WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 08-16. Research Article ISSN 2277– 7105

IN SILICO MOLECULAR DOCKING OF DI-(2-ETHYLHEXYL) PHTHALATE AND 13-HEXYLOXACYCLOTRIDEC-10-EN-2-ONE IDENTIFIED IN *AMBROSIA MARITIMA* L. (ASTERACEAE)

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Article Received on
16 September 2014,

Revised on 10 Oct 2014,
Accepted on 03 Nov 2014

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ABSTRACT

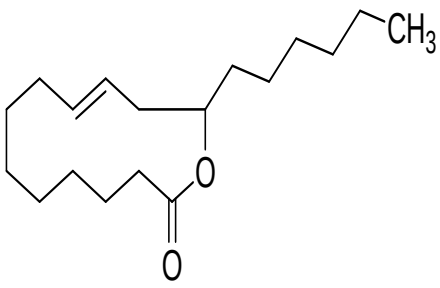
Discovery of new lead compounds to compact diseases can be traced directly to medicinal plants. *In silico* studies provide a good platform to estimate the applicability of various virtual screening methods in the assessment of a desired biological activity. This study was carried out to identify the bioactive compounds' relevant targets and to establish their therapeutic effects on molecular level. Two compounds namely, di-(2-ethylhexyl) phthalate (DEHP) and 13- hexyloxacyclotridec-10-en-2-one, were identified in the previously bio-assessed active *Ambrosia maritima*'s extract. The lead compounds' relevant targets were identified via the SwissTargetPrediction tool. The molecular docking was preformed via Auto-Dock 4.0 software. In structural bases virtual screening for target enzymes, Protein Kinase C Gamma Type (PKCG) and Cytochrome P450 19A1 (CYP19A1) ranked the top in binding probability for DEHP and 13-hexyloxacyclotridec-10-en-2-one, respectively. Molecular docking of DEHP on PKCG revealed a free binding energy of-5.75 Kcal/mol and showed three hydrogen bonds with the amino acid residue Arginine 185 and hydrophobic interactions. Docking of the other compound on CYP19A1 showed hydrogen bond with the amino acid residue Threonine 310, hydrophobic interaction and the free binding energy was -8.33 Kcal/mol. Thus, hit compounds predicted by *in silico* investigations in conjunction with ethnomedicinal

approaches are likely to confer new chemical entities with potential chemotherapeutic properties.

KEYWORDS: *Ambrosia maritima*, di-(2-ethylhexyl) phthalate, 13- hexyloxacyclotridec-10-en-2-one, Protein kinase C gamma, Cytochrome P450 19A1, SwissTargetPrediction, Auto-Dock 4.0.

INTRODUCTION

Herbal medicines have imparted wealth of information pertaining with the plants' diverse curative properties [1]. These species still, to date, provide new chemical entities, some with structural novelty, that contribute to the overall health care system [2]. The exponential increment of drug resistance in some therapeutic regimens, including those endemic, infectious and non-communicable disease therapies, has called for searching for new and effective molecules [3- 4]. There has, however, been much interest, recently, in the application of computational approach in the drug discovery process [5]. Herein, different *in silico* tools are advocated to estimate drug-protein interactions that play primer role in different biological processes such as signal transduction, cell regulation and other macromolecular assemblies [6- 7]. It is important to note that, proper selection of the potential biological target is the first and foremost task to develop a small molecular candidate ligand into a drug [8]. In this context, SwissTargetPrediction tool had recently become a premier searching engine for a wide panel of potential binding targets by offering high-throughput screening [9]. This "inverse" docking strategy; screening for ligand relevant protein targets, would fully explore the underlying mechanism of the molecules' therapeutic properties [10-11]. On the other hand, *in silico* molecular docking is of no exception in the drug discovery process; it reveals the putative binding modes and affinities of the compound to its relevant target. This molecular recognition is crucial in understanding the interaction mechanisms and to design therapeutic interventions [12]. In Sudanese traditional medicine, *Ambrosia maritima's* substantial role to compact diseases, including many infectious and tumor conditions, is witnessed [13-14] though many of its underlying phytoconstituents and their mechanisms had not been yet investigated [15]. Therefore, we anticipated that this plant contains many chemical compounds of valuable therapeutic properties. Thus, the present study aimed to reveal the potential therapeutic properties via different *in silico* tools of two candidate compounds namely, di-(2-ethylhexyl) phthalate (Fig.1) and 13- hexyloxacyclotridec-10-en-2-one (Fig.2), previous identified in *A. maritima*'s cytotoxic active extract [16], by different spectroscopical techniques [17].



