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Identification of Less Harmful Pesticides against Honey Bees: Shape-Based Similarity Analysis †

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Abstract: The high concentration of pesticide residues existing in vegetation, crops, and various edible products and the prolonged exposure to them can harm human life and contribute to the disappearance of honey bees, and several avian and animal species. The honey bees (Apis mellifera), which are efficient pollinators in addition to honey producers, are also considered important non-target test species for the terrestrial toxicity assessment of chemicals. In this context, using thiacloprid and acetamiprid as queries, we performed a 3D similarity search to select new potential products with less harmful effects against bees. For a similarity search, a small dataset of 302 compounds with pesticide activity, compiled from the literature, was used. The first 10 compounds were selected and structurally analyzed according to the TanimotoCombo metrics, and compared with each of these two queries, which is known to be effective, easily metabolized, and less toxic for bees. This approach came as a forward step in the research of pesticide ecotoxicological risk assessment for the evaluation of their potential impact on the pollinator insects and the environment.

Keywords: Apis mellifera; 3D similarity search; thiacloprid; acetamiprid

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1. Introduction

Neonicotinoids are the most commonly used insecticides for pest control. The main problems associated with the use of these insecticides, alone or in combination with other factors, are related to their negative impact against many species of insects including bees. Additionally, the application mode of insecticides (e.g., direct spray, soil and seed application, etc.) plays a key role in their impact against pollinators. Honeybees are considered the most successful and commercially valuable pollinators due to their pollination functions, maintenance of biodiversity in natural ecosystems as well as the commercial products delivered such as honey, propolis, etc. This negative neonicotinoid impact on bees has been extremely studied because the effect at different doses is still not fully understood [1]. Honeybees can be envisaged as very vulnerable to pesticides because their genome has fewer genes compared to other insects [2]. The exposure to neonicotinoids can influence the flying and the foraging ability, reproduction, and pollination for many useful insects including honeybees [3]. In particular, they affect bees by forgetting the locations of flowers or even hives, and also by increasing the fertility of queens or bumblebees [3]. In this context, the strategy of designing new neonicotinoids by modifying existing structures may be an effective way to overcome this harmful influence against pollinators.

Of the eight marketed neonicotinoids (https://www.reportbuyer.com/product/3952801/ (accessed on 26 July 2020)), thiacloprid and acetamiprid are considered to be less toxic for honeybees. These neonicotinoids are considered a group of neurotoxins, chemically sim-

ilar to nicotine, which acts specifically as nicotinic acetylcholine receptor (nAChR) antagonists [4].

Theoretical methods (QSAR, linear and nonlinear regression techniques, molecular docking, 2D and 3D similarity search, etc.) [5–7] applied in cheminformatics to discover new drugs have also been successfully employed to predict novel insecticides and pesticides with less polluting and toxic effects to fill data gaps and to reduce toxicity testing on animals. In the current work, thiacloprids and acetamiprids were used as template molecules in the 3D similarity analysis accomplished with ROCS (Rapid Overlay of Chemical Structures) [8,9] from the OpenEye package. The main goal is to find novel compounds, similar to template molecules, which are easy to metabolize and less toxic for bees.

2. Methods

From the literature [10–25], a dataset of 302 compounds with known pesticide activity was selected and used further for a 3D-similarity search. The conformational space of selected compounds was carried out with the Omega tool, OpenEye (OMEGA v.2.5.1.4, OpenEye Scientific Software, Santa Fe, NM, USA. www.eyesopen.com (accessed on 26 July 2020)) [26]. A maximum of 200 conformers for each compound was generated using default options.

The lowest energy conformers for thiacloprid and acetamiprid were also generated with Omega. The BIOVIA Discovery Studio facilities were used for structure visualization and picture delivery (Figure 1).

