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Original Article:

Investigating the Inhibitory Effects of Some Alkaloid Compounds against Aflatoxin Biosynthesis Polyketide Synthase by Molecular Docking Method

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Abstract

Introduction: Aflatoxins are toxic metabolites produced by certain fungi, primarily Aspergillus species, that contaminate various food and feed crops. Alkaloids, a diverse group of naturally occurring compounds found in plants, animals, and microorganisms, have been investigated as potential inhibitors of polyketide synthase in aflatoxin biosynthesis. The purpose of this study is to investigate the inhibitory potential of a few alkaloid compounds against aflatoxin biosynthesis polyketide synthase.

Materials and Methods: The 2D structures of 10 alkaloid compounds, used as ligands, were obtained from PubChem database and optimized using the MM2 Job command in Chem3D Pro 12.0.2.1076 software. The ligands were assessed as inhibitors against the active site of the aflatoxin biosynthesis polyketide synthase protein using AutodackVina software. The results were analyzed using Discovery Studio v16.1.0 software.

Results: Among the compounds studied, atropine, hyoscyamine, morphine, and strychnine exhibited the highest binding affinities with values of -11.9, -10.8, -11.5, and -11.1 kcal/mol, respectively, for the 3HRR (aflatoxin biosynthesis polyketide synthase) protein. Additionally, neurocaine and morphine demonstrated notable binding affinities of -11.6 and -12.9 kcal/mol, respectively, for the 3HRQ (Biosynthetic) protein. Notably, morphine displayed the strongest inhibitory activity against both proteins, with key amino acids aspartic acid (1395), alanine (1397), and valine (1394) for 3hrr protein, and glycine (1340), asparagine (1502), and arginine (1343) for 3hrq protein.

Conclusion: Aflatoxins represent serious health risks due to their potent carcinogenic and hepatotoxic properties. The findings of this study revealed the inhibitory potential of morphine. Consequently, combinations of alkaloids with a central structure similar to morphine may be promising candidates as anti-aflatoxin agents, warranting further investigation and development for drug research.

Keywords: Alkaloids, Aflatoxins, Morphine, Molecular docking simulation

1. Introduction

Aflatoxins are toxic secondary metabolites produced by certain fungi, mainly *Aspergillus flavus* and *Aspergillus parasiticus* [1]. These molds can contaminate different food crops, such as peanuts, corn, cottonseed, and tree nuts, under certain conditions of temperature, humidity, and improper capacity [2]. The biosynthesis of aflatoxins involves a complex pathway including several enzymatic reactions, like Acetate assimilation, Polyketide synthase (PKS) pathway, oxidative steps, ring formation, and tailoring reactions (These reactions involve enzymes that methylate, acetylate, or conjugate different groups to the aflatoxin molecule, leading to the production of various aflatoxin types (e.g., B1, B2, G1, G2) and export and secretion [3].