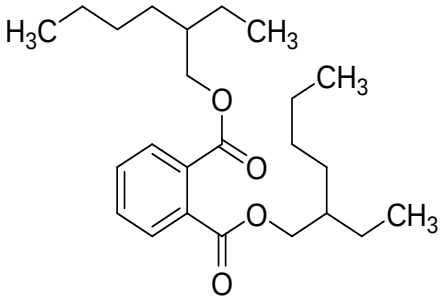


Figure (1): Di-(2-ethylhexyl) phthalate, Figure (2): 13-hexyloxacyclotridec-10-en-2-one

MATERIALS AND METHODS

Ligand Identification

As a part of our on-going drug discovery from biologically assessed plants species [16], two compounds were identified from the cytotoxic dichloromethane active extract of *Ambrosia maritima* L. namely, di(2-ethylhexyl) phthalate (DEHP) and 13-hexyloxacyclotridec-10-en-2-one, via different spectroscopical techniques [17].

Virtual Screening for Target Classes

The chemical structure of DEHP and 13-hexyloxacyclotridec-10-en-2-one was submitted in the form of canonical SMILE, retrieved from Pubchem database [18], in the SwissTargetPrediction computational tool [19].

Docking With Auto-Dock 4.0 Software

Preparation of the Ligand File

The ligand files of the two compounds were retrieved from Pubchem database in (.Pc3d) format, after wards, the two files were submitted in a Chem3D Ultra visualizing program to obtain standard 3D structures in (.mol2) format. Prior to docking, all hydrogen atoms were added to the ligand and Gastiger partial atomic charges were computed and saved. Auto-Dock 4.0 [20] automatically assigned the type of each atom and detected the root (the rigid part of the ligand) also the number of rotatable bonds that move was assigned via torsions option in the software. Finally the ligand was saved as (pdbqt) format.

Preparation of the Macromolecule File

The receptor file was retrieved from the Protein Data Bank (PDB) database in (.pdb) format [21]. The flexible residue of the protein target was selected to prepare the protein file. All polar hydrogen atoms were added and the partial kollman charge was computed. Finally, the macromolecule file was saved in (pdbqt) file format.

Preparation of the Grid Parameter File

The autogrid file was prepared with a pre-calculated dimensions; where the suitable grid size was determined, according to flexible residues, using the default grid spacing of 0.375 Å. The grid file was located in a place on the receptor surface where the expected ligand- receptor interaction occurs. Finally, the prepared gird file was saved in (.gpf) file format.

Preparation of the Docking Parameter File

After proper preparation of the input files (ligand and protein) and the calculation of the affinity maps, docking runs were conducted using Auto-Dock 4.0 software [20] using the Lamarckian Genetic Algorithm docking. The resultant structure files of Auto-Dock4.0 software was visualized using Discovery Studio Visualization (DSV) and LIGPLOT programs.

RESULTS AND DISCUSSION

The Target Binding Probabilities for Di (2-ethylhexyl) Phthalate (DEHP)

Protein kinase C gamma type (PKCG) ranked the best binging probability on the SwissTarget Prediction report (Fig.3).