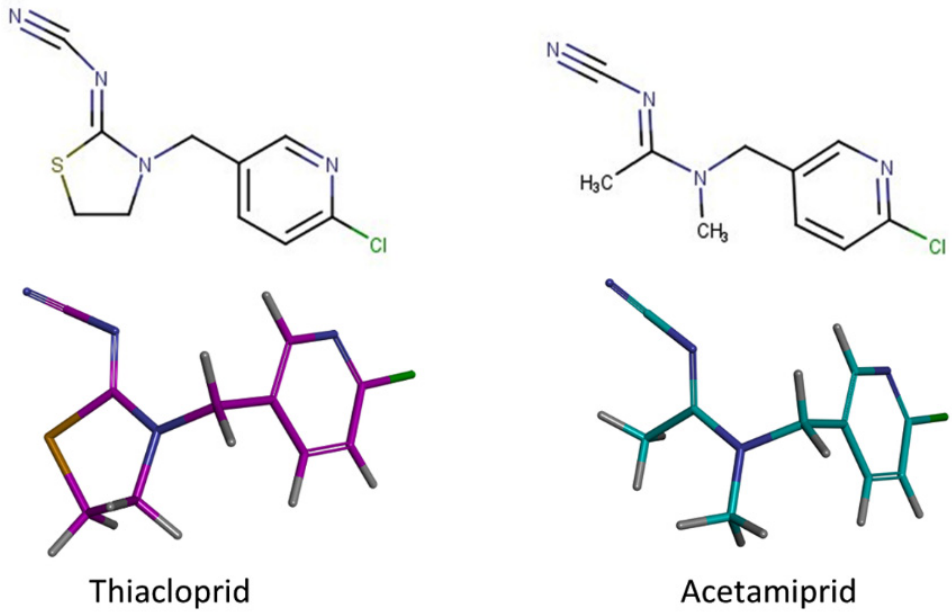

Figure 1. The 2D and 3D structure of the query compounds.

A 3D similarity search was performed with ROCS (ROCS v. 3.2.1.4, OpenEye Scientific Software, Santa Fe, NM, USA [8,9]. ROCS, a shape comparison application, is faster and more useful in handling the large conformer databases. This tool offers alignment and scoring of a database engaging thirteen similarity coefficients. The resulted aligned molecules are ordered by a TanimotoCombo ranking score (as the default option).

3. Results and Discussion

A 3D overlay with ROCS has a great advantage as it allows for optimal visualization of overlapping compounds, which leads to a better understanding of their similarity. The ROCS principles are based on the Gaussian function, which is widely used to represent shape and molecular volume.

In this light, several highly occupied regions corresponding to the pyridine ring and a yliden-cyanamide group of both template molecules were identified (Figure 2). These regions appear to have multiple hydrogen binding abilities. The pyridine ring of thiacloprids and acetamiprids and the thiazolidine ring of thiacloprid may be involved in π–π or π–σ hydrophobic bonds. As can be seen in Figure 2, the compounds prioritized by ROCS follow the same trend as the query compounds. This trend is in line with the high ShapeTanimoto similarity values (Figure 3), and implicitly the shapes (Figure 2) displayed by all the prioritized compounds.

As can be observed from Figure 3, the 3D coefficients calculated with ROCS for all ten prioritized compounds against each of the two queries showed values greater than 1.2 for TanimotoCombo, greater than 0.8 for ShapeTanimoto, and greater than 1.2 for ComboScore [27,28]. These high values indicate a very good similarity between the selected compounds and acetamiprid and thiacloprid, respectively. Four out of ten prioritized ROCS compounds, highlighted with circles in Figure 3, were considered to have a good profile through a comparison with each of the two queries.

