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Synthesis of New Fatty *N*-Acylamino Amides from 3,4-Dichloroaniline with Crop Protection Activity

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A series of biologically active *N*-acylamino amides were synthesized from readily available amino acids and common fatty acids from oil seeds, including the pharmacophoric group in the family of herbicides propanil, linuron, and diuron. The esterification followed by *N*-acylation with long-chain fatty acids was carried out with *O*-(benzotriazole-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TBTU) as activating agent. The *N*-acylamino esters were synthesized and isolated in good yields (70-98%), which after the hydrolysis step, provided the *N*-acylamino acids. Finally, new *N*-acylamino amides were obtained from the reaction with 3,4-dichloroaniline in the presence of TBTU and *N*,*N*-diisopropylethylamine (DIPEA), in yields between 24-83%. Bioassays were conducted against arthropod strawberry pests *Chaetosiphon fragaefolii*, *Duponchelia fovealis*, and *Drosophila suzukii*, without any registered compound for use in strawberry crops in Brazil. The bioassay studies showed promising results, with mortality percentages ranging from 20-80%, and the best relative mortality by *N*-oleyl glycinamide, suggesting a possible fatty chain-amino acid synergistic bioactivity. Overall, this study provides an efficient and sustainable method for synthesizing biologically active *N*-acylamino amides from abundant natural sources, with potential applications in the development of ecofriendly agrochemicals.

Keywords: *N*-acylamino amide, fatty acids, amino acids, crop protection, agrochemicals

Introduction

Crop protection compounds development is an important factor contributing to ongoing efforts to secure food supply and to fight insect vectors of many diseases worldwide. The development of pesticides is a blooming field of research, with complex demands such as resistance development by pests, and environmental, consumer, and regulatory requirements.[1,2] The global agrichemical industry innovation protocol can follow different discovery approaches, from competitor-inspired patents/literature, next generation compounds based on internal products, bioactivity hypotheses, or natural product-inspired.[1] Natural compounds are, at the moment, regarded as an important lead to new agrichemicals, although underexplored due to lack of patent protection of these compounds, posing an economical barrier for lucrative development and production.[3] There is also an economic threshold for crop targeting, which hinders the development and commercialization of active ingredients against pests in crops considered minor crops.[4] Strawberry crops are one of such minor crops in Brazil, with around 165 kt annual production,[5] even with insufficient phytosanitary support: there are currently no registered active ingredients for use in this crop against three insect species: *Chaetosiphon fragaefolii*, *Drosophila suzukii*, and *Duponchelia fovealis*.[6]

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This manuscript is part of a series of publications in the Journal of the Brazilian Chemical Society by young researchers who work in Brazil or have a solid scientific connection with our country. The JBCS welcomes these young investigators who brighten the future of chemical sciences.

From a sustainability aspect, it is known that fatty compounds from vegetable oils, such as fatty acids, esters, and other derivatives, are a class of substances that exhibit low toxicity to non-target organisms and are biodegradable in nature since they are neither toxic nor non-phytotoxic and are rapidly degraded by microorganisms in the soil. Strongly motivated by the environmentally friendly and low risk to human health of the fatty compounds, which are extracted from renewable resources, the fatty derivatives are an interesting alternative to compose the formulations of agrichemicals. This class of molecules has not been targeted only to act as adjuvants, but also as active principles in the control of pests in crops.[7-11] Mohamad *et al*.[12] studied the susceptibility of aphids to natural lauric acid-based pesticides, and the study revealed that aphid mortality was dependent on the dose of lauric acid added to the surfactant/solvent mixture, which indicates that the highest rates of mortalities were achieved when higher concentrations of lauric acid were used. In addition, the use of lauric acid together with the surfactant added in the formulation promoted the change in the surface tension of the spray drop, decreasing it, and therefore, wetting and spreading it more evenly over the leaves.[12] Furthermore, oleic acid is also classified as an insecticide, and its derivative, oleic acid, eicosyl ester, has shown larvicidal activity against *Aedes aegypti* (Diptera: Culicidae) and *Culex quinquefasciatus* (Diptera: Culicidae) larvae, since they promote significant cuticular degradation in these species.[13] Palmitic, stearic, and linoleic acids are already used in crops as natural pesticides in countries such as Brazil.[14]

