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In-silico Identification of Natural Compounds as Pesticides against Plutella Xylostella

Nergiz Gurbuz-Colak
Izmir Institute of Technology

Abstract: Plutella xylostella (diamondback moth), a major invasive pest of Brassica crops, feeds on cruciferous plants and causes serious economic loss. The moth has spread worldwide owing to its short life cycle, high fecundity, and capability to migrate long distances. Chlorantraniliprole is a human-made insecticide widely used to control P. xylostella. On the other hand, resistance to chlorantraniliprole was reported in the literature. The use of natural compounds as pesticides can eliminate resistance and reduce potential harm to humans. In the present study, natural compounds were identified as potential pesticide candidates *in silico*. To achieve this goal, the binding potentials of over 3000 natural compounds found in the MPD3 database to the diamondback moth ryanodine receptor N-terminal domain (PDB:5y9v) were scanned using AutoDock Vina. The active sites of the target proteins were identified using PyMOL software. The first filtration was applied according to the binding energies, with a threshold of -6,0 kcal/mol. Second, the binding affinities to the N-terminal region of the human ryanodine receptor 2 (PDB:4jkq) of the candidates were checked. Candidates were then filtered according to the ADME properties based on Lipinski's rule of five using DruLiTo software. Finally, toxicity (oral toxicity, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity) was evaluated using ProTox II online server. In addition, the binding energy and toxicity of chlorantraniliprole were compared. Chlorantraniliprole binds to 5y9v with a binding energy of -3,5 kcal/mol while binds to 4jkq with higher affinity (-6,8 kcal/mol). Moreover, it may cause hepatotoxicity. Dorsmanin B, chartaceone B, and 7-O-galloyltricetifavan bind to 5y9v with a binding energy of -6,1 kcal/mol, -6,0 kcal,/mol, -6,1 kcal/mol, respectively while binding to 4jkq with lower affinity (0,1 kcal/mol, -2,4kcal,/mol, -2,9 kcal/mol, respectively). In addition, these candidates did not show any toxicity. These natural compounds can be used instead of chlorantraniliprole to control *Plutella xylostella*.

Keywords: Plutella xylostella, Natural compounds, Pesticide

Introduction

Invasive insect species have a destructive effect on various aspects of human well-being, including health, food security, ecosystems, biodiversity, and the economy. The diamondback moth, scientifically known as *Plutella xylostella*, belongs to the order Lepidoptera and is one of the most notorious offenders. This moth causes significant losses worldwide by feeding on *Brassica* plants, resulting in yield losses of up to 90% and economic losses of up to US$ 5 billion. Managing this pest costs approximately $1 billion annually (Kapinder et al., 2022). Diamide pesticides have been used to control diamondback moths. Chlorantraniliprole is an effective broad-spectrum anthranilic diamide pesticide that targets ryanodine receptors (RyRs) in insects, including lepidopterians. Diamondback moths have developed resistance to this pesticide because of the excessive use of chlorantraniliprole due to its high efficiency and selectivity (Gong et al., 2014). Therefore, efforts to develop new pesticides to control diamondback moth have increased.

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Ryanodine receptors in insects are crucial intracellular calcium channels that regulate muscle contraction (Sattell et al., 2008). These receptors are targets of diamide insecticides, which have been effective against lepidopterans. However, their overuse has led to a decrease in their efficacy and insects have developed resistance to them (Sun & Xu, 2019). Humans have three isoforms of RyRs (RyRs1, RyRs2, and RyRs3) expressed in different tissues (Leeb & Brening, 1998), whereas insects have only one. Despite their shared functions, there are significant differences in the amino acid sequences of these receptors. In fact, ryanodine receptors in mammals and insects differ by approximately 45% in their sequences. This structural divergence makes them attractive targets for developing insecticides that can selectively eliminate insect populations without harming mammals. By exploiting these structural differences, scientists may develop more effective and eco-friendly insecticides to control the spread of harmful insect-borne diseases and protect crops from damage (Prestle et al., 2003).

