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Original Research Article

Pharmacodynamics Studies of Elucidated Bioactive Molecules from Metabolites of *Streptomyces longisporoflavus* Targeted against γ- Glutamyltranspeptidase (CapD enzyme) of *Bacillus anthracis*

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ABSRTACT

Inhibition of the γ-Glutamyltranspeptidase (CapD enzyme) has become a promising approach for creating potential antimicrobial drugs to combat *B. anthracis*. By targeting CapD, it is possible to interfere with the bacterium's potential to produce spores and initiate infection. The high-resolution X-ray crystal structures of the *Bacillus anthracis* transpeptidase enzyme CapD (PDB code: 3GA9 at a resolution of 2.30Å) were obtained from the Protein Data Bank (PDB). The generated coordinates were subsequently prepared for docking studies using the BIOVIA Discovery Studio Visualizer 2020. The bioactive compounds were identified from the metabolites of *S. longisporoflavus*. The canonical SMILES of the identified molecules were obtained from PubChem, and in-silico ADME screening and drug-likeness evaluation were conducted using the SwissADME free web application. The ligands and that of the standard medication (Penicillin) were prepared for docking studies with the CapD enzyme using PyRx. This process produced binding models and data on the binding affinity. The models were obtained and the examination of the interaction between the receptor and ligand were visualized using BIOVIA. The ADME study and drug-likeness properties revealed that Butanoic acid hexyl ester and 1, 1-Dibutoxybutane showed close similarities with Penicillin (standard drug), but poor binding affinity with the *CapD* enzyme. The molecular docking study revealed that Dronabinol, 5-Cholestene-3-ol and Ergost-5-en-3-ol with binding affinity of -7.7,-8.0 and -8.0 have close similarities with Penicillin (-7.4). This study aims to evaluate the therapeutic potential of *Streptomyces longisporoflavus* bioactive compounds against CapD enzyme of *B. anthracis* in the face of rising antimicrobial resistance.

Key words: CapD enzyme, *B. anthracis*, Penicillin, Bioactive molecules, Binding affinity

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Introduction

The actinomycetes, especially the species belonging to the genus *Streptomyces*, are known to be a significant source of valuable compounds. They have produced numerous antimicrobial agents that are clinically useful, such as streptomycin, actinomycin, and streptothricin.[1] *Streptomyces* are believed to be one of the major sources of bioactive compounds known and they are majorly studied for this reason.[2] They produce natural products which could be used in the production of novel compounds with actions against microorganisms.[3] As a result, they have promising antimicrobial potential and can be used as a drug or drug precursor for combatting infectious microbial agents.

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Bacillus anthracis, a Gram-positive and spore-forming bacterium is recognized as the etiological agent of anthrax. This disease is significant because of its potential as an agent of bioterrorism and its sporulation ability. *Bacillus anthracis* possesses three main virulence factors: a capsule or protective antigen, lethal toxin, and edema toxin coded on two plasmids, pXO1 and pXO2. These toxins play essential roles in the development of the disease.[4] The protective antigen attaches to receptors on the surface of cells, causing lethal toxin and edema toxin to penetrate host cells. Once inside, these toxins interfere with cellular signaling pathways, suppress immune responses, and damage the tissue.[5]

However, if any of these two plasmids are absent, the organism is unable to synthesize all of its virulence components. The resultant organism undergoes attenuation, causing a reduction in its pathogenicity in humans or animals. The pXO1 plasmid controls the synthesis of the anthrax edema and lethal toxins, whereas the pXO2 plasmid codes for the capsule.[6] The CapD enzyme, also referred to as γ-glutamyltranspeptidase, plays a crucial role in the pathogenicity of *B. anthracis* as it facilitates the processes of spore production and germination. It plays a crucial role in the biosynthesis of the bacterial cell wall and is necessary for the proper assembly of the spore coat, which protects the bacterium from adverse conditions. Furthermore, CapD is involved in the cleavage of γ-glutamyl peptides, which aids the bacterium in obtaining nutrients during infection.

Emergence of resistance among the most important bacterial pathogens is recognized as a major public health threat affecting humans worldwide.[7] In fact, the World Health Organization has named

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antibiotic resistance as one of the top public health threat of the 21st century.[7] In the year 2023, a penicillin-resistant *B. anthracis* was isolated from anthrax endemic area in East Java, Central Java and Yoggyakarta provinces of Indonesia.[8] Inhibition of the CapD enzyme has emerged as a promising approach in the production of potential antimicrobial agents against *B. anthracis*. The study of bioactive compounds from *Streptomyces longisporoflavus* against the CapD enzyme of *B. anthracis* remains relatively underexplored. By targeting CapD, it is possible to interfere with the bacterium's potential to create spores and initiate infection, which could potentially pave the way for the development of novel therapies for anthrax.