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Protein kinase C gamma type *(by homology)*	P05129	PRKCG	CHEMBL2938		89 / 9	Ser_Thr Kinase
Protein kinase C beta type *(by homology)*	P05771	PRKCB	CHEMBL3045		89 / 9	Ser_Thr Kinase
Protein kinase C alpha type	P17252	PRKCA	CHEMBL299		89 / 9	Ser_Thr Kinase
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		1392 / 6	Unclassified
Androgen receptor	P10275	AR	CHEMBL1871		75 / 1	Transcription Factor
Tyrosine-protein phosphatase non-receptor type 2 *(by homology)*	P17706	PTPN2	CHEMBL3807		16 / 9	Tyr Phosphatase
Tyrosine-protein phosphatase non-receptor type 1	P18031	PTPN1	CHEMBL335		16 / 9	Tyr Phosphatase
Protein kinase C theta type *(by homology)*	Q04759	PRKCQ	CHEMBL3920		49 / 9	Ser_Thr Kinase
Protein kinase C delta type regulatory subunit	Q05655	PRKCD	CHEMBL2996		49 / 9	Ser_Thr Kinase
Monoglyceride lipase	Q99685	MGLL	CHEMBL4191		17 / 2	Enzyme
Mu-type opioid receptor	P35372	OPRM1	CHEMBL233		73 / 5	Membrane receptor
Delta-type opioid receptor	P41143	OPRD1	CHEMBL236		73 / 5	Membrane receptor
Kappa-type opioid receptor	P41145	OPRK1	CHEMBL237		70 / 5	Membrane receptor
Nociceptin receptor *(by homology)*	P41146	OPRL1	CHEMBL2014		70 / 5	Membrane receptor
Sodium-dependent noradrenaline transporter	P23975	SLC6A2	CHEMBL222		48 / 79	Transporter

Figure (3): SwissTargetPrediction report of Di (2-ethylhexyl) phthalate. Protein Kinase C Gamma (PRKCG) scored highest binding probability.

The PKC family members are known to be involved in diverse cellular signaling pathways. They are important regulator of signaling cascades that control cell proliferation and death, and therefore represent an attractive target for cancer therapy [22].

Target Binding Probabilities for 13-Hexyloxacyclotridec-10-en-2-One

Cytochrome P450 aromatase (CYP19A1) ranked the best binging probability on the SwissTargetPrediction report (Fig. 4).

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cytochrome P450 19A1	P11511	CYP19A1	CHEMBL1978		54 / 68	Enzyme
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		70 / 11	Enzyme
Steroid 17-alpha-hydroxylase/17,20 lyase	P05093	CYP17A1	CHEMBL3522		12 / 17	Enzyme
Fatty-acid amide hydrolase 1	O00519	FAAH	CHEMBL2243		62 / 26	Enzyme
Sodium-dependent noradrenaline transporter	P23975	SLC6A2	CHEMBL222		77 / 2	Transporter
Sodium-dependent serotonin transporter	P31645	SLC6A4	CHEMBL228		66 / 2	Transporter
Sodium-dependent dopamine transporter	Q01959	SLC6A3	CHEMBL238		77 / 2	Transporter
Sodium- and chloride-dependent glycine transporter 1 *(by homology)*	P48067	SLC6A9	CHEMBL2337		60 / 2	Transporter
Sodium-dependent proline transporter *(by homology)*	Q99884	SLC6A7			47 / 2	Transporter
Sodium- and chloride-dependent neutral and basic amino acid transporter B(0+) *(by homology)*	Q9UN76	SLC6A14			47 / 2	Transporter
Sodium- and chloride-dependent glycine transporter 2 *(by homology)*	Q9Y345	SLC6A5	CHEMBL3060		47 / 2	Transporter
Androgen receptor	P10275	AR	CHEMBL1871		22 / 39	Transcription Factor
Corticosteroid 11-beta-dehydrogenase isozyme 1	P28845	HSD11B1	CHEMBL4235		82 / 15	Enzyme
Hydroxysteroid 11-beta-dehydrogenase 1-like protein *(by homology)*	Q7Z5J1	HSD11B1L			81 / 15	Enzyme
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		265 / 24	Unclassified

Figure (4): SwissTargetPrediction report of 13-Hexyloxacyclotridec-10-en-2-one. Cytochrome P450 19A1 (CYP19A1) scored highest binding probability.