Aflatoxin biosynthesis polyketide synthase (PKS) is an enzyme complex responsible for the synthesis of aflatoxins in certain species of fungi, primarily Aspergillus [4]. The PKS system involved in aflatoxin biosynthesis is a large multifunctional enzyme complex composed of several modules [5]. Each module within the PKS system performs specific enzymatic reactions, ultimately leading to the production of the aflatoxin molecule. The PKS system consists of three types of modules: ketosynthase (KS), acyltransferase (AT), and acyl carrier protein (ACP) modules [6]. The KS module catalyzes the condensation of malonyl-CoA with growing intermediates, extending the polyketide chain. The AT module selects and transfers specific acyl-CoA units to the growing polyketide chain. The ACP module carries the growing polyketide intermediate between various enzymatic domains within the PKS system [7]. During the biosynthesis process, the PKS system extends and modifies the polyketide chain iteratively until the final aflatoxin structure is formed. Additional enzymatic reactions and modifications, such as oxidation and reduction, may occur to generate the specific aflatoxin variant [8]. Aflatoxins are potent liver carcinogens classified as Group 1 human carcinogens by the IARC (International Agency for Research on Cancer). Their carcinogenicity is primarily due to metabolic activation by cytochrome P450 in the liver to form reactive exo- and endo-epoxide metabolites that covalently bind to and form DNA adducts, especially N7-guanine adduct, causing mutations like G to T transversions that can initiate cancer development through activation of oncogenes and inactivation of tumor suppressor genes [9]. In addition, aflatoxins also induce oxidative stress through generation of reactive oxygen species like superoxide anion radicals, leading to oxidative DNA damage that further promotes uncontrolled cell growth and cancer. They also impair protein synthesis and cause liver damage by inhibiting enzymes, contributing to their acute toxicity which at high doses can cause liver failure. Chronic exposure to low levels is a major risk factor for liver cancer in parts of the world where contamination is common [10]. Alkaloids are a diverse group of naturally occurring compounds that are characterized by their alkaline properties and often have pharmacological effects [11]. Some alkaloids have been found to possess anti-aflatoxin properties, meaning they can inhibit the toxic effects of aflatoxins or reduce their production [12]. One example of an alkaloid with anti-aflatoxin activity is silymarin, which is determined from the milk thistle plant (Silybum marianum). Silymarin has been shown to have hepatoprotective effects and can help prevent liver damage caused by aflatoxin exposure. It acts by inhibiting the enzymes responsible for the activation of aflatoxins and enhancing the detoxification processes in the liver [13]. Another alkaloid with anti-aflatoxin properties is berberine which is found in various plants, such as Berberis species and goldenseal (Hydrastis canadensis). Berberine has been reported to prevent the growth of aflatoxin-producing fungi and reduce the production of aflatoxins. It also has hepatoprotective effects and can mitigate the toxic effects of aflatoxins on liver cells [14]. The purpose of this research was to investigate the inhibitory effects of ten alkaloid derivatives against aflatoxin biosynthesis polyketide synthase using molecular docking method.

2. Materials and Methods

Ligands preparation

The 2D structures of derivatives and Chemical Formulas (C.F.) are reported in Table 1. The 2D Structures of ten alkaloid derivatives, used as ligands, were obtained from the PubChem database in .SDF format. The PubChem IDs and Molecular Weights (M.W.) are reported in Table 2. The 2D structures were converted from .SDF to .pdb using ChemDraw Ultra 12.0.2.1076 software. The ligands were normalized using the MM2 method by Chem3D Pro 12.0.2.1076 software. After normalization, the Total Energy (T.E.) of all derivatives was reported in Table 1. Then, AutoDockTools 1.5.7 software was used to convert the initial format of the ligands from .pdb to .pdbqt format [15]. Some alkaloid derivatives have shown promise in laboratory research in inhibiting the growth of fungi that produce aflatoxins. Aflatoxins are toxic and carcinogenic metabolites produced by certain *Aspergillus* fungus strains that can contaminate food crops such as corn, peanuts and tree nuts. Certain alkaloid derivatives appear to interfere with fungal metabolic processes required for aflatoxin biosynthesis. Some studies have found that they can downregulate or inhibit the polyketide synthase enzyme involved in the pathway leading to aflatoxin production. This helps to prevent the fungi from synthesizing toxic aflatoxins. Further research is needed, but some alkaloid derivatives show dose-dependent anti-aflatoxigenic activity without significantly inhibiting fungal growth. This suggests they may offer a means to control aflatoxin contamination in crops without completely killing off the fungi. Further controlled laboratory and field testing would be required before any alkaloid derivatives could be considered for real-world applications to prevent mycotoxin contamination in agricultural commodities. Their effects in living biological systems can be complex, so careful study and oversight are important.

Table 1. 2D structure and Chemical Formula of alkaloid compounds.

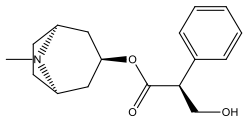
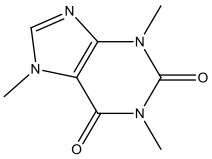
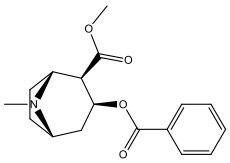
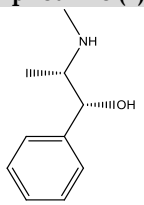
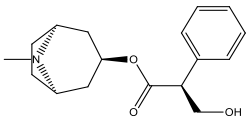
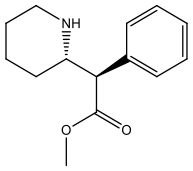
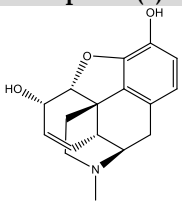
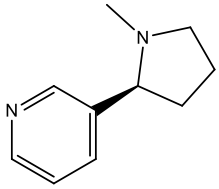
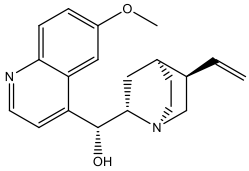
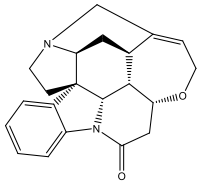
Atropine (1)	Caffeine (2)	Neurocaine (3)	Ephedrine (4)	Hyoscyamine (5)
				