Therefore, this research aims at screening bioactive molecules from secondary metabolites of *Streptomyces longisporoflavus* as drug candidates against *γ*- Glutamyltranspeptidase (CapD enzyme) of *B. anthracis.*

Materials and Methods

Production and synthesis of metabolites of S. longisporoflavus

Streptomyces longisporoflavus, isolated March 2020 from soil samples obtained from LAUTECH botanical garden according to Majolagbe *et al*., 2023[9] (The characterized isolate revealed 100% similarity to *Streptomyces longisporoflavus* with accession number- KY657268.1 available in the GenBank database as reported by Majolagbe *et al*., 2023),[9] was cultured under submerged fermentation protocol in a shake flask culture containing Yeast-malt-dextrose (YMD) broth according to the modified method of Majolagbe *et al*., (2023).[9] Synthesis of the metabolite was done using ethyl acetate and the spent medium free of biomass at ratio 2: 1 respectively. It was left overnight at 4 ^{o}C and was centrifuged at 4000 rpm for 15 minutes. The cell-free supernatant was decanted aseptically into labeled tubes and stored at 4 ^{o}C.[9, 10]

Detection of Bioactive Molecules of Streptomyces longisporoflavus

Elucidation of bioactive molecules in the Metabolites was done using Gas chromatography-Mass spectrometry (GCMS), which was carried out to detect and identify the probable bioactive compounds present in the metabolites of *Streptomyces longisporoflavus*.[12] Gas Chromatography Model: 7890A A system hyphenated to a mass spectrometer (5975 C) utilizing an auto-injector for a 10-milliliter syringe and a triple-axis detector. The carrier phase used was helium gas. For five minutes, the temperature in the column was raised from 35 °C to 150 °C at a rate of 4 °C per minute. After that, it was elevated to 250 °C at a rate of 20 °C per minute and maintained for five minutes. The elution period was 47.5 minutes. Solution software provided by the supplier was used to control the system and retrieve data. The compound was identified by comparing the GC-MS data to those obtained from NIST library (NISTII).[9] Each molecule was taken as a potential drug candidates for Pharmacokinetics and pharmacodynamics study.

ADME Properties and Drug-likeness Prediction

The Absorption-Distribution-Metabolism-Excretion (ADME) properties of the bioactive compounds of *S. longisporoflavus* were studied in silico. The canonical smiles of the compounds were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/).[17] The evaluation of the in-silico ADME properties and drug-likeness screening was done using the free web tool Swiss ADME developed by the Swiss Institute of Bioinformatics and freely available at https//:www.swissadme.ch.[13] Computed were basic physicochemical parameters such atom counts, polar surface area (PSA), molecular weight (MW), and molecular refractivity (MR). The rules of five (RO5) screening, as outlined by Lipinski (2001), Ghose (1999), Veber (2002), Egan (2000), and Muegge (2001), were used to implement drug-likeness candidature. The Abbot Bioavailability scores were computed to determine the likelihood of a molecule having an oral bioavailability of at least 10% based on factors such as total charge, TPSA, and violation of the Lipinski's filter. The lipophilicity of the compounds was assessed using iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT models from which a consensus log Po/w value was determined.[13] The solubility (log S) of the ligands was implemented by three different models: ESOL, Ali, and SILICOS-IT.[13]

Molecular Docking

The high-resolution X-ray crystal structures of *Bacillus anthracis* transpeptidase enzyme (protein) in its liganded state (PDB code: 3GA9; resolution: 2.30 Å) were obtained from the Protein Data Bank (PDB). The PDB coordinates obtained were prepared for docking studies by eliminating ligands, heteroatoms, and water molecules using BIOVIA.[14]

Ligands which include penicillin which is the control drug candidate currently in circulation for the treatment of anthrax caused by *Bacillus anthracis*, [15] and the 5 bioactive molecules found in *Streptomyces longisporoflavus* were obtained from the PubChem database and prepared using AutoDock tools.[9]

3GA9 was prepared for docking studies as earlier stated. The ligands were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[17] and processed using Open Babel (https://openbabel.org/). The prepared ligands were docked with 3GA9 using Pyrx (https://sourceforge.net/projects/pyrx/files/) resulting in the generation of binding models and binding affinity data. The binding models were loaded into the BIOVIA System [14] which was used to visualize in 2D and 3D, the Receptor-Ligand interaction and analyze the amino residues involved in the binding interactions.