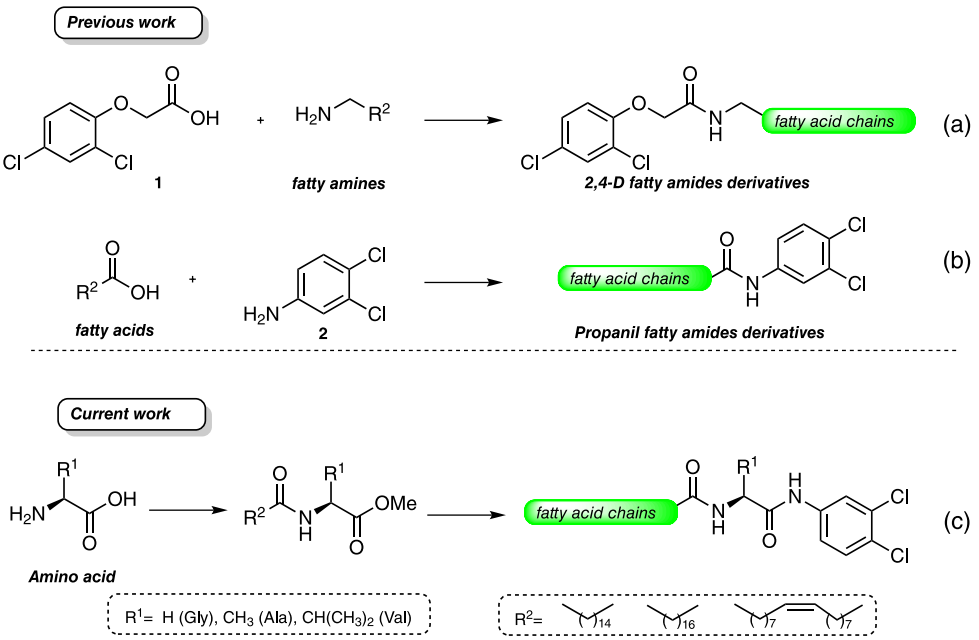
The modulation of physicochemical properties by fatty chain incorporation, besides its intrinsic bioactivity, has been explored recently in herbicide formulations, with applications such as herbicidal ionic liquids (HIL).[11,15-19] Based on the potential for the technological application of lipophilic derivatives in the agro-industrial sector, our research group has been investing efforts in the study of compounds derived from vegetable oils aimed at this sector. In recent work, esters and fatty amides were synthesized from the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (**1**), and in this same context, fatty amides were synthesized from 3,4-dichloroaniline (**2**), a functional group common to the herbicides linuron, diuron and propanil (Scheme 1).[8] In this study, the pre-emergent herbicidal activity of the synthesized compounds was evaluated *in vitro* against onion (*Allium cepa*, "Anasac Jardin") and lettuce (*Lactuca sativa*, "Vilmorin Jardin") seeds as mono- and dicots. Motivated by the positive results of germination inhibition obtained from the new esters and fatty amides, the next question evaluated was the influence of the alkyl chains on the environmental behavior of the new compounds, incorporated into the basic structure of the herbicides 2,4-D and propanil by through covalent bonds. Kinetic studies and nuclear magnetic resonance (NMR) experiments conducted in the study showed that the new fatty derivatives were significantly more susceptible to degradation, suggesting that the modifications carried out may add interesting properties to the new herbicidal compounds, such as modulation of solubility, volatility, and surfactant properties. The results obtained by the authors showed that the incorporation of the fatty chains, both in the form of esters and amides, resulted in the acceleration of the degradation of the compounds studied, which is an environmentally desirable property to compounds aimed at the agrochemical sector.[8]

In this context, considering previous works on the synthesis of lipophilic compounds and the precedent insecticidal activity of fatty compounds against arthropods, the present study investigated the synthesis of new fatty *N*-acylamino amides from available natural products: amino acids (glycine, *L*-alanine, and *L*-valine) and fatty acids family such as palmitic (C16:0), stearic (C18:0), and oleic (C18:1) with the pharmacophoric group 3,4-dichlorophenyl, present in the family of herbicides propanil, linuron and diuron (Scheme 1).

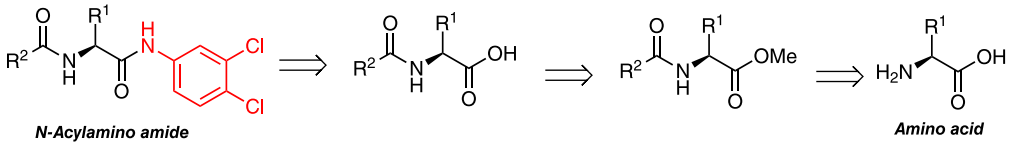
All synthesized compounds were studied against three representatives of strawberry pest arthropods. *Chaetosiphon fragaefolii*, (Cockerell) (Hemiptera: Aphididae) causes phloem sap suction and tissue death, and indirectly virus transmission.[20] The female *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) has a serrated ovipositor that is rarely found in other drosophilids, a fact that allows the species to oviposit in green and ripe fruits, accelerating decomposition of these fruits.[21] *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae) has a cryptic habit, developing caterpillars sheltering in the basal region of the strawberry plant, and when perforating the base of the plants by piercing, they cause premature death of the plants.[22] The attacks of these pests represent a phytosanitary challenge due to cases of resistance to insecticides, allied to the lack of commercially available active ingredients.[4,6]