$C_{17}H_{23}NO_3$	$C_8H_{10}N_4O_2$	$C_{17}H_{21}NO_4$	$C_{10}H_{15}ON$	$C_{17}H_{23}NO_3$
Methylphenidate (6)	**Morphine (7)**	**Nicotine (8)**	**Quinine (9)**	**Strychnine (10)**
				
$C_{14}H_{19}NO_2$	$C_{17}H_{19}NO_3$	$C_{10}H_{14}N_2$	$C_{20}H_{24}N_2O_2$	$C_{21}H_{22}N_2O_2$

Table 2. AutoDockVina results of alkaloid compounds (1-10) (ASP: Aspartic acid, THR: Threonine, GLN: Glutamine, ASN: Asparagine, HIS: Histidine, ALA: Alanine, ILE: Isoleucine, ARG: Arginine, SER: Serine, GLU: Glutamic acid, VAL: Valine, LYS: Lysine, TYR: Tyrosine, GLY: Glycine, PHE: Phenylalanine)-(M.W: Molecular Weight, T.E: Total Energy, D.F.R: Distance From RMSD, B.M.R: Best Mode RMSD)

Compounds		1	2	3	4	5	6	7	8	9	10
PubChem CID		174174	2519	446220	9294	154417	4158	5288826	89594	3034034	441071
M.W (g/mol)		289.4	194.19	303.35	165.23	289.4	233.31	285.34	162.23	324.4	334.4
T.E (Kcal/mol)		39.8312	22.4832	53.2449	2.0364	39.8312	14.8166	39.2056	19.7290	47.3351	334.4
3hrr	Affinity (Kcal/mol)	-11.9	-7.8	-8.2	-7.2	-10.8	-8.5	-11.5	-5.1	-6.9	-11.1
	D.F.R	22.08	16.22	3.03	40.32	66.46	59.28	32.85	36.74	32.64	33.08
	B.M.R	23.95	17.14	4.60	41.90	68.45	60.93	34.74	38.40	34.97	36.67
	H-Bonds	ASP:1441 THR:1433 GLN:1406	GLN:1406 THR:1442	ASN:1568 HIS:1345	ALA:1536 ILE:1576	ARG:1425 SER:1426 GLU:1415	THR:1442 ALA:1397	ASP:1395 ALA:1397 VAL:1394	HIS:1538	GLN:1367 LYS:1307	ARG:1500 ARG:1627 ARG:1631
3hrq	Affinity (Kcal/mol)	-7.7	-5.0	-11.6	-8.0	-7.0	-8.7	-12.9	-5.0	-8.4	-5.7
	D.F.R	51.64	18.48	43.49	29.64	46.24	27.76	13.67	17.56	31.12	35.49
	B.M.R	53.97	19.36	45.24	30.25	49.11	30.46	15.13	18.99	33.17	37.08
	H-Bonds	GLN:1367 GLN:1364	ASN:1568	HIS:1602 LYS:1591 ASP:1604	ASP:1604 LYS:1591	HIS:1541 HIS:1538	ASN:1518 TYR:1566	GLY:1340 ASN:1502 ARG:1343	PHE:1501	GLU:1515 ASN:1487	ARG:1631

Receptor preparation

The 2D structure of Aflatoxin biosynthesis polyketide synthase (PDB IDs: 3HRR and 3HRQ) were obtained from Protein Data Bank (https://www.rcsb.org/) with a resolution of 1.90°A for 3HRR and 1.80°A for 3HRQ (A: Angstrom) and in .pdb format. Then, using Discovery Studio Client 4.5 software, the ligands and water molecules were separated from the proteins, and the structures were stored separately as a .pdb files. Finally, the structures were prepared by adding polar hydrogens using AutoDockTools-1.5.6 software and saved in .pdbqt format [16].