Results and Discussion

Gas chromatography- Mass spectrometry

Figure 1 shows the GCMS result of the elucidated bioactive molecules carried out to detect and identify the probable bioactive compounds present in the metabolites of *Streptomyces longisporoflavus*.[12] Butane-1,1- dibutoxy-2 (1H) also known as 1,1-Dibutoxylbutane was the most prominent compound in the analyzed sample because it has the highest percentage area of 51.86 % at 19.32 min, followed by 5-cholestene-3-ol with a percentage peak area of 33.60 % at 44.40 min, Butanoic acid hexyl ester with a 10.88 % peak area at 9.40 min, Dronabinol with a 2.34 % peak area at 40.59 min, and Ergost-5-en-3-ol with 1.87 % peak area at 43.80 min as shown in Table 1. Compounds identified as being present in the metabolites (Butane-1,1-dibutoxy-2 (1H), 5-Cholestene-3-ol, Dronabinol, Ergost-5-en-3-ol, and Butanoic Acid Hexyl Ester) possess antimicrobial properties that can serve as therapeutic agents for pathogenic organisms.

Table 1: Observed bioactive molecules present in the metabolite of *Streptomyces longisporoflavus*

S/No.	Probable Compound	% Peak Area	Retention time (min)
1	Butane-1,1-dibutoxy-2 (1H)	51.86	19.32
2	5- Cholestene -3-ol	33.60	44.40
3	Butanoic acid hexyl ester	10.88	9.40
4	Dronabinol	2.34	40.59
5	Ergost-5-en-3-ol	1.87	43.80

ADME Properties of Bioactive Compounds with Penicillin

The ADME properties of the identified bioactive compounds of *S. longisporoflavus* were compared to the properties of Penicillin as the control, as presented in the tables below. The main physicochemical characteristics of the bioactive compounds are outlined in Table 2. This table shows the molecular formula, molecular weight, number of heavy atoms, number of aromatic heavy atoms, fraction csp3, number of rotatable bonds, number of H-bond acceptors, number of H-bond donors, molar refractivity, and Topological Surface Area (TPSA) of

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Butanoic acid hexyl ester, I,1- Dibutoxyl ethane, Dronabinol, Ergost-5-en-3-ol, and Penicillin (a standard drug used against *B. anthracis*). The identified compounds had molecular weights ranging from 145.16 to 400.68 g/mol. Ergost-5-en-3-ol has the highest molecular weight however it can still show a good level of distribution as it is within the normal range of <500 g/mol.

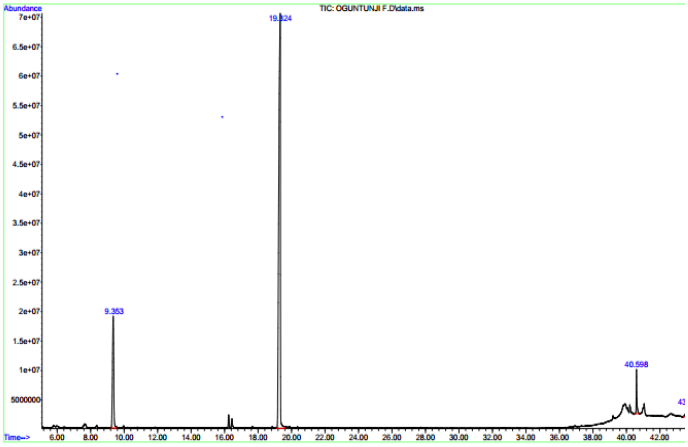

Figure 1: Chromatogram of *Streptomyces longisporoflavus* metabolite showing the bioactive molecules

Table 3 shows the lipophilicity of the bioactive molecules. According to Lipinski's rule of 5 for drug-likeness, a lipophilicity range of 0 to 5 is generally regarded as optimum for drug development. [18, 19] In the present study, only Butanoic acid hexyl ester, 1,1-Dibutoxybutane and penicillin showed optimal lipophilicity with Butanoic acid hexyl ester in a very close similarity to penicillin (1.73 and 1.29 respectively). This indicates that these compounds will be readily absorbed across the membranes and enter the systemic circulation, resulting in a high level of bioavailability.

