-ligand interaction diagrams indicate that the affinity of the studied indole-based derivatives is shown to be governed by hydrogen bonding (H-bond) which occurs either with a nitrogen or oxygen atom of the indole group and hydrophobic and pi interactions specifically within the binding pocket. Upon comparison of with compounds previously assessed for their HIV-1 gp 120 and FabH inhibition abilities, respectively, it is found that the new compounds compare with the reference compounds. Results of the analysis show that the indole-based analogs can be a starting point for synthesis of drugs designed for both HIV therapy and as antibacterial agents.

Further analysis of the indole-based derivatives such as molecular dynamic simulations can be done to better assess their potential as drug candidates for both HIV-1 gp 120 and FabH inhibition. Exploration of the other potential applications of the synthesized compounds can also be beneficial to serve as starting points for the development of drugs. In particular, the indole-based derivatives can be docked as anti-leishmanial agents by specifically targeting pteridine reductase and as anti-inflammatory agents by specifically targeting cyclooxygenase 2.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, methodology, validation, and formal analysis was done by OBA, and RIT. Writing—original draft preparation was done by OBA. Writing—review and editing and supervision was done by OBA, and RIT. All authors have read and agreed to the published version of the manuscript.

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