Many of the human microsomal P450s aromatase catalyze the metabolism of a wide variety of compounds including xenobiotic and drugs, also involves in the synthesis of estrogens from androgens making it a unique target for estrogen-dependent breast cancer [23].

Molecular Docking

Docking of DEHP (entry code CID_8343) on PKCG (entry code 2UZP) revealed good biochemical interactions with free energy binding value of -5.75 Kcal/mol (Fig. 5).

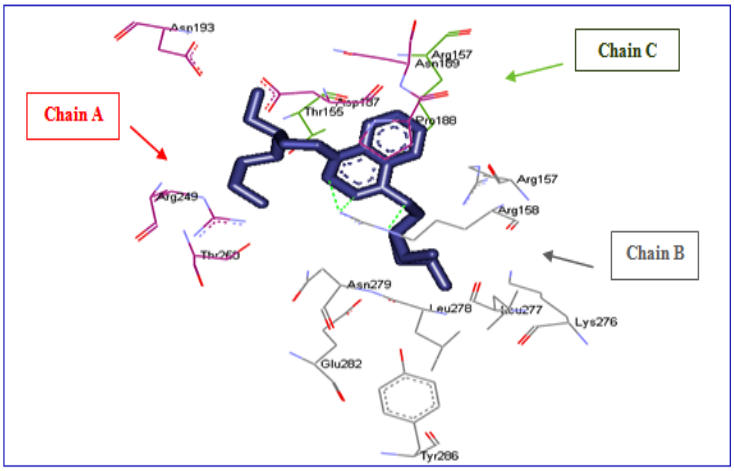


Figure (5): Docking of Di (2-ethylhexyl) phthalate on protein kinase C gamma type. Amino acid residues in chain A, B and C are colored red, green and grey, respectively.

From results depicted in figure (5), three hydrogen bonds are formed with the amino acid residue Arginine at 185 in chain (B) of the trimeric Protein kinase C gamma type (PKCG). The ligand is completely surrounded by an envelope of hydrophobic residues and embedded in the enzyme (Fig. 6).

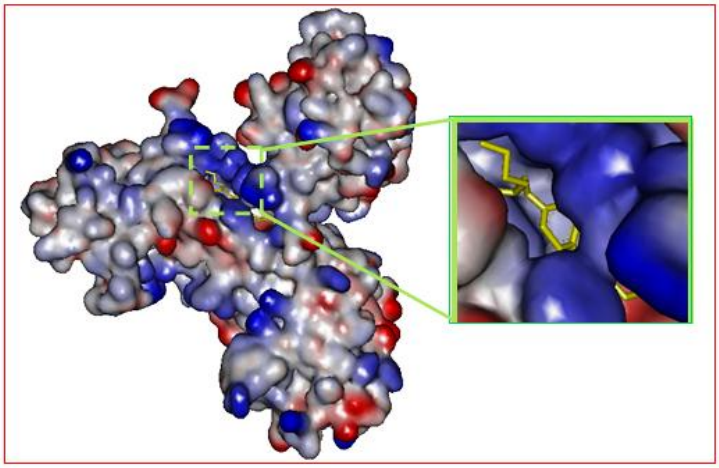


Figure (6): Schematic diagram depicting the trimeric complex structure of protein kinase C gamma (PKCG) type with three chains. The ligand, in "stick" yellow colour, embedded in the enzyme.

On the other hand, docking of 13-hexyloxacyclotridec-10-en-2-one (entry code CID_5369119) on to CYP19A1 (entry code 3S79) revealed a good biochemical interaction with a free binding energy score value of -8.33Kcal/mol (Fig. 7).