Table 4 shows the water solubility of the bioactive molecules. Solubility is a physicochemical property of drugs that significantly affects their absorption, distribution, and formulation.[13] In order to enhance the absorption, it is expected that drugs are present in the form of aqueous solution at the absorption site.[20] In the present study, Butanoic acid hexyl ester, 1,1-Dibutoxybutane and penicillin are soluble, this indicates that these compounds (with optimal lipophilicity and solubiliity) can easily penetrate the central nervous system (CNS). [21] In this study, it was observed that Butanoic acid hexyl ester, 1,1-Dibutoxybutane and penicillin have the potential to be easily absorbed into the systemic circulation in the gastrointestinal tract and permeate the BBB while Ergost-5-en-3-ol and 5-Cholestene-3-ol showed low absorption and no BBB permeate. .This can be a cause of concern for possible CNS-related side effects. However, as the computational method does not measure the extent of permeation, the effect may not be so significant to cause severe toxic effects on the CNS.

Table 5 shows the pharmacokinetics of the bioactive molecules. Orally administered drugs should have high absorption in the gastrointestinal tract (GIT) for optimal pharmacokinetics. The BBB, or Blood Brain Barrier, plays a crucial role in limiting the penetration of drugs into the central nervous system (CNS).[21] It was observed in this study that Butanoic acid hexyl ester, 1,1-Dibutoxybutane and penicillin have potential to be well absorbed into the systemic circulation in the GIT and permeate the BBB while Ergost-5-en-3-ol and 5-Cholestene-3-ol showed low absorption and no BBB permeate. This could be a cause of concern for possible CNS-related side effects. However, as the computational method does not measure the extent of permeation, the degree of permeability may not be significant enough to create a noticeable adverse effect on the central nervous system (CNS). P-gp usually acts as an efflux transporter that pumps xenobiotics or drugs back into the GIT lumen and consequently reduce plasma and tissue concentrations of the drug.[22, 23] In this study, all the compounds were predicted to be non-substrate of P-gp.

Understanding the interactions between compounds and the cytochrome P450 (CYP) system is very essential to characterise the pharmacokinetics of candidate drugs. These interactions play a vital role in the transformation and elimination of drugs from the body system. [13] When drugs inhibit certain isoforms of this enzyme system, it can lead to poor elimination and drug-induced toxicity. Therefore, candidate drugs need to have minimal inhibitory activity against these enzyme isoforms. This study revealed that Ergost-5-en-3-ol and Penicillin did not show any potential to inhibit any of the five P450 isoforms. This suggests that these compounds would be efficiently metabolized in the liver and easily eliminated from the body.

Table 6 shows the overall drug-likeness property which assesses quantitatively how much of the physicochemical and structural characteristics of the compounds comply or are consistent with the majority of well-known drugs. This was predicted using five computational "rule of 5" filters – Lipinski (Pfizer), Ghose (Amgen), Verber (GSK), Egan (Pharmacia), and Muegge (Bayer) – to allow for con sensus in the prediction[11]. The Lipinski's rule is the first-ever implemented "rule of 5" for drug-likeness, and the result of this study showed that Butanoic acid hexyl ester, 1,1-Dibutoxybutane and penicillin screened showed compliance with the Lipinski's rule. On using multiple filters (to increase the precision of the prediction), 1,1-Dibutoxybutane and penicillin showed no violation of any rules for all 5 filters. A violation of Muegge and Ghose was observed for Butanoic acid hexyl ester, 1,1-Dibutoxybutane, Ergost-5-en-3-ol and 5-Cholestene-3-ol while only Ergost-5-en-3-ol and 5-Cholestene-3-ol showed violation of Egan. This finding suggests that Dronabinol and Penicillin have better drug-like characteristics relative to the other compounds.

Table 7 shows the medicinal chemistry of the bioactive compounds. They were screened for BRENK and PAIN alerts. None of the compounds in this study showed any PAIN alerts. This shows absence of any structurally promiscuous moiety (also referred to as frequent litters). These structural fragments or components are recognized for generating false-positive responses in in-silico assays regardless of the protein target that is being studied.[24]

It is important to treat such alerts with caution. BRENK alerts, however, indicate fragments of compounds that may potentially be harmful, chemically reactive, and metabolically unstable. This study revealed that Butanoic acid hexyl ester and Penicillin did not exhibit any BRENK alert, but 1,1-Dibutoxybutane, Ergost-5-en-3-ol, 5-Cholestene-3-ol, and Dronabinol each exhibited only one BRENK alert. This may not pose any risk as it depends on the nature of the structural moiety producing the alert. However, this factor should be taken into account in the prioritization of compounds for drug-like properties.

