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In silico Analysis of Glycogen Synthase Kinase 3-beta (GSK-β) as Aflatoxin Binder

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Aflatoxins are secondary metabolites of certain fungi like *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nomius* and *Aspergillus parasiticus*. Food products like cereals, milk, milk products, nuts, oilseeds and spices are reported for aflatoxins contamination. Among the fourteen aflatoxin types reported so far, aflatoxins B1, B2, G1, G2, M1, and M2 are commonly studied. Aflatoxins are known to cause various diseases including aflatoxicosis in livestock and domestic animals and cancer in humans. Recently aflatoxin B1 has reported binding with Glycogen synthase kinase 3-beta (GSK-3â). This prompted to carry out the present study, where Glycogen synthase kinase 3-beta (GSK-3â) was evaluated on the docking behaviour of 13 aflatoxin analogues using Patch Dock. In addition, molecular physicochemical, drug-likeness, ADME (Absorption, Distribution, Metabolism and Excretion analyses) were also carried out. The molecular physiochemical analysis revealed that aflatoxin analogue showed nil violation and complied well with the Lipinski's rule of five. ADME analysis indicates all thirteen aflatoxin analogue predicated to have high gastro-intestine (GI) absorption property. Docking studies, with GSK-3â, revealed that aflatoxin G2 analogue showed the largest atomic contact energy (-224.82 kcal/mol) and Aflatoxin P1 analogue had the least (-160.33 kcal/mol). In addition, aflatoxin P1 analogue has interacted with Asp200 amino acid residue of GSK-3â. Thus, the present study showed the potential of GSK-3â as aflatoxin binder.

Keywords: Aflatoxins contamination; Aflatoxin analogues; ADME; Docking; Ligand; Glycogen synthase kinase 3-beta (GSK-3β)

Mycotoxins are natural fungal secondary metabolites with low-molecular-weight (i.e., small molecules) produced by ûlamentous fungi and the most important genera of mycotoxin-producing fungi are *Aspergillus, Fusarium, Stachybotrys, Claviceps, Penicillium* and *Alternaria*[1]. The principal classes of mycotoxins, which cause health problems in animals and human beings, include aflatoxin, trichothecenes, fumonisins, zearalenone and ochratoxin A[2]. Aflatoxins are potent mycotoxin (fungal toxin) capable of causing death on humans and animals. There are several types of aflatoxins,

among them, aflatoxins B1, B2, G1, G2, M1, and M2 are commonly studied[3]. It is highly pathogenic and leads to carcinogenic, mutagenic, hepatotoxic and immunosuppressant effects in animals and humans[4–6]. Particularly in children, it causes stunted growth, immunosuppression, liver cancer and even death; when they consume milk from dairy cattle that feed an aflatoxin contaminated feed[7,8]. Moreover, aûatoxin B1 (AFB1) is the most potent carcinogenic (biological) agent which has been reported by several researchers throughout the world[9]. Furthermore, in dairy cattle, after the consumption of Aflatoxin contaminated feed, AFB1 and AFB2 will be biotransformed into hydroxylated metabolites like aûatoxin M1 (AFM1) and M2 (AFM2) respectively; that later contaminates milk and milk products[10–12].

Glycogen Synthase Kinase 3-beta (GSK-3â) was first identified as a negative regulator of glycogenesis and subsequently reported to regulate varies signalling pathway and cellular function. GSK-3â is an important enzyme in the process of neurogenesis, neuronal migration, differentiation and survival of immature brain[13,14]. Numerous studies have been reported for the binding ability of GSK-3â with certain chemical compounds and plant extracts. In addition to this few potential inhibitors have been reported for this enzyme[15-16]. Recently aflatoxin B1 has reported binding with GSK-3â [17]. This incited to carry out the present study, where GSK-3â was evaluated on the docking behaviour of 13 aflatoxin analogues using Patch Dock as well as physicochemical, drug-likeness and ADME (Absorption, Distribution, Metabolism and Excretion) analyses were also carried out.