2-Chloro-5-(4,5-dihydroimidazol-1-ylmethyl)pyridine, **I**,(green circles) was the second compound prioritized by thiacloprid and the seventh by acetamiprid. (2S)-1-[(6-Chloropyridin-3-yl)methyl]imidazolidine-2-carbaldehyde, **II**, (blue circles) was the third compound prioritized in accordance with thiacloprid, and the fourth by acetamiprid. 4-{[(6-Chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-5H-furan-2-one, **III**, (purple circles) was the fourth compound prioritized toward thiacloprid and the third toward acetamiprid. 2-Chloro-5-{[(7R)-7-methyl-2H,3H,5H,6H,7H-imidazo [1,2-a]pyridin-1-yl]methyl}pyridine, **IV**, (cyan circles) was the third compound prioritized by thiacloprid and the eighth by acetamiprid. Based on the good qualities demonstrated by ROCS analysis, these four compounds will be subjected, in further studies, to molecular docking and molecular dynamic simulation.

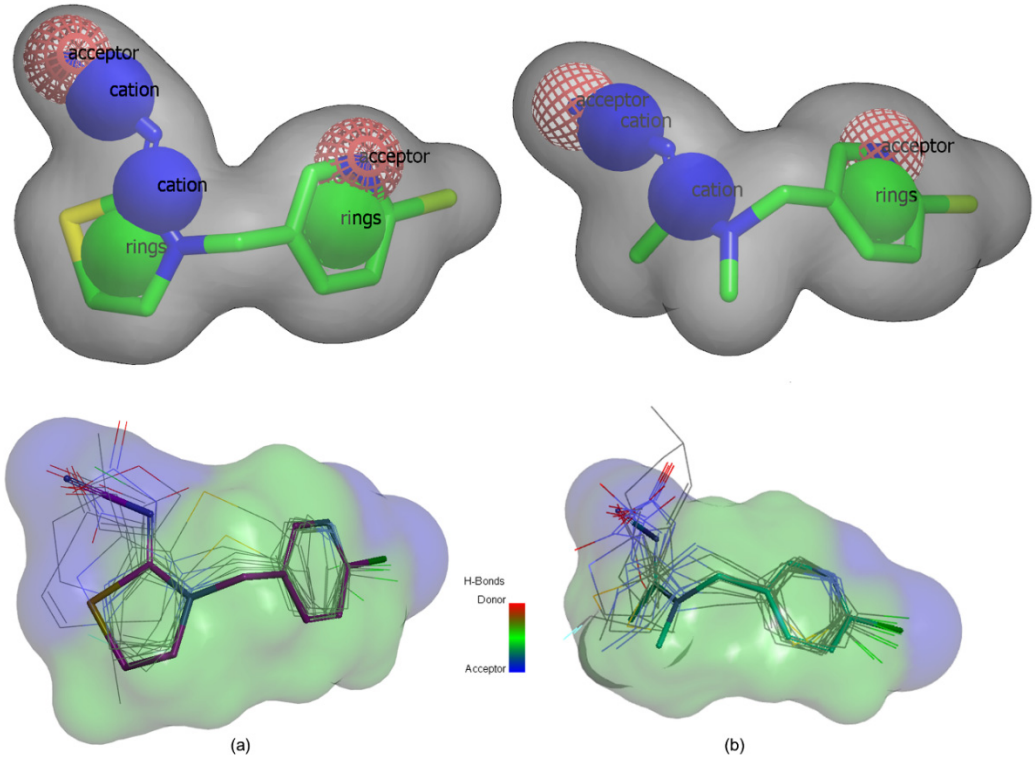

Figure 2. Molecular shapes of queries molecules. The Rapid Overlay of Chemical Structures (ROCS) overlapping of the top compounds ranked by TanimotoCombo against thiacloprid (**a**) and acetamiprid (**b**); the surface around queries is rendered.