Molecular docking

The necessary .config files for molecular docking were prepared. The size and location of the "grid box" were determined using AutoDockTools-1.5.6 software. The dimensions and centers (_X, _Y and _Z) of the grid box were set to cover all parts of the enzymes (*3HRR:* X center: -6.806, Y center: -14.917, Z center: 4.972; and *3HRQ:* X center: -215.639, Y center: -198.167, Z center: -140.361). Then, the required information was recorded and stored in the .config file and a specific .config file was generated for each ligand (ten ligands). It should be noted that this was done separately for each receptor. The overall findings obtained through molecular docking were examined using AutoDockTools-1.5.6 software. In order to analyze the results in greater detail, the ligand-receptor complex corresponding to the conformation with the highest binding energy was prepared and assessed for each alkaloid compound using Discovery Studio Client 4.5 software [17].

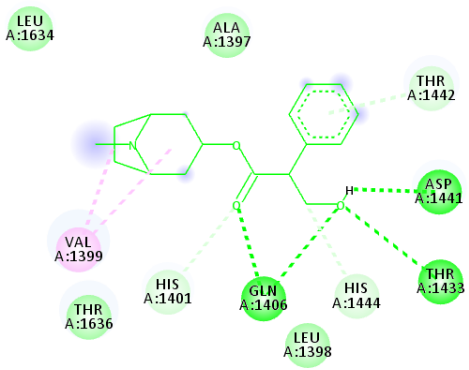
3. Results

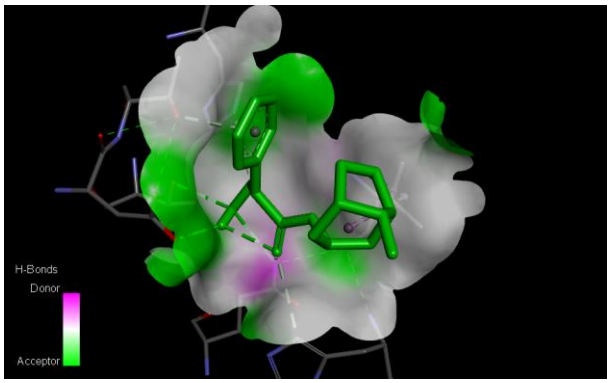
As shown in Table 2, all PubChem CID, molecular weight, total energy, affinities of 3HRR/3HRQ, along with the distances from RMSD and the best mode RMSD of the compounds, were calculated.

3HRR: As shown in Fig 1, the compounds Atropine, Hyoscyamine, Morphine and Strychnine exhibited the highest affinities among the compounds with -11.9, -10.8, -11.5 and -11.1 kcal/mol, respectively, Meanwhile, compound Atropine interacted with amino acids aspartic acid (1441 hydrogen bonds), threonine (1433 hydrogen bonds) and glutamine (1406 hydrogen bonds); compound Hyoscyamine with amino acids arginine (1425 hydrogen bonds), serine (1426 hydrogen bonds) and glutamic acid (1415 hydrogen bonds); compound Morphine with the amino acids aspartic acid (1395 hydrogen bonds), alanine (1397 hydrogen bonds) and valine (1394 hydrogen bonds); and finally compound Strychnine with the amino acid arginine (1500, 1627 and 1631 hydrogen bonds) inhibited 3HRR protein.

3HRQ: As shown in Fig 2, the compounds Neurocaine and Morphine showed the highest affinities among the compounds with -11.6 and -12.9 kcal/mol, respectively. Meanwhile, compound Neurocaine interacted with amino acids Histidine (1602 hydrogen bonds), Lysine