MATERIAL AND METHODS

Ligand preparation

Thirteen types of Aflatoxins were prepared as a ligand for this study, as illustrated in Table 1. The ligands were namely (I) Aflatoxin B1; (II) Aflatoxin G1; (III) Aflatoxin M1; (IV) Aflatoxin B2; (V) Aflatoxin G2; (VI) Aflatoxin M2; (VII) Tetrahydrodeoxy aflatoxin B1; (VIII) Compound 2; (IX) Compound 8; (X) Compound 11; (XI) Aflatoxin Q1; (XII) Aflatoxin P1; (XIII) Aflatoxin B1-8,9-epoxide (18). The chemical structures of these thirteen Aflatoxin analogues were first drawn in ChemBio Draw Ultra 12.0 (*www.cambrid gesoft.com*) and subsequently the molecular mechanics (MM2) energy minimization of ligands were carried out by ChemBio 3D Ultra 12.0, according to the reported procedure[19]. These minimized energy structures were further utilized for Patch Dock study.

Identification and preparation of target protein

The three dimensional (3D) structure of the target protein Glycogen synthase kinase 3-beta (GSK-3â), PDB 3F88 with resolution of 2.6 A^0 was obtained from the Research Collaborator for Structural Bioinformatics (RCSB) Protein Data Bank (*www.rcsb.org*)[20]. The first task was preparing the protein by removing another chains (B, C and D) on it other than the A chain, by using UCSF Chimera software (*www.cgi.ucsf.edu/chimera*), followed by removing water particles (without hydrogen bonds) which are crystallographically observed[21]. Then prepared protein was uploaded to the Patch Dock server with each pre-prepared ligands for docking analysis.

Molecular descriptors calculation

Smiles of our prepared ligands are then copied to an online software to calculate the thirteen descriptors by using the online database of Molinspiration (*www.molinspiration.com*). The molecular descriptors studied in this study are log P, molecular weight, polar surface area, number of atoms, number of rotatable bond, number of O or N, number of OH or NH, ion channel modulator, drug-likeness including G protein coupled receptors ligand, kinase inhibitor, nuclear receptor ligand, and number of violations to Lipinski's rule[20].

Analysis of ADME

ADME (Absorption, Distribution, Metabolism and Excretion) analysis was carried out for all the 13 aflatoxin analogues using Swiss ADME. A standard default protocol was adopted for the same[20,22].

Docking studies

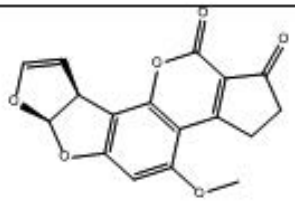
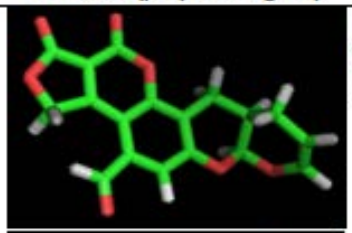
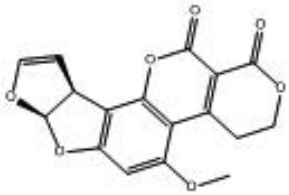
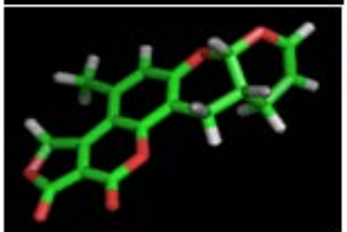
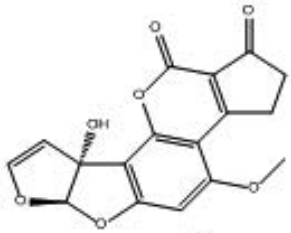
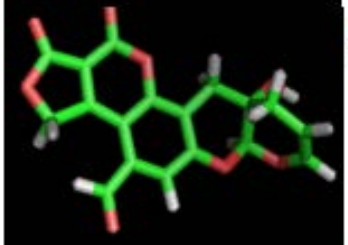
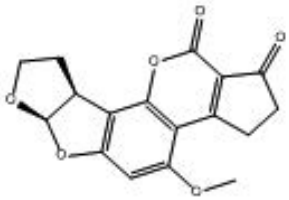
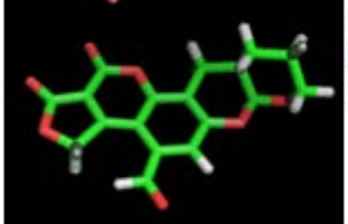
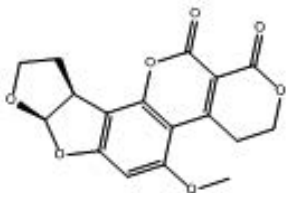
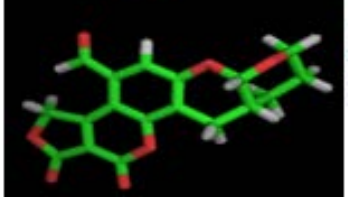
To conduct docking studies all the ligand and protein were uploaded to an online server called PatchDock (*http://bioinfo3d.cs.tau.ac.il/PatchDock*). The docking results were sent to our pre-registered email address and all the data are obtained from it. Patch Dock uses geometry-based molecular docking algorithm method to recognize the binding scores, by binding residues atomic contact energy of the given ligands. The ACE and other information are copied and the first

