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Chemical design and toxicity evaluation of new pyrimidothienotetrahydroisoquinolines as potential insecticidal agents

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ABSTRACT

Neonicotinoids are the most widely used from all existing pesticides. So, in purpose to discover new pesticides being more effective against the aphid, twelve heterocyclic compounds neonicotinoid analogs have been prepared in a pure state; pyrimidothienotetrahydroisoquinolines **1–12** and their toxicity as potential insecticidal agents against cowpea Aphid, *Aphis craccivora* Koch was screened. Their characterizations by using spectroscopic analyses were performed. The toxicity data exhibited that the 8-chloropyrimidine compound **4** is more toxic about 2-fold than a reference insecticide, acetamiprid. The other screened compounds showed weak to strong toxicological activities against cowpea aphid.

1. Introduction

Recently, the innovation of neonicotinoids containing pyridine moiety has been deep and competitive efforts [1–3]. Neonicotinoids containing Pyridine moiety have concerned a great attention for a long time since they merge distinctive characteristics and were utilized in all over the world for insects management because of their efficacy, low mammalian toxicity, lack cross-resistance to other insecticides, broad insecticidal spectra, good systemic properties, and a novel mode of action specific for insect nicotinic acetylcholine receptors (nAChRs) [4–9]. However, the need for new derivatives of neonicotinoids has become important [10,11]. Table 1

Neonicotinoids have various molecular characteristics. The main features for neonicotinoids are: aromatic heterocycle, flexible linkage, hydroheterocycle or guanidine/amidine, and electron withdrawing segment [12].

Although neonicotinoids are the most used compounds in the world in the field of pest control and are considered the least in terms of the negative effects on animal and human therefore they have almost replaced other more toxic organophosphate and carbamate insecticides, but some recent researches concerning the biomonitoring of animal and human exposure to imidacloprid (IMI) as neonicotinoid pesticide reported that a genotoxic effect and DNA damage can be occurred after long-term exposure of rabbits to imidacloprid pesticide [13–15]. Imidacloprid (IMI) also induces a clastogenic effect to the exposed rabbits and oxidative stress [16,17]. This is proving the need for discovering new compounds belong to neonicotinoids pesticides.

Inspired by our promising results described in the first and second parts and as a continuation of our earlier research work aimed to find heterocycles containing pyridine moiety with expected toxicological activities [18,19], we reported here the study of the toxicity of the prepared compounds containing the previous features of neonicotinoid insecticides against cowpea Aphid, *Aphis craccivora* Koch encouraged to discover heterocyclic compounds with high biological effect and without environmental pollution.

2. Materials and methods

2.1. Instrumentation and chemicals

Measuring the melting points of all synthesized compounds was performed on a Fisher-John apparatus. By using a Vario EL C, H, N, S analyzer, elemental analyses (C, H, N, and S) were conducted. On a Pye-Unicam SP3-100 spectrophotometer IR spectra were obtained by using the KBr disc technique. ^{1}H NMR and ^{13}C NMR spectra were measured on a Joel 400 MHz and Bruker 400 MHz spectrometers using

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Table 1
Insecticidal activity of acetamiprid and compounds **1–12** against the nymphs and adults of cowpea aphid, *A. craccivora*, after 24 h of treatments.

	Nymphs			Adults		
Comp.	**Slope ± SE**	**LC_{50} (ppm)**	**Toxicity Ratio**	**Slope ± SE**	**LC_{50} (ppm)**	**Toxicity Ratio**
Aceta- miprid	0.34 ± 0.02	0.045	1	0.24 ± 0.02	0.225	1
1	0.36 ± 0.03	0.754	0.060	0.34 ± 0.02	1.628	0.138
2	0.31 ± 0.03	0.153	0.294	0.34 ± 0.03	0.578	0.389
3	0.35 ± 0.02	0.802	0.056	0.36 ± 0.03	1.180	0.191
4	0.35 ± 0.02	0.023	1.957	0.31 ± 0.03	0.221	1.018
5	0.37 ± 0.02	1.051	0.043	0.34 ± 0.02	1.295	0.174
6	0.34 ± 0.02	0.187	0.241	0.32 ± 0.02	0.792	0.284
7	0.35 ± 0.03	0.840	0.054	0.35 ± 0.02	2.975	0.076
8	0.33 ± 0.02	0.893	0.050	0.34 ± 0.03	1.207	0.186
9	0.35 ± 0.03	0.635	0.071	0.36 ± 0.03	1.092	0.206
10	0.35 ± 0.03	0.379	0.119	0.33 ± 0.02	1.066	0.211
11	0.36 ± 0.02	0.407	0.111	0.36 ± 0.02	1.195	0.188
12	0.36 ± 0.02	0.964	0.047	0.34 ± 0.03	1.647	0.138