3hrr+Atropine





3hrr+Hyoscyamine

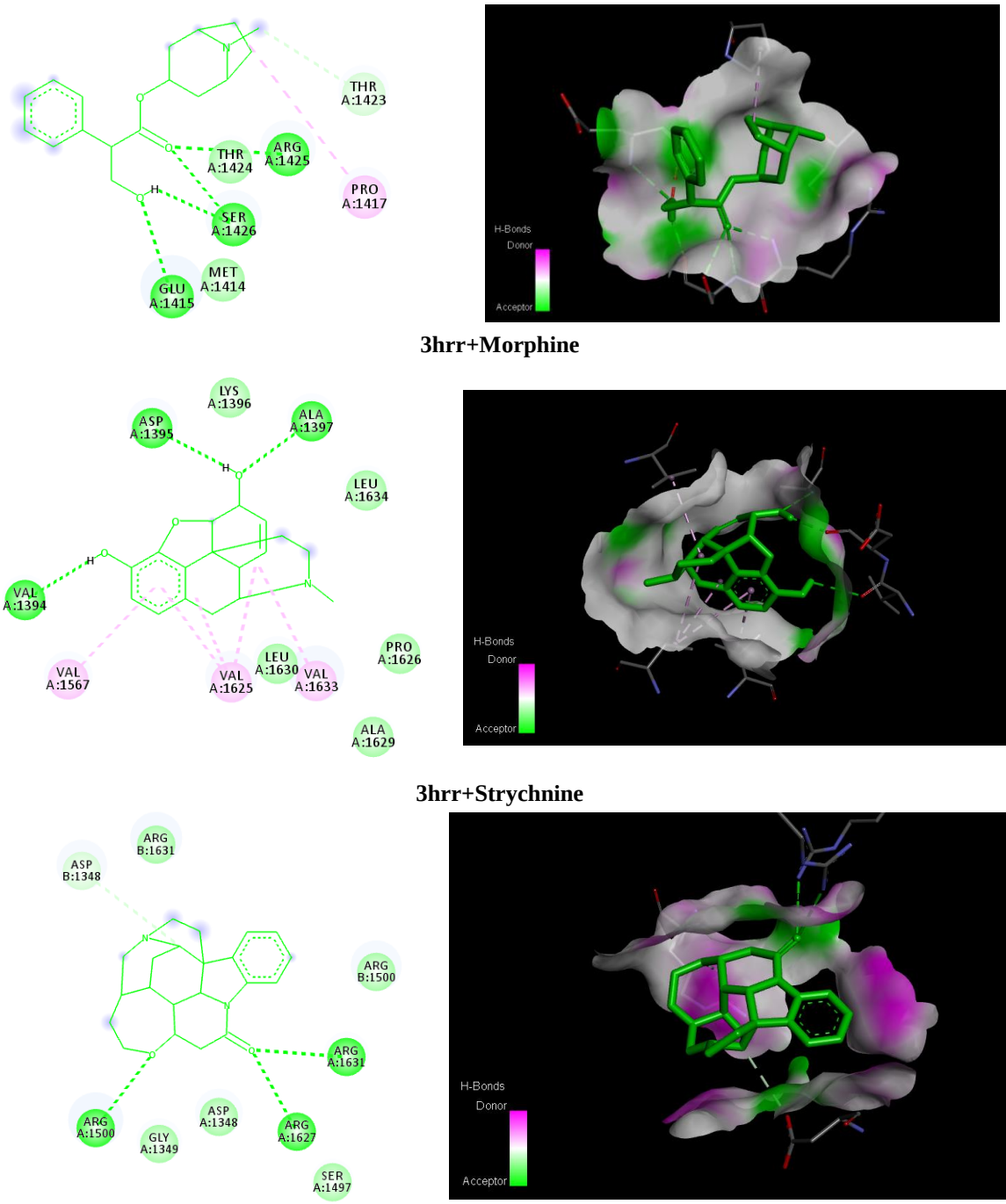


Figure 1. 2D and 3D results of AutoDockVina for 3hrr.

(1433 1591 hydrogen bonds) and Aspartic acid (1604 hydrogen bonds); and compound Morphine with amino acids Glycine (1340 hydrogen bonds), Asparagine (1502 hydrogen bonds) and Arginine (1343 hydrogen bonds) inhibited 3HRQ protein.