solution selected from the top of lists of several solutions (the docked protein-ligand complex) and downloaded in a database (pdb) file format. Finally, analysis of the protein for having a binding site for the Aflatoxin analogues were done by PyMOL software (www.pymol.org), by opening and measuring the bond distance and also identification and labelling the amino acids which binds with the particular ligand[20,21].

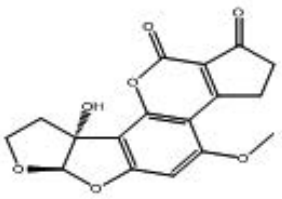
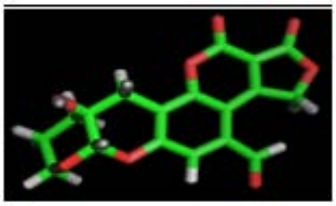
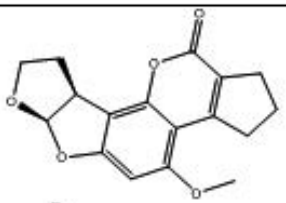
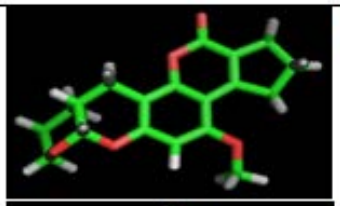
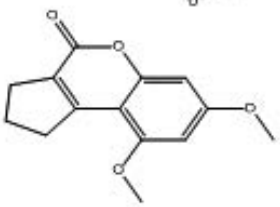
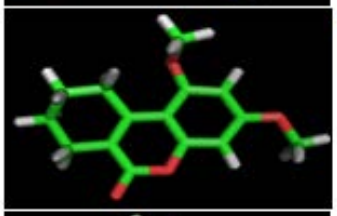
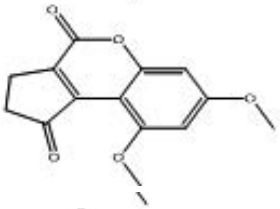
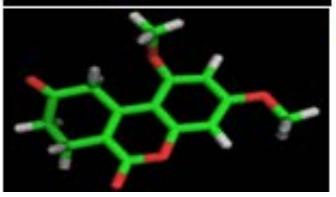
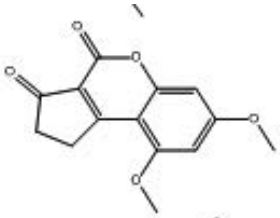
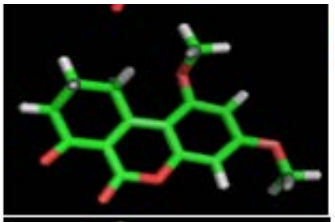
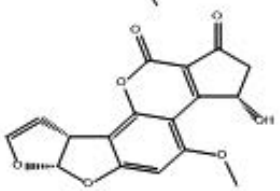
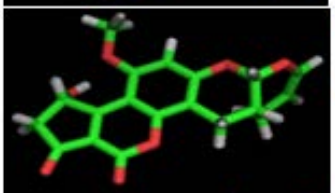
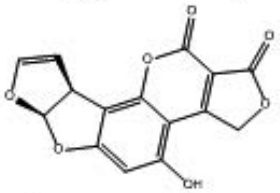
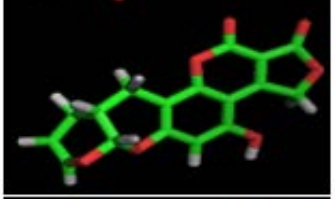
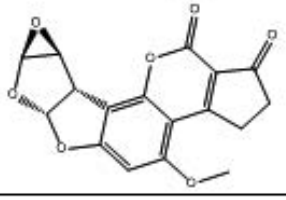
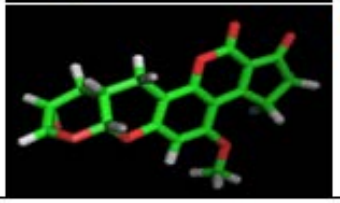
RESULTS AND DISCUSSION