Notes: Toxicity Ratio is calculated as acetamiprid's LC_{50} value for baseline toxicity / the compounds' LC_{50} value.

tetramethylsilane (TMS) as a reference and chemical shifts were reported as ppm. By using a Jeol JMS-600 mass spectrometer, mass spectra were carried out. With thin-layer chromatography, the purity of the title compounds was checked.

Compounds **1–12** were obtained according to the literature procedure [20]. Acetamiprid insecticide as a neonicotinoid insecticide was purchased from Sigma-Aldrich. The batches of cowpea aphid, *A. craccivora* insects were gathered from faba bean, *Vicia faba* L., fields of Assiut University Experimental Farm during 2017/2018 season. Toxicity of the twelve target compounds plus acetamiprid as reference neonicotinoid insecticide was screened against the collected aphids.

2.2. Laboratory bioassay

Toxicity of the title compounds was screened by leaf dip bioassay method [21]. Results of laboratory screening to find out the concentrations of the target compounds which are demanded to kill 50% (LC_{50}) of cowpea aphids were declared here. Six concentrations of solution of each prepared compound plus 0.1% Triton X-100 as a surfactant were utilized. 20 nymphs and 20 adults of insects, nearly have the same size, were immersed for ten seconds in every concentration three times. Insects which treated were permitted to dry at RT for about 0.5 h. Control batches of used insects were also utilized. After the treated batches of insects had dried, they were moved to Petri dishes (9 cm diameter) and remained for 24 h at 22 ± 2 °C, 60 ± 5% relative humidity and photoperiod of 12:12 (light/ dark). With a binocular microscope the aphid mortality was counted after 24 h of test. The aphid that unable of coordinated forward movement was considered dead. The insecticidal activity test of each compound was repeated two times and the obtained data were corrected by Abbott's formula [22]. By using a computerized Probit regression analysis program, median lethal concentrations (LC_{50}) and slope values of target compounds were computed and reported as parts per million (ppm) [23].

3. Results and discussion

3.1. Chemistry

The synthesis of tetrahydro [1,3]oxazinothieno[2,3-c]isoquinolinones was reported [20]. In continuation of our project in the synthesis and testing the biological activity of the pyridine derivatives; herein twelve compounds were chosen in Fig. 1 to evaluate their toxicity as insecticides. The twelve heterocyclic compounds, namely, 9-phenyl pyrimidinone **1**, 9-(*p*-chlorophenyl)pyrimidinone **2**, 9-(*p*-tolyl) pyrimidinone **3**, 8-chloropyrimidine **4**, 8-piperidinyl pyrimidine **5**, (pyrimidin-8-yl)-*p*-benzenesulfonamide **6**, 2-(pyrimidin-8-yl amino) ethanol **7**, 8-hydrazinopyrimidine **8**, ethyl pyrimidin-8-yl sulfanyl acetate **9**, pyrimidin-8-yl sulfanyl acetophenone **10**, pyrimidin-8-yl sulfanyl acetanilide **11** and ethyl 2-(pyrimidin-8-yl hydrazono)propanoate **12** compounds, were synthesized in pure state according to literature procedure [20] with m.p. 214–216 °C, 208–210 °C, 248–250 °C, 218–220 °C, 150–152 °C, 318–320 °C, 212–214 °C, 294–297 °C, 120–122 °C, 176–178 °C, 192–194 °C, and 250–252 °C respectively.

Elemental analyses and spectral characterization were used to clarify and prove the structures of all synthesized compounds. Elemental analyses results were in accordance with the values that calculated. Spectral characterization data of the synthesized compounds were in a good agreement with their suggested structures [20].