Excessive use of chemical pesticides has been linked to numerous detrimental effects on the environment and inhabitants. In addition to harming humans and animals, they also have a negative impact on soil fertility. Moreover, their overuse has led to the emergence of pesticide resistance in insects, making them less effective in combating pest infestations (Kapinder et al., 2022). Recently, concerns regarding the harmful effects of chemical pesticides on the environment and human health have been growing. This has led researchers to explore the use of natural compounds as alternative pesticides. Natural compounds offer several advantages, including being environmentally friendly, sustainable, target-specific, inexpensive, and safer (Borges et al., 2021). However, it is challenging to evaluate the biological activity of the vast number of secondary metabolites produced by plants.

In the current study, the insecticidal properties of natural compounds present in the MPD3 database were assessed for their ability to interact with the ryanodine receptor of the diomandback moth. Through rigorous screening, a group of candidates was selected based on multiple criteria. This study identified natural pesticides that may be effective in combating diomandback moths. These compounds should be further evaluated to determine their efficacy as pesticides. The findings of this study underscore the value of computational techniques for the discovery of pesticides and highlight the potential of natural compounds as a source of novel insecticides.

Method

This study aimed to analyze the binding energies of natural compounds using molecular docking. A total of 3,150 compounds used in this study were obtained from the MPD3 database in .sdf format and subjected to docking studies with the diamondback moth ryanodine receptor N-terminal domain (PDB:5y9v). AutoDock Vina in PyRx virtual screening software was used to determine the binding energies of these compounds. The ligand files (compound files) were then converted to. pdbqt format after energy minimization. The crystal structure of the N-terminal domain of the ryanodine receptor of diamondback moth and human ryanodine receptor 2 (RyR2) (PDB: 4jkq) were obtained from the "RCSB Protein Data Bank" and used as a rigid molecule after protein preparation using "BIOVIA Discovery Studio 2021" software. PyRx virtual screening software was employed to conduct docking studies, and the candidates were filtered based on their binding energies with a threshold of -6 kcal/mol. The drug-likeness of the compounds was evaluated using the DruLiTo software according to Lipinski's Rule of Five (Ro5). Moreover, their physicochemical properties were evaluated to ensure their suitability as pesticide candidates. The toxicity of the compounds was assessed using the ProTox II online server to ensure safety for agricultural use. In addition, the binding energies and toxicities of chlorantraniliprole and the candidate molecules were compared. LigPlot+ software was used to evaluate the interaction of the pesticide candidates with the target protein.

Results and Discussion

In the present study, the N-terminal domain of the ryanodine receptor of the diamondback moth was used to identify candidate pesticides derived from natural compounds. Given the crucial function of ryanodine receptors in muscle contractions and the significant dissimilarity in amino acid sequences between insects and humans, they have become attractive targets for pesticides (Prestle et al., 2003). To identify potential candidates, a molecular docking technique was employed with a binding energy threshold of -6 kcal/mol. The binding energies of the candidates were compared those with of human RyR2 and chlorantraniliprole insecticides already used in the management of diamondback moths. Candidates that showed higher affinity for RyRs than

human RyR2 and chlorantraniliprole are presented in Table 1. A total of 92 out of 3,150 natural compounds bound to the N-terminal domain of RyRs with a binding energy of -6 kcal/mol or lower.

Table 1. Candidates after the first filtration based on binding energies.