Aflatoxins are potent mycotoxins which are now threatening both the feed and livestock sector throughout the world. In recent years', numbers of aflatoxin binders have been reported in the literature[23]. For instance, few commercial available aflatoxin binders are aflabalan, astraben-20, anzymit, flow guard, formycin, mycoflix

Table 1. Thirteen aflatoxin analogues selected for the present study

Ligand names*	Two dimensional (2D) structure	Three dimensional (3D) structure (prepared Ligand)	SMILES**
I.			O=C1OCC(C2=C(C=C3OC4C(CC3=C2O5)CC=CO4)C=O)=C1C5=O
II.			O=C1OCC(C2=C(C=C3OC4C(CC3=C2O5)CC=CO4)C)=C1C5=O
III.			O=C1OCC(C2=C(C=C3OC4C(CC3=C2O5)(O)CC=CO4)C=O)=C1C5=O
IV.			O=C1OCC(C2=C(C=C3OC4C(CC3=C2O5)CCCO4)C=O)=C1C5=O
V.			O=C1OCC(C2=C(C=C3OC4C(CC3=C2O5)CCCO4)C=O)=C1C5=O

Note: *- (I) Aflatoxin B1; (II) Aflatoxin G1; (III) Aflatoxin M1; (IV) Aflatoxin B2; (V) Aflatoxin G2; (VI) Aflatoxin M2; (VII) Tetrahydrodeoxy aflatoxin B1; (VIII) Compound 2; (IX) Compound 8; (X) Compound 11; (XI) Aflatoxin Q1; (XII) Aflatoxin P1; (XIII) Aflatoxin B1-8,9-epoxide. **- Simplified molecular input line entry system (SMILES)

VI.			O=C1OCC(C2=C(C=C3OC4C(CC3=C2O5)(O)CCCO4)C=O)=C1C5=O
VII.			O=C1C2=C(CCC2)C3=C(C=C4OC5C(CC4=C3O1)CCCO5)OC
VIII.			COC1=CC2=C(C(CCCC3)=C3C(O2)=O)C(OC)=C1
IX.			COC1=CC2=C(C(CC3=O)=C(CC3)C(O2)=O)C(OC)=C1
X.			COC1=CC2=C(C(CCCC3=O)=C3C(O2)=O)C(OC)=C1
XI.			O=C1CC(O)C(C2=C(C=C3OC4C(CC3=C2O5)CC=CO4)OC)=C1C5=O
XII.			O=C1OCC(C2=C(C=C3OC4C(CC3=C2O5)CC=CO4)O)=C1C5=O
XIII.			O=C1CCC(C2=C(C=C3OC4C(CC3=C2O5)CC6C(O6)O4)OC)=C1C5=O

plus, mycosorb, red crown and SA-20[24–26]. Thus, in the present study Glycogen synthase kinase 3-beta (GSK-3â) was evaluated on the docking behaviour of 13 aflatoxin analogues using PatchDock. Table 1 represents the thirteen ligands structure and SMILES selected for the present study.