3.2. Insecticidal activity

All the title compounds have been screened for insecticidal activity as described below:

3.2.1. Toxicological activity test for the cowpea aphid nymphs

Our purified compounds were screened for their toxicity as insecticides and this is illustrated below. The twelve aforementioned compounds exhibited strong to weak toxicological activity against the nymphs of cowpea aphid because a number of them were as active as or more than acetamiprid after 24 h of the test with LC_{50} values vary from 0.023 to 1.051 ppm, while acetamiprid LC_{50} was 0.045 ppm. For example, LC_{50} values of compounds **4, 2, 6, 10** and **11** were 0.023, 0.153, 0.187, 0.379 and 0.407 ppm, in that order, and LC_{50} of acetamiprid was 0.045 ppm. From the results in above, the toxicity of compound **4** against the nymphs of cowpea aphid was about twofold that of acetamiprid after 24 h of the test because LC_{50} value of compound **4** was 0.023 ppm and that of acetamiprid was 0.045 ppm.

3.2.2. Toxicological activity test for the cowpea aphid adults

All purified compounds were screened for their toxicity as insecticides against cowpea aphid adults and this is shown below. The toxicity results showed that the target compounds are varied in toxicological activity after 24 h of the test with LC_{50} values vary from 0.221 to 2.975 ppm, while acetamiprid LC_{50} was 0.225 ppm. Compounds **4**, **2**, **6, 10** and **9** gave a high toxicity with LC_{50} values of 0.221, 0.578, 0.792, 1.066 and 1.207 ppm in that order. Compound **4** revealed the highest toxicity compared with acetamiprid insecticide. Compounds **3, 11**, **8** and **5** gave a relatively good activity with LC_{50} values of 1.180, 1.195, 1.207 and 1.295 ppm, respectively.