	Binding energies (kcal/mol)			Binding energies (kcal/mol)	
Compound name	5y9v	4jkq	Compound name	5y9v	4jkq
Chlorantraniliprole	-3.5	-6.8	Chushizisin I	-6.2	17.2
Chartaceone B	-6.0	-2.4	Epoxyazadiradione	-6.0	2.6
7-O-galloyltricetifavan	-6.1	-2.9	Chrotacumine C	-6.1	-2.2
Alvaradoin J	-6.0	9.8	Eucalmaidin B	-6.0	-2.2
Cepharadione B	-6.2	-5.7	Eucalmaidin E	-6.1	6.7
23,24-Dihydrocucurbitacin B	-6.2	11.5	Sibiricaxanthone A	-6.5	2.2
23,24-Dihydrocucurbitacin E	-6.0	10.4	Kadcoccilactone D	-6.0	1.2
28-deoxonimbolide	-6.3	-2.6	Kadcoccilactone G	-6.6	13.3
Rhinacanthin N	-6.7	5.5	Fuscaxanthone G	-6.1	-3.1
Rhinacanthin Q	-6.5	-3.6	Garcihombronane D	-6.7	-0.5
Artobiloxanthone	-6.2	-0.9	Garcihombronane E	-6.2	-1.4
Longikaurin B	-6.1	-4.9	Rhinacanthin B	-6.3	-5.7
Bruceantin	-6.0	5.9	Ovaliflavanone C	-6.4	-6.3
Calceolarioside A	-6.3	-2.5	Vittarin F	-6.1	-3.1
Calceolarioside B	-6.2	2.5	Styraxlignolide B	-6.1	3.4
Isorhoifolin	-6.4	13.8	Withanolide F	-6.5	1.4
Maytenfolone-A	-6.2	17.7	Isoglycyrol	-6.4	-6.0
Melianin C	-6.4	19.5	Cudraflavanone A	-6.2	5.5
Nigrasin D	-6.0	0.7	Physalin H	-6.2	25.0
Neodiosmin	-6.2	16.3	Geyerline	-6.1	16
Prochaetoglobosin I	-6.0	0.2	Withanolide E	-6.8	-1.7
Solamargine	-6.2	24.0	Newbouldiaquinone A	-6.6	-4.7
Withaferin A	-6.4	-1.5	Enantiomultijugin	-6.0	-4.7
(R)-cryptopleurine	-6.0	-5.3	Azadirachtin I	-6.1	4.9
4',5'-Dihydro-11,5'-dihydroxy-4'-Methoxytephrosin	-6.3	0.9	Bartericin B	-6.0	6.2
Mansonone H	-6.0	-5.7	Alvaradoin L	-6.1	-3.4
Tephrosin	-6.3	2.2	Nigrolineaxanthone I	-6.0	-5.5
Acumitin	-6.0	-4.7	Salvianolic acid J	-6.6	3.8
Alpha-Toxicarol	-6.5	-0.8	Physalin F	-6.1	6.5
Antofine	-6.1	-5.4	Sophoraisoflavanone C	-6.0	-4.8
Chicoric acid	-6.0	-3.7	Quresimine A	-6.2	12.2
Inophyllum E	-6.1	2.0	Daphnodorin F	-6.7	3.6
Sanggenon M	-6.0	0.5	Ponganone V	-6.1	-4.8
Subtrifloralactone A	-6.1	6.5	Physangulide	-6.8	26.6
Subtrifloralactone B	-6.0	3.3	6-Farnesyl-3',4',5,7-tetrahydroxyflavanone	-6.0	7.3
Subtrifloralactone C	-6.0	7.2	Dorsmanin B	-6.1	0.1
Subtrifloralactone D	-6.7	-5.5	Tanariflavanone B	-6.0	6.1
Subtrifloralactone E	-6.3	-5.6	Lumaflavanone A	-6.2	8.6
Subtrifloralactone F	-6.4	-4.6	Physalin J	-6.0	18.9
Subtrifloralactone G	-6.3	-5.0	(2S)-5,7,3',4'-Tetrahydroxyflavanone 7-glucuronide	-6.0	-2.9
28-hydroxyisoiguesterin	-6.2	-0.4	5,7,3',4'-Tetrahydroxyflavanone 7-alpha-L-arabinofuranosyl-(1->6)-glucoside	-6.2	8.5
Gedunin	-6.1	7.4	Ergotamine	-6.9	35.8
Salannin	-6.0	14.8	Desmodianone A	-6.3	0.8
Chaetoglobosin V	-6.3	23.9	Bolusanthol C	-6.1	-5.1
Lysicamine	-6.1	-5.8	Daphnodorin M	-6.2	24.2
Nimocinol	-6.2	-3.3	Balsaminone B	-6.8	1.2