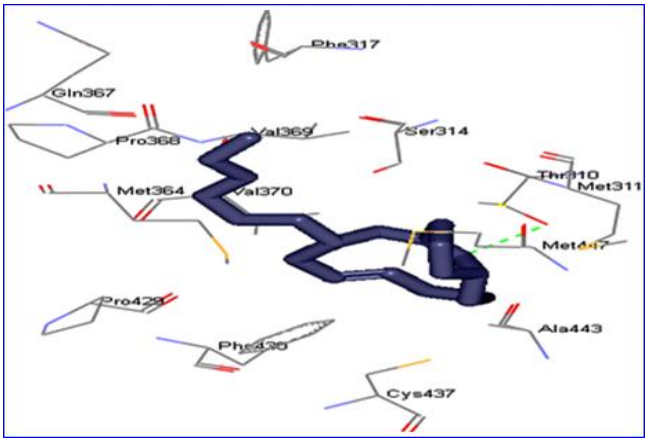


Figure (7): Docking of 13-Hexyloxacyclotridec-10-en-2-one on Cytochrome P450 19A1.

The docking result (Fig. 7) depicts the biochemical interactions of the ligand with the enzyme target; hydrogen-bonding (green color) is formed with the amino acid residue Threonine at

position 310. Furthermore, the compound is completely surrounded by an envelope of hydrophobic residues (Fig. 8).

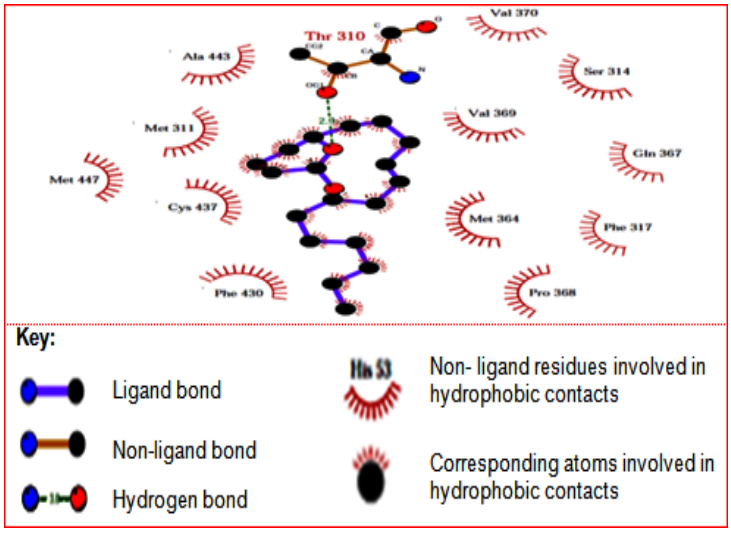


Figure (8): Schematic diagram depicting the biochemical interactions of 13-Hexyloxacyclotridec-10-en-2-one with Cytochrome P450 19A1; hydrogen-bonding (green color) with Threonine at position 310 and hydrophobic interaction.

Interestingly, the ligand binding site is only one amino acid residue shift from the binding site of Exemestane (Fig. 9), a preeminent anticancer drug where its cyclic ketone functional group binds to the amino acid residue Asparagine (309) with a hydrogen bond [23]. To this end, it is likely that 13-hexyloxacyclotridec-10-en-2-one possess chemotherapeutic activity.

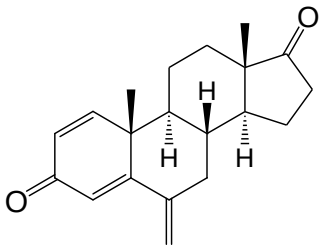


Figure (9): Exemestane

CONCLUSION

The reputed antitumor activity of *A. maritima* hopefully pertained with these two promising antitumor agents, and, apart from their anticarcinogenic properties, other therapeutic properties might be present. The resulting listing of the other targets, though undoubtedly leaving out some considerably promising research results, should be enough to answer the questions of the diverse therapeutic properties of these compounds. This could hopefully serve as a base for further drug research and development.

ACKNOWLEDGMENTS

Grateful thanks to Dr. Haidar Abdalgadir taxonomist, Herbarium, Medicinal and Aromatic Plant Research Institute (MAPRI) Sudan, for the identification of the plant species. The author, also, acknowledges Dr. Mohammed A. Salih, Department of Bioinformatics, Africa City of Technology (ACT), Sudan, for his help in the *in silico* virtual screening.

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