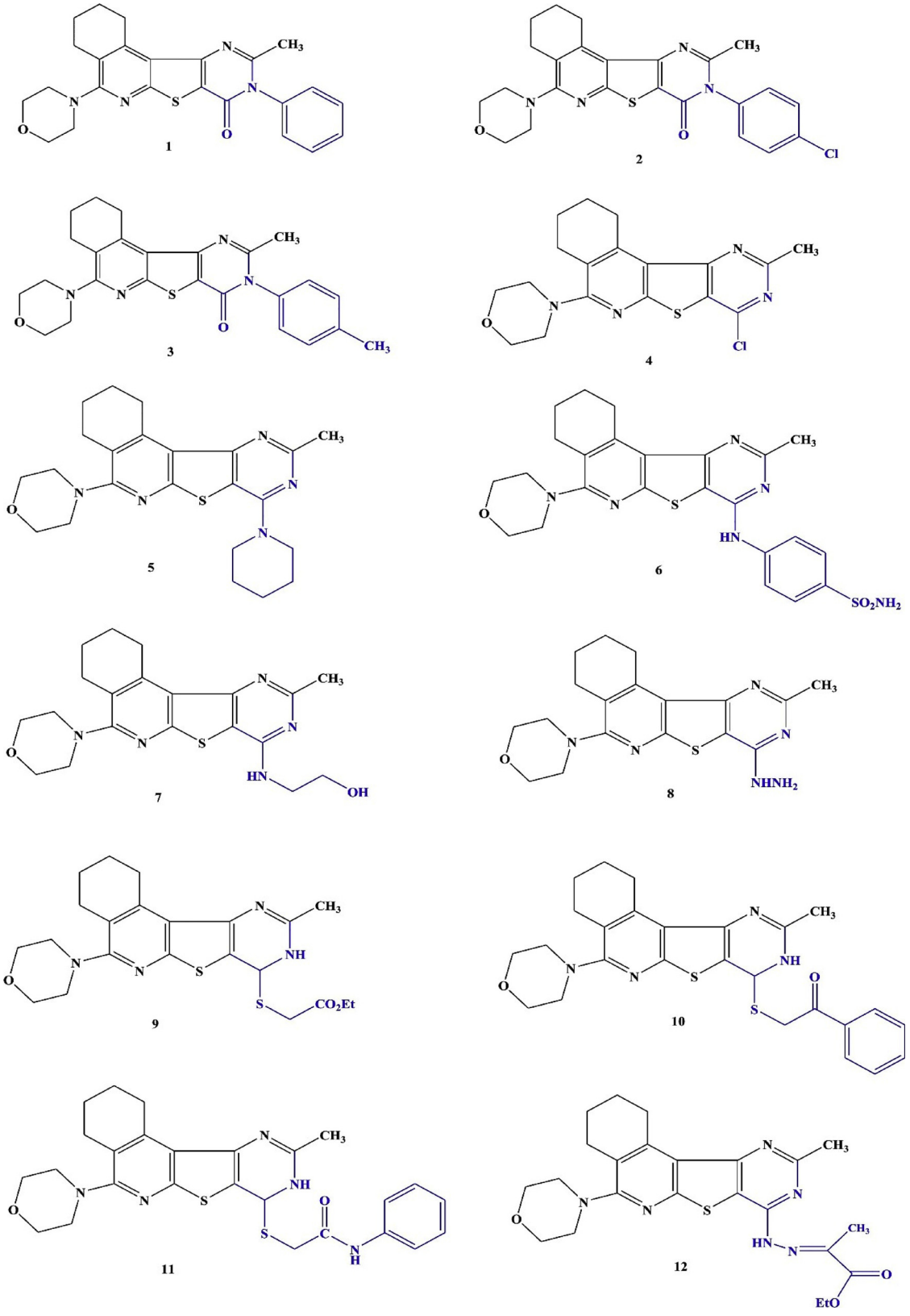

Fig. 1. Target compounds that tested for their insecticidal activity.

4. Structure-action relationship

As a continuation of this work, the structure-activity relationships (SAR) were reported here according to the toxicity values in table below and Fig. 2 as well. It is shown that the chloropyrimidine derivative **4** is more active against cowpea aphid than the other pyrimidothienotetrahydroisoquinoline synthesized derivatives. The high activity associated with compounds **2** and **6** may be due to the presence of the chlorophenyl and benzene sulfonamide moiety respectively in their structure in addition to the general characteristics of the prepared