3hrq+Neurocaine

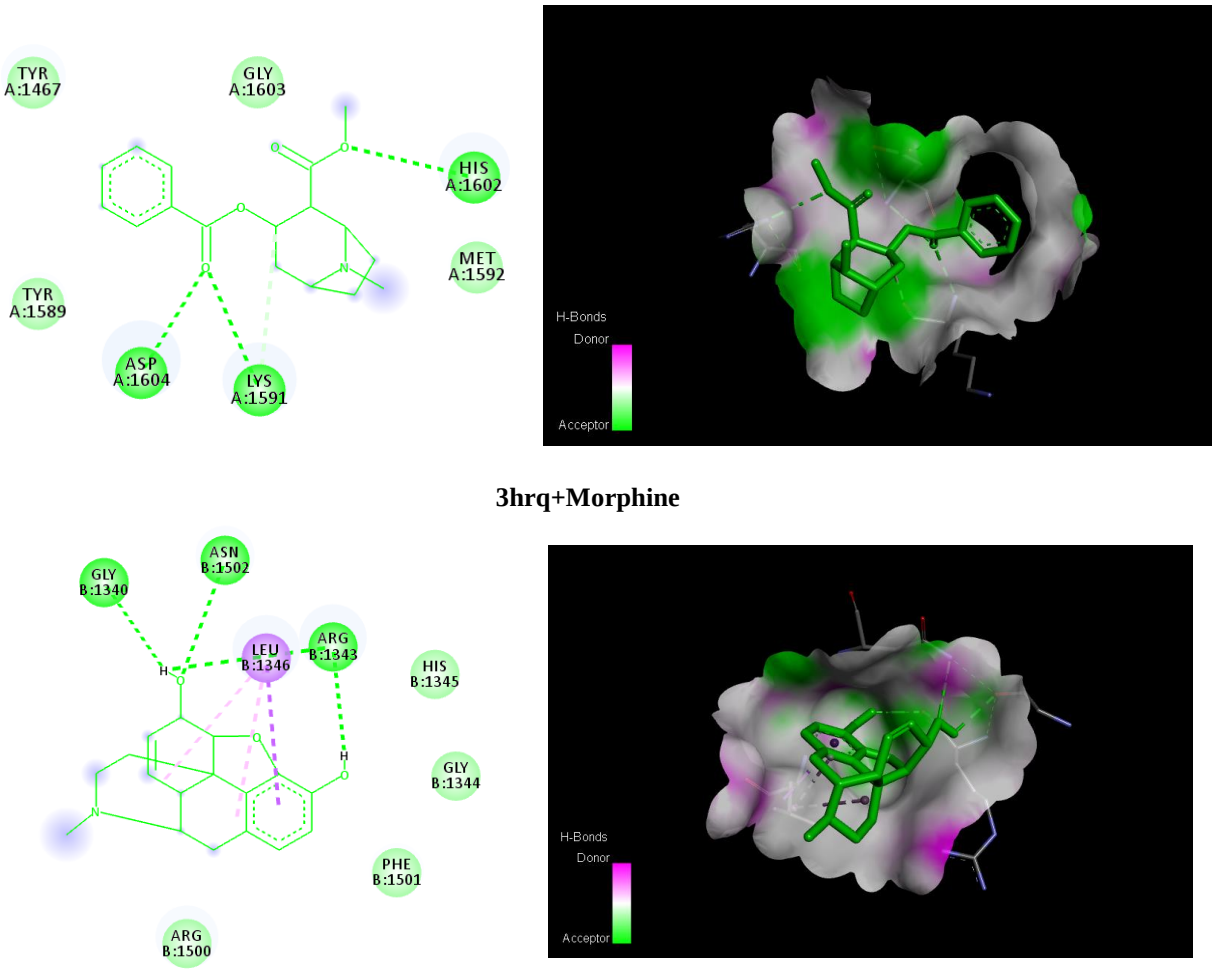


Figure 2. 2D and 3D results of AutoDock Vina for 3hrq

4. Discussion

The biosynthesis of aflatoxins, highly toxic compounds produced by certain molds, involves a complex enzymatic pathway, with polyketide synthases (PKSs) playing a crucial role. The importance of aflatoxin biosynthesis PKS lies in its direct involvement in the production of aflatoxins, which are known for their high toxicity and carcinogenic properties [18]. PKS enzymes are responsible for the synthesis of the polyketide backbone, which serves as the core structure of aflatoxins. These enzymes catalyze the iterative condensation of malonyl-CoA units, leading to the formation of a polyketide chain. The polyketide intermediate undergoes further enzymatic reactions, resulting in the synthesis of diverse aflatoxin variants, including B1, B2, G1, and G2. Understanding the role of PKS enzymes in aflatoxin biosynthesis is crucial for elucidating the molecular mechanisms underlying toxin production [19]. The toxicity and carcinogenicity of aflatoxins underscore the importance of studying aflatoxin biosynthesis PKS. These compounds can cause severe health effects, including liver damage, immunosuppression, growth impairment, and an increased risk of liver cancer in humans and animals [20]. By targeting the enzymes involved in aflatoxin biosynthesis, including PKS, it may be possible to develop intervention strategies to inhibit or disrupt aflatoxin production. This could involve the development of enzyme inhibitors or genetic engineering approaches to manipulate the expression or activity of PKS enzymes. Such interventions could have significant implications for reducing aflatoxin contamination in food and feed, thereby protecting human and animal health [21]. Finding anti-aflatoxin molecules is of paramount importance due to the significant health risks associated with aflatoxin contamination [22]. Anti-aflatoxin alkaloids are a group