The biological activity of an insecticide depends on its intrinsic activity and bioavailability, which are in turn influenced by its structural properties. The relationship between the structure and biological activity of an experimentally designed pesticide is contingent on its bioavailability. While absorption, distribution, metabolism, and excretion (ADME) properties and mammalian toxicity are typically utilized as criteria for evaluating pharmaceuticals, they may also be applied to pesticides. Understanding and evaluating the parameters that limit the bioavailability of a pesticide during its development provides critical information for the selection of pesticide candidates (David, 2016). Thus, the second filtering process was conducted based on the ADME properties, considering Lipinski's Rule of Five (Ro5). Ro5 is used to determine the oral bioavailability and membrane permeability of a compound. Molecular descriptors are incorporated into this rule. Therefore, the candidate's molecular weight must be less than 500 Da, its LogP (hydrophobicity) must be less than 5, the number of hydrogen bond donors (HBD) must be less than 5, and the number of hydrogen bond acceptors (HBA) must be less than 10. Candidates that comply with these criteria were deemed to have acceptable solubility and cell permeability (Avram et al., 2014). The ADME properties of the filtered candidates are presented in Table 2.

Table 2. ADME properties of the candidates.

Compound Name	MW (g/mol)	LogP	HBA	HBD
Chlorantraniliprole	483,15	4,72	20	2
Chartaceone B	458,5	4,54	6	3
7-O-galloyltricetifavan	442,37	2,7	10	7
Alvaradoin J	528,51	1,07	39	5
Cepharadione B	323,33	3,23	20	0
23,24-Dihydrocucurbitacin B	560,72	3,72	56	3
23,24-Dihydrocucurbitacin E	558,7	4,41	54	3
28-deoxonimbolide	452,54	4,22	37	0
Rhinacanthin N	460,48	4,86	31	2
Rhinacanthin Q	474,5	5,16	33	1
Artobiloxanthone	464,44	4,68	28	4
Longikaurin B	406,47	0,95	37	3
Bruceantin	548,58	1,15	47	3
Calceolarioside A	478,45	0,13	37	7
Calceolarioside B	478,45	0,13	37	7
Isorhoifolin	578,52	-1,1	43	8
Maytenfolone-A	470,68	5,94	50	1
Melianin C	620,77	6,46	56	0
Nigrasin D	454,47	3,08	34	4
Neodiosmin	608,55	-1,9	46	8
Prochaetoglobosin I	482,66	6,8	41	2
Solamargine	868,06	1,14	89	9
Withaferin A	470,6	3,35	44	2
(R)-cryptopleurine	377,48	4,87	31	0
4',5'-Dihydro-11,5'-dihydroxy-4'-methoxytephrosin	474,46	1,85	36	3
Mansonone H	258,27	2,33	18	1
Tephrosin	410,42	3,11	29	1
Acumitin	466,48	5,47	27	3
Alpha-Toxicarol	410,42	3,71	29	1
Antofine	363,45	4,48	29	0
Chicoric acid	474,37	1,23	30	6
Inophyllum E	402,44	5,24	26	0
Sanggenon M	436,45	4,19	31	3
Subtrifloralactone A	454,56	3,18	40	1
Subtrifloralactone B	454,56	3,18	40	1
Subtrifloralactone C	470,56	2,3	41	2
Subtrifloralactone D	456,57	3,01	42	2
Subtrifloralactone E	456,57	3,01	42	2
Subtrifloralactone F	472,57	1,98	43	3
Subtrifloralactone G	472,57	1,98	43	3
28-hydroxyisoiguesterin	420,58	6,14	39	2
Gedunin	482,57	4,56	40	0
Salannin	596,71	5,29	52	0