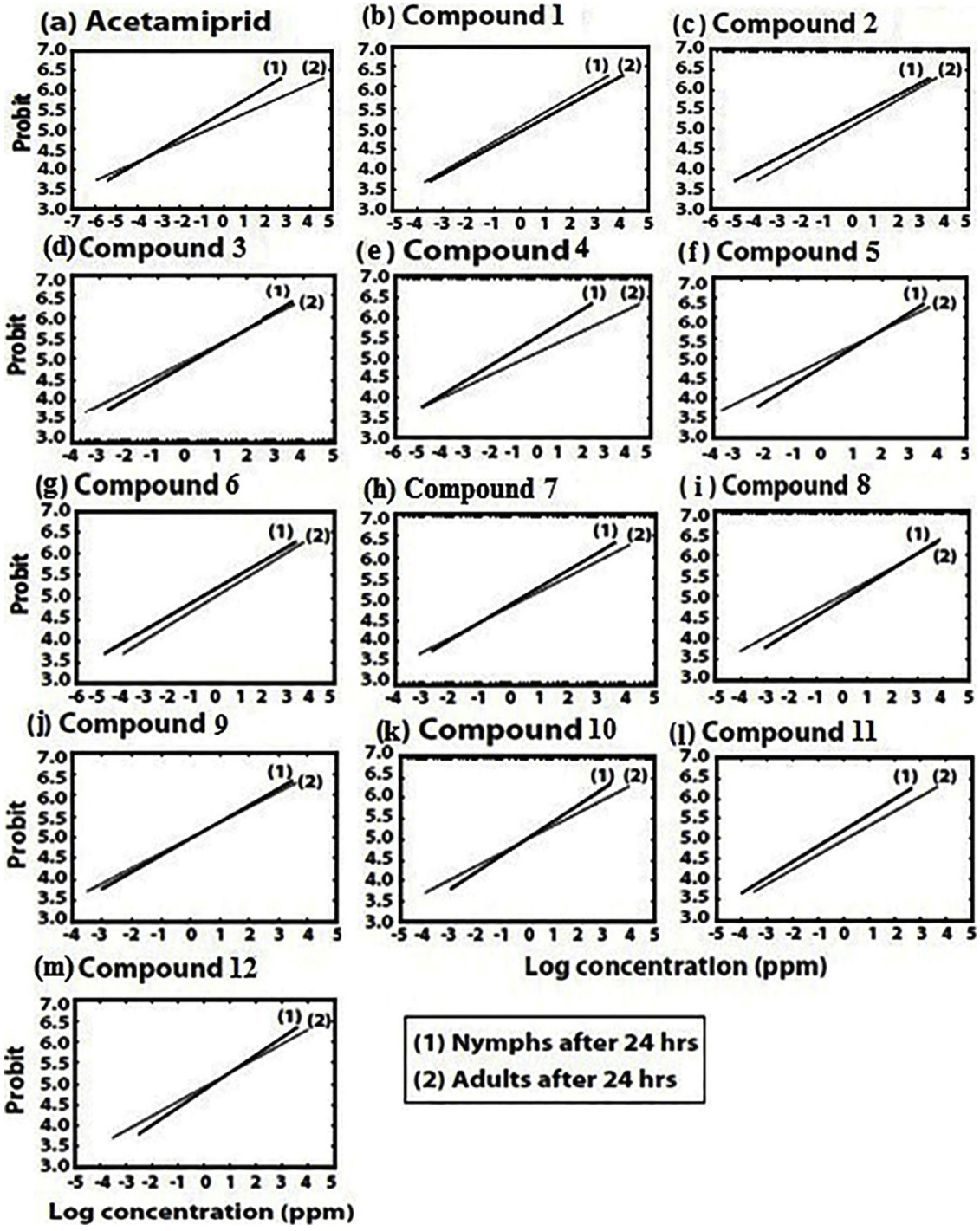

Fig. 2. Insecticidal activities of acetamiprid and compounds **1–12** against the nymphs and adults of cowpea aphid, *A. craccivora* after 24 h of treatment.

compounds. Also, toxicity of tetrahydroisoquinoline compounds **10** and **11** is higher than that of **9** analog, this may be due to the presence of sulfanyl acetophenone and sulfanyl acetanilide moiety respectively in compounds **10** and **11** which may cause the insecticidal activity. The presence of phenyl group may reflect better activity than the tolyl group and this is shown in compounds **1** and **3**. Also, compound **7** which contains amino ethanol group possess higher insecticidal activity than compound 8 which contains hydrazino group. Finally, the aphicidal activity of compound **12** is higher than that of compound **5** and this may be due to the presence of hydrazono propanoate moiety in compound **12** than piperidinium group in compound **5**.

5. Conclusion

A series of pyrimidothienotetrahydroisoquinolines which are analog to neonicotinoid insecticides and contain pyridine moiety were chemically prepared. The toxicity of these compounds was estimated against cowpea aphid, *A. craccivora* and showed that a number of the prepared compounds possess good toxicological activities as insecticides, whereas some of them revealed reasonable aphicidal activities. Especially, chloropyrimidine compound **4** had the best insecticidal activity since it exceeded the aphicidal activity of a commercial pesticide, acetamiprid. The activity concerning compound **4** may be due to the existence of the chlorine atom attached to the pyrimidine cycle in

its molecular structure. Our research demonstrated that the new pyrimidothienotetrahydroisoquinoline derivatives containing pyridine moiety could effectively control cowpea aphids, *A. craccivora*, and this emphasis our studies done before [18,19]. These results are hopeful and valuable for additional work on the improvement of new and other potent pesticides.

Disclosure of potential conflicts of interest

We have no conflicts of interest to disclose.

References

[1] N. Zhang, M. Tomizawa, J.E. Casida, α-Nitro ketone as an electrophile and nucleophile: synthesis of 3-substituted 2-nitromethylenetetrahydrothiophene and tetrahydrofuran as Drosophila nicotinic receptor probes, J. Org. Chem. 69 (3) (2004) 876–881, https://doi.org/10.1021/jo035457g.

[2] H. Zhang, T. Breeze, A. Bailey, D. Garthwaite, R. Harrington, S.G. Potts, Arthropod pest control for UK oilseed rape-comparing insecticide efficacies, side effects and alternatives, PLoS One 12 (1) (2017), https://doi.org/10.1371/journal.pone.0169475 e0169475.