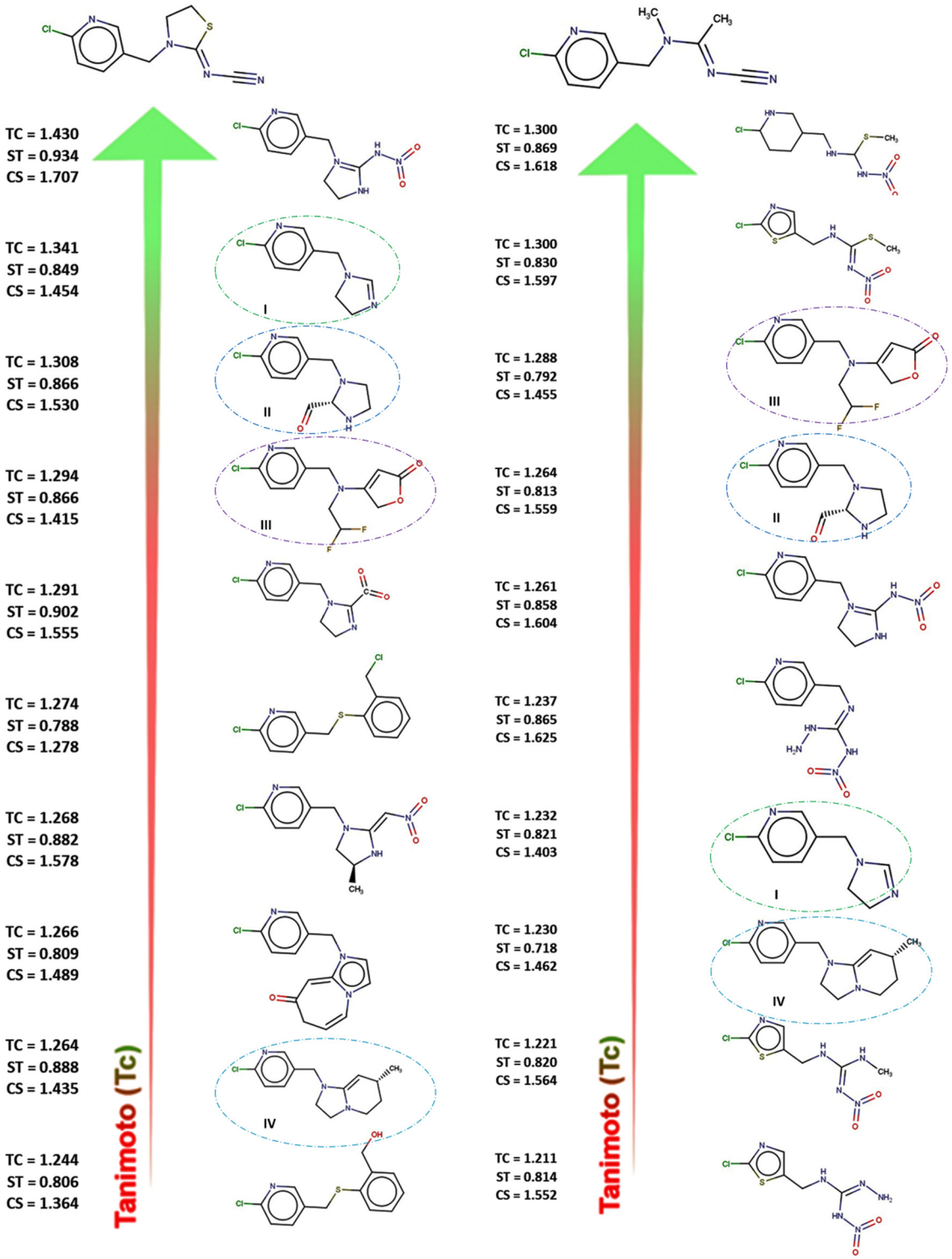

Figure 3. The chemical structures of the top ten compounds ranked by TanimotoCombo; against thiacloprid (**left**) and acetamiprid (**right**).

The computed pharmacokinetic proprieties of the selected four compounds and the two queries are listed in Table 1. These were performed with a freely accessible web server pkCSM (http://biosig.unimelb.edu.au/pkcsm/ (accessed on 28 September 2020)). The pkCSM program affords a fast and easy method to the early assessment of compounds [29]. Regarding the CNS (central nervous system) permeability, it could be observed that all four selected compounds showed logPS values lower than −3, being considered unable to penetrate the CNS of insects.

Table 1. Pharmacokinetic properties of thiacloprid, acetamiprid, and the selected compounds *.