Figure 2 shows good in vivo drug absorption and permeation according to Lipinski's rule of 5.[1]

Molecular Docking of Ligand to the Binding Site of CapD

Bacillus anthracis, the Gram-positive bacterium and causative agent of anthrax, possesses a polysaccharide capsule that shields it from the host immune system. This capsule plays a vital role in the organism's virulence (disease-causing capacity).[25] CapD (capsular depletion protein D) exhibit an active binding site as shown in Figure 3 and it emerges as a crucial player in this process, influencing both capsule assembly and disassembly.[5] Currently, penicillin remains the standard treatment for anthrax.[26]

Docking Penicillin with the CapD (Figure 4) shows that penicillin binds with some key amino residues (Ser372, Pro427, Gly428, and Gly429) which contribute to the CapD transpeptidation reaction by activating Thr352) formerly reported to be coding for the virulence nature of the Cap- D,[27] therefore, ligands binding to the Thr^{352} residue on the CapD have high potential of inactivating *Bacillus anthracis* and attenuating its virulence. It is deduced from the 2D visualization of the docking of penicillin with CapD that the Thr^{352} residue on the CapD was bound by penicillin. Thus, it is safe to say that the ability of penicillin to bind to the Thr^{352} increased its effectiveness against *B. anthracis*. This serves as a model with which the effectiveness of the compounds found in the *S. longisporoflavus* can be established.

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Table 2: Physicochemical properties of the bioactive molecules and Penicillin (standard drug)

Physicochemical properties	Butanoic acid hexyl ester	1,1-Dibutoxylbutane	Dronabinol	Ergost-5-en-3-ol	5-Cholestene-3-ol	Penicillin
Formula	C_9H_7NO	$C_{12}H_{26}O_2$	$C_{21}H_{30}O_2$	$C_{28}H_{48}O$	$C_{27}H_{46}O$	$C_{16}H_{18}N_2O_4S$
Molecular weight (g/mol)	145.16	202.33	314.46	400.68	386.65	334.39
No of heavy atom	11	14	23	29	28	23
No of arom. heavy atom	10	0	6	0	0	6
Fraction Csp3	0	1	0.62	0.93	0.93	0.44
No of rotatable bond	0	10	4	5	5	5
No of H-bond acceptors	1	2	2	1	1	4
No of H-bond donors	1	0	1	1	1	2
Molar refractivity	44.57	61.97	97.91	128.42	123.61	89.86
TPSA (A^o)	32.86	18.46	29.46	20.23	20.23	112.01

Table 3: Lipophilicity of the bioactive molecules and Penicillin (standard drug)

Lipophilicity	Butanoic acid hexyl ester	1,1-Dibutoxylbutane	Dronabinol	Ergost-5-en-3-ol	5-Cholestene-3-ol	Penicillin
Log Po/w (iLOGP)	1.56	3.74	3.9	4.92	4.96	1.58
Log Po/w (XLOGP3)	1.26	3.85	6.97	8.8	8.72	1.83
Log Po/w (WLOGP)	1.53	3.75	5.74	7.63	7.39	0.48
Log Po/w (MLOGP)	1.65	2.89	4.39	6.54	6.34	1.55
Log Po/w (SILICOS-IT)	2.64	3.47	5.41	6.63	6.4	1.02
Consensus Log Po/w	1.73*	3.54	5.28	6.9	6.76	1.29*

Table 4: Water solubility of the bioactive molecules and Penicillin (standard drug)

Water solubility	Butanoic acid hexyl ester	1,1-Dibutoxylbutane	Dronabinol	Ergost-5-en-3-ol	5-Cholestene-3-ol	Penicillin
Log S (ESOL)	-2.21	-2.86	-6.11	-7.54	-7.4	-2.93
Solubility (mg/ml)	9.02E-01	-2.79E-01	2.44E-04	1.16E-05	1.54E-05	3.94E-01
Class	Soluble	Soluble	Poorly soluble	Poorly soluble	Poorly soluble	Soluble
Log S(Ali)	-1.55	-3.93	-7.4	-9.11	-9.02	-3.8
Solubility (mg/ml)	4.10E+00	2.36E-02	1.24E-05	3.13E-07	3.65E-07	5.27E-02
Class	Very soluble	Soluble	Poorly soluble	Poorly soluble	Poorly soluble	Soluble
Log S (SILICOS-IT)	-3.58	-3.81	-5.93	-5.79	-5.78	-2.94
Solubility (mg/ml)	3.78E-02	3.10E-02	3.69E-04	6.42E-04	6.48E-04	3.88E-01