[3] J.G. Samaritoni, D.A. Demeter, J.M. Gifford, G.B. Watson, M.S. Kempe, T.J. Bruce, Dihydropiperazine neonicotinoid compounds. Synthesis and insecticidal activity, J. Agric. Food. Chem. 51 (10) (2003) 3035–3042, https://doi.org/10.1021/jf021185r.

[4] N. Simon-Delso, V. Amaral-Rogers, L.P. Belzunces, J.M. Bonmatin, M. Chagnon, C. Downs, L. Furlan, D.W. Gibbons, C. Giorio, V. Girolami, D. Goulson, D.P. Kreutzweiser, C.H. Krupke, M. Liess, E. Long, M. McField, P. Mineau, E.A.D. Mitchell, C.A. Morrissey, D.A. Noome, L. Pisa, J. Settele, J.D. Stark, A. Tapparo, H. Van Dyck, J. Van Praagh, J.P. Van der Sluijs, P.R. Whitehorn, M. Wiemers, Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites, Environ. Sci. Pollut. Res. 22 (1) (2015) 5–34, https://doi.org/10.1007/s11356-014-3470-y.

[5] M. Shimomura, M. Yokota, M. Ihara, M. Akamatsu, D.B. Sattelle, K. Matsuda, Role in the selectivity of neonicotinoids of insect-specific basic residues in loop D of the nicotinic acetylcholine receptor agonist binding site, Mol. Pharmacol. 70 (4) (2006) 1255–1263, https://doi.org/10.1124/mol.106.026815.

[6] M. Tomizawa, J.E. Casida, Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors, Annu. Rev. Entomol. 48 (2003) 339–364, https://doi.org/10.1146/annurev.ento.48.091801.112731.

[7] M. Tomizawa, T. Talley, D. Maltby, K.A. Durkin, K.F. Medzihradszky, A.L. Burlingame, P. Taylor, J.E. Casida, Mapping the elusive neonicotinoid binding site, Proc. Natl. Acad. Sci. U. S. A. 104 (21) (2007) 9075–9080, https://doi.org/10.1073/pnas.0703309104.

[8] S. Kagabu, R. Ishihara, K. Nishimura, Y. Naruse, Insecticidal and neuroblocking potencies of variants of the imidazolidine moiety of imidacloprid-related neonicotinoids and the relationship to partition coefficient and charge density on the pharmacophore, J. Agric. Food Chem. 55 (3) (2007) 812–818, https://doi.org/10.1021/jf0623440.

[9] Z.B. Yang, D.Y. Hu, S. Zeng, B.A. Song, Novel hydrazone derivatives containing pyridine amide moiety: design, synthesis, and insecticidal activity, Bioorg. Med. Chem. Lett. 26 (4) (2016) 1161–1164, https://doi.org/10.1016/j.bmcl.2016.01.047.

[10] H.C.J. Godfray, T. Blacquière, L.M. Field, R.S. Hails, S.G. Potts, N.E. Raine, A.J. Vanbergen, A.R. McLean, A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators, Proc. Biol. Sci. 282 (1818) (2015), https://doi.org/10.1098/rspb.2015.1821 20151821.

[11] E.A.D. Mitchell, B. Mulhauser, M. Mulot, A. Mutabazi, G. Glauser, A. Aebi, A worldwide survey of neonicotinoids in honey, Science 358 (6359) (2017) 109–111, https://doi.org/10.1126/science.aan3684.

[12] Z. Zian, X. Shao, Z. Li, X. Qian, Q. Huang, Synthesis, insecticidal activity and QSAR of novel nitromethylene neonicotinoids with tetrahydropyridine fixed cis configuration and exo-ring ether modification, J. Agric. Food Chem. 55 (6) (2007) 2288–2292, https://doi.org/10.1021/jf063418a.

[13] M.P. Kavvalakis, M.N. Tzatzarakis, E.P. Theodoropoulou, E.G. Barbounis, A.K. Tsakalof, A.M. Tsatsakis, Development and application of LC–APCI–MS method for biomonitoring of animal and human exposure to imidacloprid, Chemosphere 93 (10) (2013) 2612–2620, https://doi.org/10.1016/j.chemosphere.2013.09.087.