Chaetoglobosin V	528,64	4,67	42	4
Lysicamine	291,3	3,46	17	0
Nimocinol	452,58	5,21	40	1
Chushizisin I	476,52	4,39	35	3
Epoxyazadiradione	466,57	4,63	39	0
Chrotacumine C	499,51	3,12	38	2
Eucalmaidin B	498,48	0,02	42	7
Eucalmaidin E	512,59	0,88	50	5
Sibiricaxanthone A	538,46	-1,96	39	9
Kadcoccilactone D	512,63	5,46	47	0
Kadcoccilactone G	530,65	3,21	50	2
Fuscaxanthone G	478,58	6,7	39	2
Garcihombronane D	470,68	6,58	50	2
Garcihombronane E	470,68	6,58	50	2
Rhinacanthin B	408,49	5,12	33	0
Ovaliflavanone C	352,38	4,34	25	1
Vittarin F	436,45	4,05	31	3
Styraxlignolide B	532,49	0,2	40	4
Withanolide F	470,6	3,54	44	3
Isoglycyrol	366,36	4,51	22	1
Cudraflavanone A	422,47	5,2	32	3
Physalin H	562,99	0,97	41	2
Geyerline	710,81	1,95	63	2
Withanolide E	486,6	2,75	45	3
Newbouldiaquinone A	410,38	4	20	1
Enantiomultijugin	422,43	4,01	28	0
Azadirachtin I	618,67	1,1	54	3
Bartericin B	408,49	4,58	33	3
Alvaradoin L	470,47	1,4	35	5
Nigrolineaxanthone I	392,4	4,73	25	2
Salvianolic acid J	538,46	2,73	34	6
Physalin F	526,53	0,77	40	1
Sophoraisoflavanone C	476,6	6,91	41	3
Quresimine A	502,64	3,2	49	2
Daphnodorin F	542,49	3,99	32	6
Ponganone V	382,41	4,48	28	0
Physangulide	522,63	0,86	51	5
6-Farnesyl-3',4',5,7-tetrahydroxyflavanone	492,6	7,18	42	4
Dorsmanin B	392,5	4,9	4	1
Tanariflavanone B	490,59	6,93	40	3
Lumaflavanone A	506,59	5,05	41	2
Physalin J	526,53	0,77	40	1
(2S)-5,7,3',4'-Tetrahydroxyflavanone 7-glucuronide	464,38	-0,22	32	7
5,7,3',4'-Tetrahydroxyflavanone 7-alpha-L-arabinofuranosyl-(1->6)-glucoside	528,51	-1,85	45	9
Ergotamine	581,66	2,2	44	3
Desmodianone A	436,5	5,38	34	3
Bolusanthol C	408,49	5,18	33	3
Daphnodorin M	542,49	3,6	32	5
Balsaminone B	506,46	1,55	31	4

A crucial step in the development of pesticides is to conduct risk assessment to evaluate their potential adverse effects on human health. In this study, an *in silico* toxicity analysis was conducted to assess the toxicity of the identified pesticide candidates. Oral toxicity was evaluated to determine the acute toxicity of the compounds and hepatotoxicity was evaluated to determine whether they could cause liver failure. Additionally, carcinogenicity was evaluated to determine whether the compounds could trigger tumor formation, and immunotoxicity was evaluated to determine whether they could have adverse effects on the immune system. Mutagenicity was evaluated to determine whether the compounds caused DNA or cell damage. Finally, cytotoxicity of the candidates was evaluated to determine whether they could cause any deficits. The toxicity profiles of the pesticide candidates are presented in Table 3 (Banerjee et al., 2018).

Table 3. Toxicity assessment of the candidates.

*Color code; dark green and green: non-toxic, yellow: acceptable, orange and red: toxic

When their binding energies, ADME properties, and toxicity were evaluated, dorsmanin B, chartaceone B, and 7-O-galloyltricetifavan were the leading candidates in the fight against the diamondback moth. The interaction of these compounds with the N-terminal domain of the diamondback moth ryanodine receptor is shown in Figure 1A-C. Dorsmanin B binds to RyRs through hydrophobic interactions with amino acids Gln69, Leu71, Leu79, Val80, and Arg96 on RyRs. Chartaceone B binds to RyRs through hydrogen bonding with His95 and Thr97 on RyRs and hydrophobic interactions with the amino acids Thr19, Glu20, Glu68, Gln69, and Arg96. 7-O-galloyltricetifavan binds to RyRs through hydrogen bonding with Gln69 and Arg96 on RyRs, and hydrophobic interactions with amino acids Leu71, Leu79, His95, Thr97, and Leu99.