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Class	Soluble	Soluble	Moderately soluble	Moderately soluble	Moderately soluble	Soluble

Table 5: Pharmacokinetics of the bioactive molecules and Penicillin (standard drug)

Pharmacokinetics	Butanoic acid hexyl ester	1,1-Dibutoxylbutane	Dronabinol	Ergost-5-en-3-ol	5-Cholestene-3-ol	Penicillin
GI absorption	High	High	High	Low	Low	High
BBB permeant	Yes	Yes	Yes	No	No	No
P-gp substrate	No	No	No	No	No	No
CYPIA2 inhibitor	Yes	Yes	No	No	No	No
CYP2C19 inhibitor	No	No	Yes	No	No	No
CYP2C9 inhibitor	No	No	Yes	No	Yes	No
CYP2D6 inhibitor	No	No	Yes	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No
Log Kp (skin permeation) cm/s	-6.29	-4.8	-3.27	-2.5	-2.47	-7.04

Table 6: Drug-likeness of the bioactive molecules and penicillin (standard drug)

Drug-likeness	Butanoic acid hexyl ester	1,1-Dibutoxylbutane	Dronabinol	Ergost-5-en-3-ol	5-Cholestene-3-ol	Penicillin
Lipinski	Yes: 0 violation	Yes: 0 violation	Yes;1 violaton	Yes; 1 violation	Yes; 1 violation	Yes; 0 violation
Ghose	No: 2 violatons	Yes	No; 1 violation	No; 2 violations	No; 2 violations	Yes
Veber	Yes	Yes	Yes	Yes	Yes	Yes
Egan	Yes	Yes	Yes	No; 1 violation	No; 1 violation	Yes
Muegge	No; 1 violation	Yes	No; 1 violation	No; 2 violations	No; 2 violations	Yes
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.56

Table 7: Medicinal chemistry of the bioactive molecules and Penicillin (standard drug)

Medicinal chemistry	Butanoic acid hexyl ester	1,1-Dibutoxylbutane	Dronabinol	Ergost-5-en-3-ol	5-Cholestene-3-ol	Penicillin
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert
Brenk	0 alert	1 alert	1 alert	1 alert	1 alert	0 alert
Leadlikeness	No; 1 violation	No; 3 violations	No; 1 violation	No; 2 violations	No; 2 violations	Yes
Synthetic accessibility	1.36	3.11	4.27	6.17	5.98	3.98

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Table 8: Binding affinity of the bioactive compounds and Penicillin

Ligand	Binding affinity
Butanoic acid hexyl ester	-5.1
1,1-Dibutoxylbutane	-4.9
Dronabinol	-7.7*
Ergost-5-en-3-ol	-8.0*
5-Cholestene-3-ol	-8.0*
Penicillin	-7.4

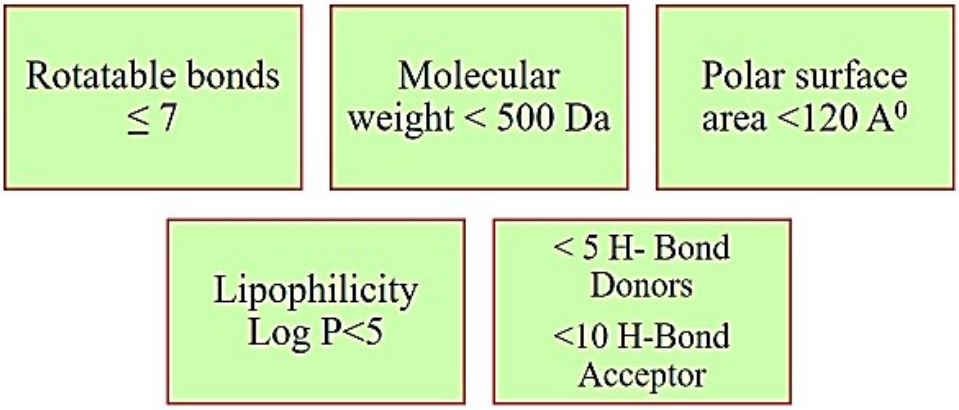

Figure 2: Good *In vivo* Drug absorption and Permeation *Lipinski's Rule of 5.* [17]