To study the physiochemical and drug-likeness properties of these thirteen ligands (aflatoxin analogues) Lipinski's rule of five/Thumb of five was applied. According to previous studies violation of the Lipinski's rule of five is when logA is >5, MW >500, number of N, O (hydrogen bond receptor) is >10, number of OH and NH (hydrogen bond donor) is >5 and number of the rotatable bond (rotb) is >15[21]. With regard physiochemical properties, all the 13 aflatoxin analogues showed nil violation and complied well with the Lipinski's rule of five as shown in Table 2.

Table 2. Molecular physicochemical descriptors analysis of thirteen aflatoxin analogues using Molinspiration online software tool

Ligand Names	Log A[a]	TPSA[b]	Natoms[c]	MW[d]	nON[e]	nOHNH[f]	Nviolations[g]	Nrotb[h]	Volume[i]
I.	1.48	74.98	23	321.28	6	0	0	1	253.24
II.	1.52	84.22	24	328.28	7	0	0	1	262.22
III.	-0.9	95.21	24	328.28	7	1	0	1	260.93
IV.	1.57	74.98	23	314.29	6	0	0	1	259.42
V.	1.61	84.22	24	330.29	7	0	0	1	268.41
VI.	1	95.21	24	330.29	7	1	0	1	267.12
VII.	2.23	57.91	22	300.31	5	0	0	1	257.24
VIII.	2.38	48.68	18	246.26	4	0	0	2	219.24
IX.	1.72	65.75	19	260.25	5	0	0	2	221.42
X.	1.72	65.75	19	260.25	5	0	0	2	221.42
XI.	0.49	95.21	24	328.28	7	1	0	1	261.28
XII.	1.2	85.98	22	298.25	6	1	0	0	235.71
XIII.	1.11	87.51	24	328.28	7	0	0	1	257.62

Note: a- Octanol-Water partition coefficient, b- Polar surface area, c -Number of non-hydrogen atoms, d- Molecular weight, e- Number of hydrogen bond acceptors [O and N atoms], f -Number of hydrogen bond donors [OH and NH groups], g- Number of Rule of 5 violations, h- Number of rotatable bonds, i- Molecular volume

Table 3. Drug-likeness property analysis of thirteen aflatoxin analogues using Molinspiration online software tool

Ligand Names	GPCR* ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
I.	-0.26	-0.60	-0.43	0.09	-0.36	0.09
II.	-0.24	-0.51	-0.38	0.01	-0.38	-0.01
III.	-0.16	-0.52	-0.42	0.21	-0.29	0.18
IV.	-0.22	-0.60	-0.53	0.10	-0.19	0.23
V.	-0.19	-0.49	-0.47	0.03	-0.02	0.14
VI.	-0.13	-0.48	-0.47	0.26	-0.16	0.29
VII.	-0.37	-0.72	-0.59	-0.05	-0.36	0.11
VIII.	-0.78	-0.79	-0.82	-0.26	-0.85	-0.19
IX.	-0.61	-0.71	-0.69	-0.05	-0.65	-0.04
X.	-0.55	-0.63	-0.70	-0.03	-0.59	0.00
XI.	-0.07	-0.52	-0.34	0.21	-0.22	0.17
XII.	-0.23	-0.53	-0.41	0.23	-0.33	0.22
XIII.	-0.23	-0.56	-0.36	0.17	-0.23	0.28

Note: *GPCR- G Protein coupled receptors

In the case of drug-likeness if the score > 0 was considered active, -5.0 to -0.0 as moderate active and < -5.0 as inactive[21]. All the 13 ligands showed active to moderate active score towards all the six descriptions. Interestingly, none of them showed inactive score as shown in Table 3.

Similarly, Absorption, Distribution, Metabolism and Excretion (ADME) is simple and essential analysis tool. Recent days, it is commonly accepted in the early stage of drug development program, because of its unique characteristic nature. Table 4 shows the ADME profile of the thirteen selected aflatoxin analogues; all the ligands are predicted to have high gastrointestinal (GI) absorption effect.