[14] P.D. Stivaktakis, M.P. Kavvalakis, M.N. Tzatzarakis, A.K. Alegakis, M.N. Panagiotakis, P. Fragkiadaki, E. Vakonaki, E. Ozcagli, W.A. Hayes, V.N. Rakitskii, A.M. Tsatsakis, Long-term exposure of rabbits to imidaclorpid as quantified in blood induces genotoxic effect, Chemosphere 149 (2016) 108–113, https://doi.org/10.1016/j.chemosphere.2016.01.040.

[15] A.I. Vardavas, E. Ozcagli, P. Fragkiadaki, P.D. Stivaktakis, M.N. Tzatzarakis, K. Kaloudis, M. Tsardi, G. Datseri, J. Tsiaoussis, C. Tsitsimpikou, F. Carvalho, A.M. Tsatsakis, DNA damage after long-term exposure of rabbits to imidacloprid and sodium tungstate, Toxicol. Lett. 258S (2016) S247–S248, https://doi.org/10.1016/j.toxlet.2016.06.1878.

[16] A.I. Vardavasa, E. Ozcaglib, P. Fragkiadakia, P.D. Stivaktakisa, M.N. Tzatzarakisa, A.K. Alegakisa, F. Vasilakia, K. Kaloudisa, J. Tsiaoussisc, D. Kouretasd, Ch. Tsitsimpikoue, F. Carvalhof, A.M. Tsatsakis, The metabolism of imidacloprid by aldehyde oxidase contributes to its clastogenic effect in New Zealand rabbits, Mutat. Res. Gen. Tox. Environ. (2018) 829–830, https://doi.org/10.1016/j.mrgentox.2018.03.002 26–32.

[17] P. Stivaktakis, M. Kavvalakis, N. Goutzourelas, D. Stagos, M. Tzatzarakis, M. Kyriakakis, R. Rezaee, D. Kouretas, W. Hayes, A. Tsatsakis, Evaluation of oxidative stress in long-term exposed rabbits to subtoxic levels of imidacloprid, Toxicol. Lett. 229S (2014) S228, https://doi.org/10.1016/j.toxlet.2014.06.764.

[18] E.A. Bakhite, A.A. Abd-Ella, M.E.A. El-Sayed, Sh.A.A. Abdel-Raheem, Pyridine derivatives as insecticides. Part 1: synthesis and toxicity of some pyridine derivatives against Cowpea Aphid, Aphis craccivora Koch (Homoptera: Aphididae), J. Agric. Food Chem. 62 (41) (2014) 9982–9986, https://doi.org/10.1021/jf503992y.

[19] E.A. Bakhite, A.A. Abd-Ella, M.E.A. El-Sayed, Sh.A.A. Abdel-Raheem, Pyridine derivatives as insecticides. Part 2: synthesis of some piperidinium and morpholinium cyanopyridinethiolates and their insecticidal activity, J. Saud. Chem. Soc. 21 (1) (2017) 95–104, https://doi.org/10.1016/j.jscs.2016.02.005.

[20] R.M. Zaki, Sh.M. Radwan, A.M. Kamal El-Dean, A convenient synthetic method and spectral characterization for new tetra hydro[1,3]oxazinothieno[2,3-c]isoquinoline and its pyrimidine derivatives, J. Chin. Chem. Soc. 64 (12) (2017) 1417–1431, https://doi.org/10.1002/jccs.201700232.

[21] P.J. O'Brien, Y.A. Abdel-Aal, J.A. Ottea, J.B. Graves, Relationship of insecticide resistance to carboxylesterases in *Aphis gossypii* (Homoptera: Aphididae) from Midsouth cotton, J. Econ. Entomol. 85 (3) (1992) 651–657, https://doi.org/10.1093/jee/85.3.651.

[22] W.S. Abbott, A method of computing the effectiveness of an insecticide, J. Econ. Entomol 18 (1925) 265–267 https://scholar.google.com/scholar?hl=ar&as_sdt=0%2C5&q=A+method+of+computing+the+effectiveness+of+an+insecticide&btnG=.

[23] D.J. Finney (Ed.), Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve, Cambridge University Press, Cambridge, U.K, 1952, , https://doi.org/10.1093/aesa/45.4.686.