of naturally occurring compounds that have been found to possess inhibitory or detoxifying effects against aflatoxins. These alkaloids can be derived from various sources, including plants and microorganisms, and have been studied for their potential in reducing aflatoxin contamination and its harmful effects [23]. Some examples of anti-aflatoxin alkaloids include: Coumarins are a class of compounds found in a variety of plants. They have been investigated for their ability to inhibit aflatoxin production by interfering with the growth and metabolism of aflatoxin-producing fungi [24]. Flavonoids are widely distributed plant compounds known for their antioxidant and antimicrobial properties. Certain flavonoids, such as quercetin and apigenin, have shown inhibitory effects on aflatoxin synthesis and can reduce aflatoxin production by aflatoxin-producing fungi [25]. Curcumin is a natural compound found in turmeric. It has been studied for its potential to inhibit aflatoxin production and reduce the toxic effects of aflatoxins. Curcumin exhibits antioxidant, anti-inflammatory, and hepatoprotective properties, which can help mitigate aflatoxin-induced liver damage [26]. Resveratrol is a polyphenolic compound found in grapes, berries, and other plants. It has been shown to possess antimicrobial and antifungal properties, which can inhibit the growth of aflatoxin-producing fungi. Additionally, resveratrol has been reported to have hepatoprotective effects and can mitigate aflatoxin-induced liver damage [27]. In these cases, the purpose of this research was to identify the inhibitors of aflatoxin biosynthesis polyketide synthase by natural compounds such as alkaloids. In this study, we investigated Atropine, Caffeine, Neurocaine, Ephedrine, Hyoscyamine, Methylphenidate, Morphine, Nicotine, Quinine and Strychnine as PksA inhibitors. According to the results, atropine, hyoscyamine, morphine and strychnine exhibited the highest affinity among the compounds with a number of amino acids like aspartic acid, threonine, glutamine, arginine, serine, glutamic acid, aspartic acid, alanine, valine and arginine for inhibited 3HRR protein. According to the reviewed sources, there were very limited studies on the effects of the alkaloids studied in this research on aflatoxin. Atropine is primarily known for its use as an anticholinergic medication to block the effects of acetylcholine in the body. Hyoscyamine is a naturally occurring tropane alkaloid found in plants such as Datura stramonium and Hyoscyamus niger. Morphine is an opioid analgesic commonly used for pain management. Strychnine is a natural alkaloid derived from the seeds of Strychnos nux-vomica and is known for its toxicity to humans and animals [28]. According to Durrieu et al.'s 2023 research on screening the anti-aflatoxin B1 activity of Peruvian plant extracts, the findings indicated that the effectiveness of certain plant extracts in inhibiting AFB1 production was associated with their composition, particularly their polyphenol content and subsequent antioxidant activity. Aloysia citrodora (AC) exhibited the highest inhibition with 78% reduction in AFB1 synthesis, followed by Annona muricata (AM) at 69%, Uncaria tomentosa (UT) at 58%, and Myrciaria dubia (MD) at 32%. Similarly, Dysphania ambrosioides (DA) and Minthostachys mollis (MM) extracts displayed inhibitory effects on AFB1 synthesis, with DA inhibiting 47% and MM inhibiting 89% of production. However, these extracts showed limited antioxidant activity (IC50 DDPH > 400 mg/L), suggesting an alternative mode of inhibition [12]. Also, compounds Neurocaine and Morphine had the highest affinity among the compounds with a number of amino acids like Histidine, Lysine, Aspartic acid, Glycine, Asparagine and Arginine to inhibit 3HRQ protein. In vitro studies have shown that berberine, an alkaloid found in plants like barberry, can inhibit the production of aflatoxins by the fungus Aspergillus flavus. It functions by suppressing the expression of genes involved in aflatoxin biosynthesis [29]. In vivo animal studies found that berberine administration reduced aflatoxin B1-induced toxicity in mice. It decreased liver damage, oxidative stress and levels of DNA adduct formation caused by the mycotoxin [30]. Other alkaloids from plants like piperine from black pepper and capsaicin from chili peppers have also exhibited protective effects against aflatoxin toxicity in animal models. They help reduce liver damage and biochemical changes induced by aflatoxins. Sanguinarine, an alkaloid from bloodroot, inhibited aflatoxin production in A. flavus cultures. It functions by disrupting fungal morphology and downregulating aflatoxin gene clusters [31]. In vitro and in vivo research indicates alkaloids from Sophora flavescens like matrine and oxymatrine protect against aflatoxin B1-induced liver damage in cells and animal tissues by antioxidant and anti-inflammatory mechanisms. Cell culture studies show alkaloids from Coptis chinensis like berberine, coptisine and palmatine can protect liver cells from aflatoxin B1 toxicity by reducing oxidative stress, DNA damage and apoptotic effects [32]. Some limitations that could be relevant to this study and might have impacted the findings include the following: molecular docking is inherently predictive and relies on computational algorithms that may not fully account for the complexities of biological systems. The accuracy of docking results can be influenced by the choice of docking software, scoring functions, and the conformational flexibility of the target protein and ligands. While molecular docking provides insights into possible binding interactions, the findings should ideally be complemented with experimental validation, such as enzyme activity assays or cell-based studies, to confirm the inhibitory effects of the alkaloid compounds. The study may have focused