The docking study and binding site analysis with GSK-3â, showed that ligand of Aflatoxin G2 exhibited the highest atom contact energy (ACE) of -224.82 kcal/mol, on other hand Aflatoxin P1 showed the minimum ACE (-160.33 kcal/mol). The

Table 4. ADME (Absorption, Distribution, Metabolism and Excretion) analysis of thirteen aflatoxin analogues using Swiss ADME online tool

Ligand Names	GI absorption	BBB permeability	P-gp substrate	*CYP1A2 inhibitor	*CYP2C19 inhibitor	*CYP2C9 inhibitor	*CYP2D6 inhibitor	*CYP3A4 inhibitor	# Log K_p
I.	High	Yes	No	No	Yes	No	Yes	Yes	-7.05
II.	High	No	No	No	Yes	No	No	No	-7.05
III.	High	No	No	No	No	No	Yes	No	-7.92
IV.	High	Yes	No	No	Yes	No	Yes	Yes	-7.27
V.	High	No	No	No	Yes	No	Yes	No	-7.41
VI.	High	No	Yes	No	No	No	Yes	Yes	-8.14
VII.	High	Yes	Yes	Yes	Yes	No	Yes	Yes	-6.56
VIII.	High	Yes	No	Yes	Yes	No	No	No	-6.20
IX.	High	Yes	No	Yes	Yes	No	No	No	-6.95
X.	High	Yes	No	Yes	No	No	No	No	-6.92
XI.	High	No	No	No	No	No	No	No	-7.94
XII.	High	No	No	No	No	No	Yes	No	-7.20
XIII.	High	No	No	No	No	No	Yes	Yes	-7.71

Note: *CYP-Cytochrome P450, #Log Kp -Skin Permeation (cm/s).

Table 5. The interaction energy analysis of thirteen aflatoxin analogues with Glycogen synthase kinase (GSK) using Patch Dock.

Ligand Names	ACE* (kcal/mol)	Interaction	Bonding distance (A)
I.	-168.64	No	Nil
II.	-197.50	Gln185	1.91
III.	-216.37	No	Nil
IV.	-210.99	Val 135	3.52
V.	-224.82	Gln 185	2.22
VI.	-168.64	Gln 185	3.54
VII.	-197.56	Gln 185	2.32
VIII.	-195.41	No	Nil
IX.	-183.87	No	Nil
X.	-163.27	No	Nil
XI.	-195.41	Glu 137	2.61
		Gln185	3.16
XII.	-160.33	Asp 200	2.60
XIII.	-202.42	Val 135	2.88

Note: *- Atomic contact energy.

binding energy calculation showed the following order: Aflatoxin G_2 ÂAflatoxin M_1 ÂAflatoxin B_2Â AFB1-8,9-epoxyÂTetrahydreoxy Aflatoxin B_1 Â Aflatoxin G_1Â Compound 2= AFQ1Â Compound 8ÂAflatoxin $B_{1=}$Aflatoxin M2 Â Compound 11 Â AFP1. Further, five ligands (Aflatoxin G1, G2, M2, Tetrahydreoxy Aflatoxin B_1 and AFQ1) have shown to interact with Gln185 amino acid residue of GSK-3â. Interesting another five ligands (Aflatoxin B1, M1 and Compound 2, 8, 11) does not show interaction with amino acid residue of GSK-3â (Table 5). Present result infers that the interaction is better for the bio transformed analogues of Aflatoxin (Aflatoxin Q1, Aflatoxin P1, Aflatoxin M2, Tetrahydrodeoxy aflatoxin B1 and AFB1-8, 9-epoxide), rather than the naturally occurring like AFB1, AFGB2 and AFG2. Thus, the present finding was in good agreement with earlier reports[17,27].

CONCLUSION

In the present study, all the 13 tested ligands have shown to dock with the target protein Glycogen synthase kinase 3-beta (GSK-3â). Thus, the present study showed the potential of GSK-3â as aflatoxin binder, especially for the bio transformed analogues of Aflatoxin (Aflatoxin Q1, Aflatoxin P1, Aflatoxin M2, Tetrahydrodeoxy aflatoxin B1 and AFB1-8, 9-epoxide).

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