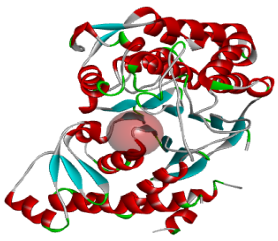

Figure 3: CapD enzyme showing the active binding site

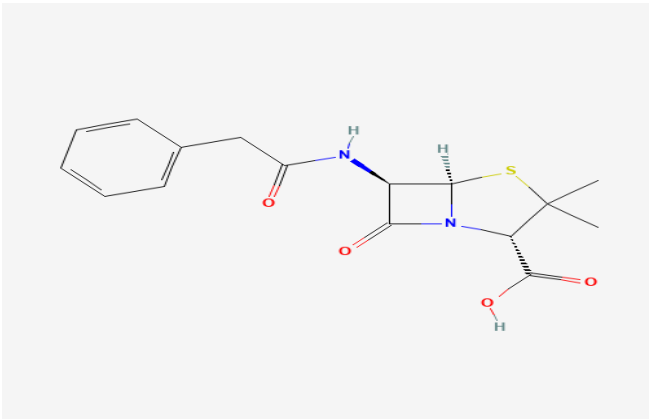

Figure 4: Penicillin 2D structure

Figure 5 shows the 2D visualization of Penicillin docked with CapD enzyme. Figure 6 shows Ergost-5-en-3-ol docked with enzyme CapD. Figure 7 shows Dronabinol docked with enzyme CapD. Figure 8 shows Butanoic acid hexyl ester docked with enzyme CapD. Figure 9 shows 5-cholestene-3-ol docked with enzyme CapD. Figure 10 shows 1,1-Dibutoxybutane docked with enzyme CapD. Comparing the 2D visualization of penicillin to that of the 5 bioactive molecules found in *Streptomyces longisporoflavus,* Dronabinol, 5-cholestene-3-ol and Ergost-5-en-3-ol has docking scores of -7.7, -8.0, and -8.0 respectively similar to Penicillin with docking score of -7.4 as shown in Table 8

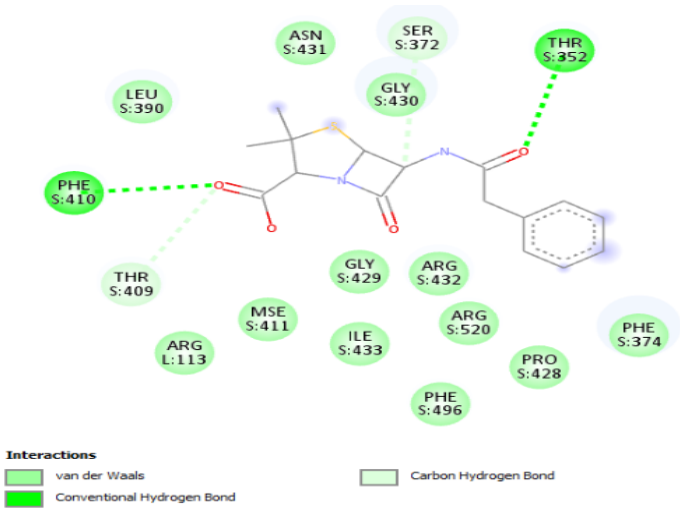

Figure 5: 2D visualization of Penicillin docked with CapD enzyme

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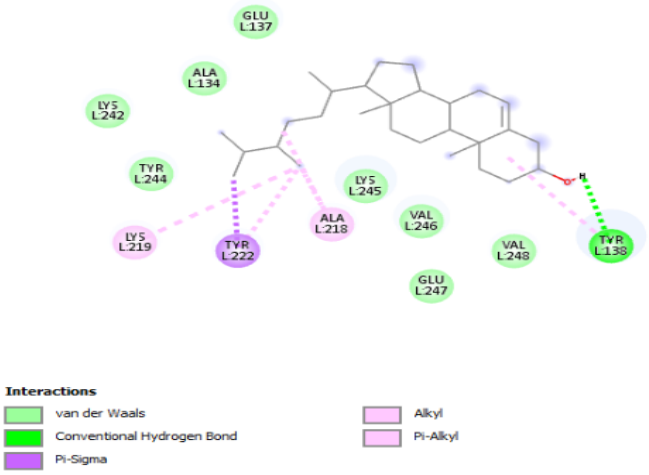