		Thiacloprid	Acetamiprid	I	II	III	IV
Molecule properties:	MW	252.73	222.679	195.653	225.679	288.681	263.772
	LogP	2.12088	2.06628	1.5789	0.6652	2.2428	2.7338
	#RBN	2	2	2	3	5	2
	#Acceptors	4	3	3	4	4	3
	#Donors	0	0	0	1	0	0
	Surface Area	102.944	93.827	82.063	93.244	113.179	113.057
Toxicity	Max. tolerated dose (human) MRTD (log mg/kg/day)	0.488	0.766	0.555	0.1	0.304	0.192
	hERG I inhibitor	No	No	No	No	No	No
	hERG II inhibitor	No	No	No	No	No	No
	Oral Rat Acute Toxicity (LD50)	3.085	2.906	2.675	2.793	2.969	2.864
	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	0.699	0.795	0.904	1.186	1.567	0.877
	Hepatotoxicity	Yes	Yes	Yes	Yes	No	Yes
	Skin Sensitization	No	No	Yes	No	No	No
	T. Pyriformis toxicity pIGC50 (log ug/L)	1.132	0.986	0.869	0.026	0.807	1.025
	Minnow toxicity LC50 (log mM)	1.441	1.566	2.072	2.601	1.892	1.568
Distribution	VDss (human) (LogL/kg)	−0.134	−0.217	0.161	0.749	−0.074	0.475
	Fraction unbound (human)	0.486	0.507	0.576	0.897	0.507	0.444
	BBB permeability (log BB)	0.114	0.132	0.192	−0.264	0.289	0.593
	CNS permeability (logPS)	−2.922	−2.867	−3.199	−3.442	−3.688	−3.475
Excretion	Total Clearance (log mL/min/kg)	0.201	0.193	0.489	0.9	0.484	0.203
	Renal OCT2 substrate	No	No	No	No	No	No

* MW—Molecular Weight; RBN—Rotatable Bonds; VDss—volume of distribution at steady state; BBB—blood–brain barrier; VDss is considered low if below 0.71 L/kg (log VDss < −0.15) and high if above 2.81 L/kg (log VDss > 0.45). For a given compound: a logBB > 0.3 is considered to readily cross the blood–brain barrier while molecules with logBB < −1 are poorly distributed to the brains, a MRTD of less than or equal to 0.477 log(mg/kg/day) is considered low, and high if greater than 0.477 log(mg/kg/day), a LC50 value below 0.5 mM (logLC50 < −0.3) is regarded as high acute toxicity, a pIGC50 (negative logarithm of the concentration required to inhibit 50% growth in log ug/L) is predicted, while a value >−0.5 log ug/L is considered toxic. Compounds with a logPS > −2 are considered to penetrate the central nervous system (CNS), while those with logPS < −3 are considered as unable to penetrate the CNS.

4. Conclusions

In this study, thiacloprid and acetamiprid were used as queries in order to find new potential compounds with less harmful effects against bees. Four compounds (2-chloro-5-(4,5-dihydroimidazol-1-ylmethyl)pyridine, (2S)-1-[(6-chloropyridin-3-yl)methyl] imidazolidine-2-carbaldehyde, 4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-5H-furan-2-one, and 2-chloro-5-{[(7R)-7-methyl-2H,3H,5H,6H,7H-imidazo[1,2-a]pyridin-1-yl]methyl}pyridine) were selected as similar in shape and volume to both queries, which are known for their reduced toxic effect against bees [30,31]. This approach is a first attempt to find novel compounds with an enhanced safety profile against pollinator insects and the environment.

Author Contributions: L.C. and A.B. (Alina Bora) conceived of the presented idea, designed, and accomplished the computational framework including editing; A.B. (Ana Borota) performed some computational determinations; S.F.-T. selected and provided the pesticide dataset. All authors contributed to the writing of the paper and approved the content. All authors have read and agreed to the published version of the manuscript.

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