on a limited range of alkaloid compounds. The diversity in structure and pharmacological profiles among alkaloids could mean that not all relevant compounds were investigated, potentially overlooking more effective inhibitors. The reliability of docking studies heavily depends on the quality of the 3D structure of the target protein. If the polyketide synthase structure used was derived from a template or was a model rather than experimentally determined, inaccuracies could affect the docking predictions. Molecular docking typically occurs under idealized conditions, which might not accurately reflect the physiological environment, including factors such as pH, ionic strength, and the presence of other biomolecules that could affect binding interactions. Proteins are dynamic entities, and docking studies may not account for the full range of conformational changes that may occur during substrate binding or catalysis. These dynamics can significantly influence binding affinities and the inhibitory potential of the compounds. The study may not have differentiated between selective inhibition of the target polyketide synthase and potential off-target effects on other enzymes or pathways, which could have implications for the safety and effectiveness of these compounds if developed as therapeutic agents [33].

5. Conclusion

The results of the study indicated that Morphine, an alkaloid compound, exhibited the most significant inhibitory effects on aflatoxin biosynthesis polyketide synthase compared to other compounds tested. This promising finding suggests that Morphine could serve as a valuable lead compound in the development of new therapeutic agents aimed at mitigating aflatoxin production. Aflatoxin poses serious health risks due to its carcinogenic and hepatotoxic nature. Given Morphine's central role in the study, exploring its derivatives—those compounds that retain the core structure of morphine but may incorporate modifications to enhance efficacy, bioavailability, or specificity—could yield novel inhibitors against aflatoxin synthesis. By systematically modifying the morphine scaffold, researchers may identify variants that exhibit improved binding affinities or selectivity for the target enzyme, potentially leading to more effective strategies for controlling aflatoxin contamination. Furthermore, the structural diversity offered by the alkaloid family opens avenues for the synthesis of a range of Morphine-derived compounds. These derivatives could be designed to optimize their pharmacokinetic properties and reduce any potential side effects associated with morphine itself. The development of such compounds could lead to innovative therapeutic options that not only inhibit aflatoxin synthesis but also prove safe and effective for use.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Author's contributions

Conceptualization: Mansour Bayat, Yasin SarveAhrabi; Methodology: Yasin SarveAhrabi, Mansour Bayat, Sarina Nejati khoei; Investigation: Mansour Bayat, Yasin SarveAhrabi, Sarina Nejati khoei; Writing – original draft: Mansour Bayat, Yasin SarveAhrabi; Writing – review & editing: Yasin SarveAhrabi, Mansour Bayat; Funding acquisition: Mansour Bayat, Yasin SarveAhrabi; Resources: Mansour Bayat, Yasin SarveAhrabi; Supervision: Mansour Bayat, Yasin SarveAhrabi.

Conflict of interest

The authors declared no conflicts of interest.

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