Figure 6: Ergost-5-en-3-ol docked with enzyme CapD

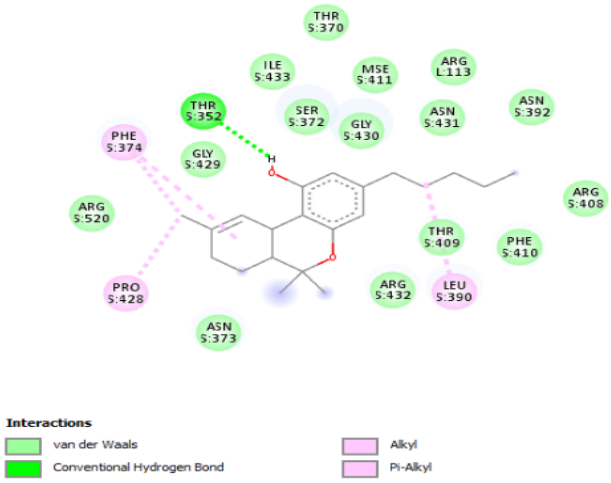

Figure 7: Dronabinol docked with enzyme CapD

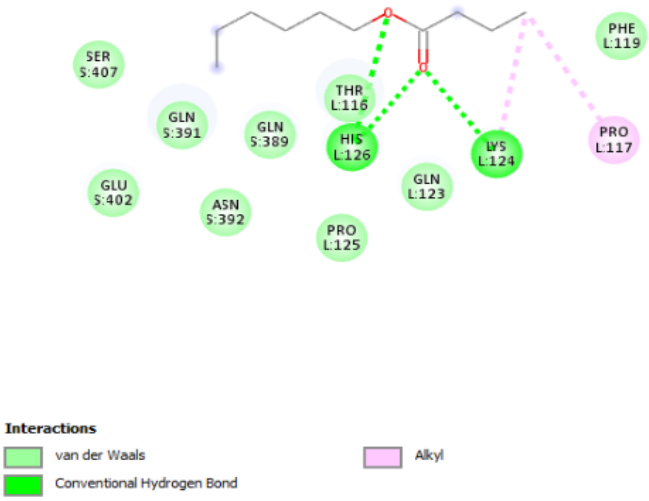

Figure 8: Butanoic acid hexyl ester docked with enzyme CapD

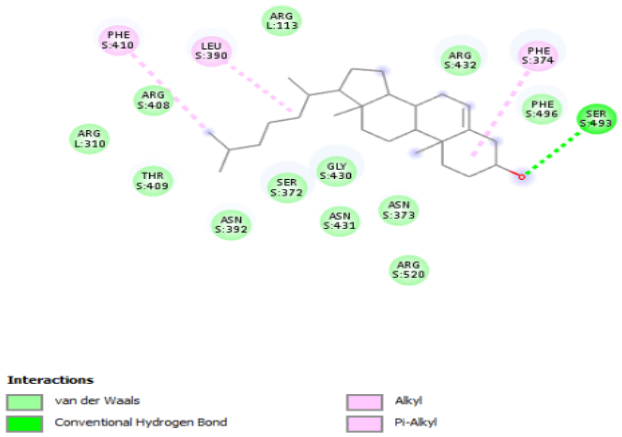

Figure 9: 5- Cholestene -3-ol docked with enzyme CapD

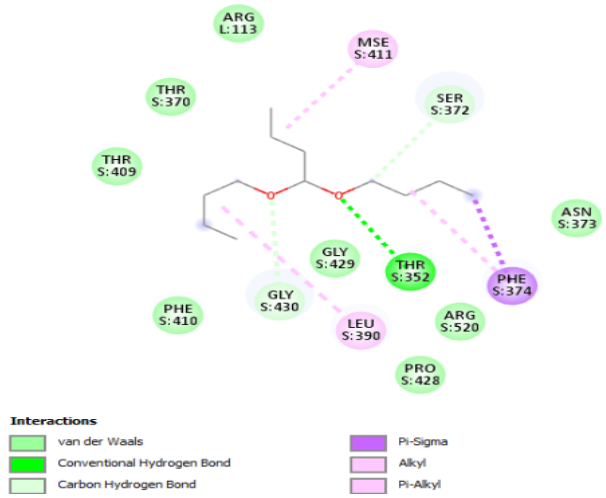

Figure 10: 1,1- Dibutoxylbutane docked with enzyme CapD

Conclusion

This study has revealed the potential of bioactive compounds from natural sources like *S. longisporoflavus* as therapeutic agents that can be used in combatting anthrax disease caused by *Bacillus anthracis* with specific action targeted against the γ-glutamyltranspeptidase (CapD) enzyme. In addition, Dronabinol, 5-cholestene-3-ol and Ergost-5-en-3-ol are promising therapeutic options when combined with other promising candidates with better ADME properties. Given the results this study has shown, future studies should consider exploring synergistic effects of these bioactive molecules against the virulence factors of pathogens like *Bacillus anthracis*.

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.

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