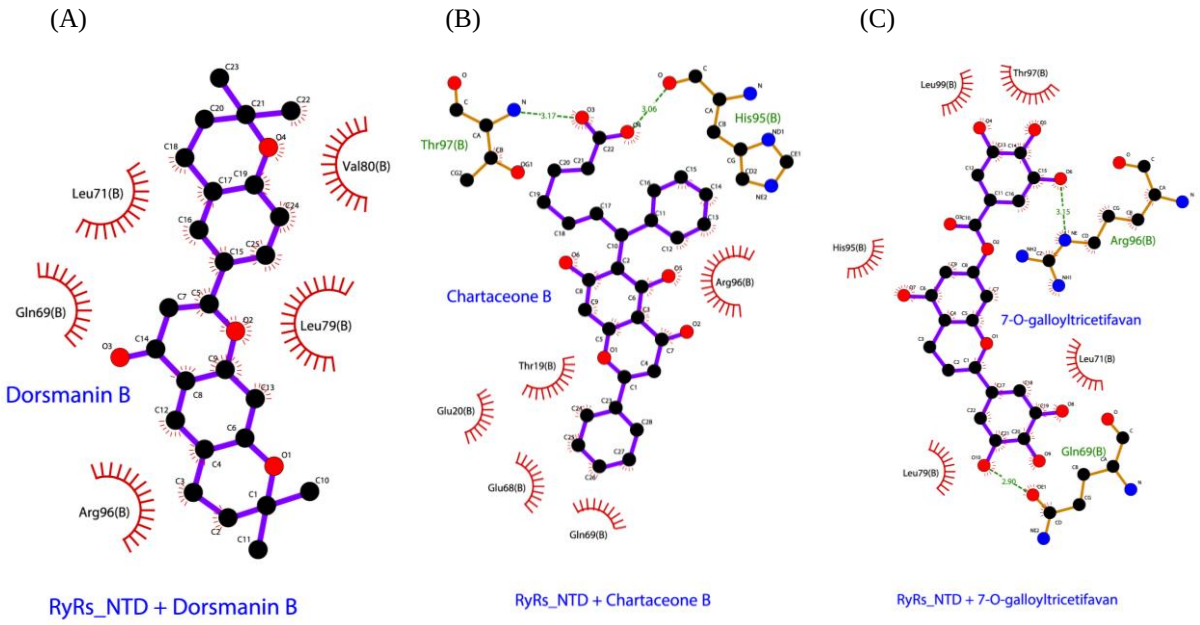

Figure 1. Interactions between candidates and diamondback moth RyRs N terminal domain (A) Dorsmanin B and RyRs NTD (B) Chartaceone B and RyRs NTD and (C) 7-O-galloyltricetifavan and RyRs NTD

Conclusion

The diamondback moth is a destructive insect species that causes significant losses to agriculture and human well-being. To manage this pest, researchers have developed new pesticides, including chlorantraniliprole, an effective broad-spectrum anthranilic diamide pesticide that targets insect ryanodine receptors. However, excessive use of chlorantraniliprole has led to the development of resistance in diamondback moths. To address this issue, researchers are exploring the use of natural compounds as alternative pesticides. This study aimed to identify natural compounds that may be effective in combating diamondback moths by assessing their binding energies with the ryanodine receptor of diamondback moths using molecular docking. A total of 3,150 compounds were screened, and 28 candidates were selected based on their binding energies and other criteria. These compounds showed promising insecticidal properties and were evaluated as potential pesticides. Three natural compounds, including dorsmanin B, chartaceone B, and 7-O-galloyltricetifavan, have demonstrated potential as pesticide candidates against diamondback moth.The study highlights the potential of natural compounds as a source of novel insecticides and the value of computational techniques for the discovery of pesticides.

Recommendations

This study was conducted using computational models, rather than practical trials. The next step involves the formulation of conclusive pesticide compositions based on the candidates identified in the investigation. These compositions will then be evaluated for efficacy in a controlled laboratory setting through in vitro testing.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the author.

Acknowledgements or Notes

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Author Information

Nergiz Gurbuz Colak
Izmir Institute of Technology
Izmir, Turkey
Contact e-mail: *nergizgurbuz@iyte.edu.tr*

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