Results and Discussion

The retrosynthetic pathway to the fatty *N*-derivatives was based on the insertion of the pharmacophoric group common to the herbicides linuron, diuron, and propanil, from the functionalization of the *N*-acyl derivatives with 3,4-dichloroaniline according to Scheme 2.



Scheme 1. Previous[8] and current work. (a) Synthesis of fatty acid amides from herbicide 2,4-D (**1**); (b) synthesis of fatty acid amides from fatty acids and 3,4-dichloroaniline (**2**), the pharmacophoric unit present in the propanil, diuron, and linuron herbicide's family; (c) synthesis of new *N*-acylamino amides from amino acids, fatty acids and 3,4-dichloroaniline.

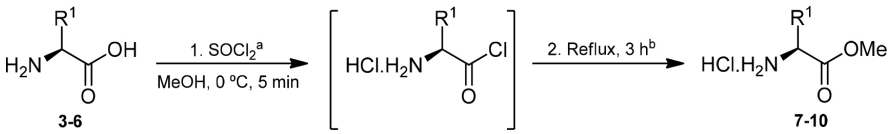


Scheme 2. Retrosynthesis of the new fatty derivatives proposed in this work.

Amino esters synthesis

The amino acid esterification reactions occurred according to previous works[8,23,24] by conversion of the carboxylic acid into its respective acid chloride, generated *in situ* in the presence of thionyl chloride ($SOCl_2$) in methanol. The suspension was cooled to 0 °C, followed by the slow addition of freshly distilled thionyl chloride ($SOCl_2$, 30 mmol, 1.5 equiv.). After completing the addition, the mixture was stirred and refluxed for 3 h. The amino esters chlorides were isolated after the evaporation and submitted to purification by recrystallization in methanol, in yields from 81 to 96% (Table 1). The amino esters **7-10** were characterized by ^{1}H and ^{13}C NMR spectroscopy. In all compounds, the main chemical shift observed in the ^{1}H spectrum corresponds to a singlet at approximately 3.73 ppm, corresponding to the methoxyl group $–OCH_3$ introduced in the esterification step, confirming the formation of the compounds.

Table 1. Synthesis of amino esters monohydrochlorides **7-10**



entry	Amino acid	R^1	Amino ester.HCl	Yield / %
1	glycine (**3**)	H	**7**	96
2	L-alanine (**4**)	CH_3	**8**	93
3	L-valine (**5**)	$CH(CH_3)_2$	**9**	81
4	L-serine (**6**)	CH_2OH	**10**	92

[a]Reaction conditions: **3-6** (1 equiv.) in MeOH (1 mol L^{-1}) cooled to 0 °C, followed by slow addition (5 min) of freshly distilled $SOCl_2$ (1.5 equiv.); [b]After completing the addition, the mixture was stirred and refluxed for 3 h.

Additionally, all the spectra showed signals in the region between 3.76 and 4.10 ppm, referring to α-carbonyl hydrogens whose coupling depends on the substituent in the starting amino acid structure. Another characteristic signal of this class of compounds, present in all spectra acquired in DMSO-d_6, is the broad singlet above 8.00 ppm, attributed to the 3 hydrogens of the $–NH_3^+$ group (for details, see Supplementary Information (SI) section, Figures S1-S8).

Synthesis of *N*-acylamino esters **12-15**

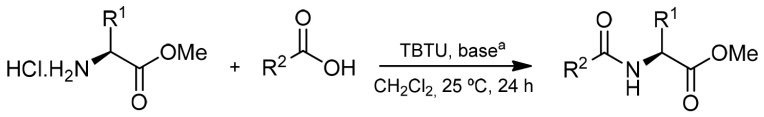
The *N*-acylation step with fatty acids palmitic (**11a**; C16:0), stearic (**11b**; C18:0), and oleic (**11c**; C18:1) was investigated from amino esters **7-10**. The reactions were carried out using uronium salts, such as *O*-(benzotriazole-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium (TBTU) tetrafluoroborate, as a coupling agent, widely used in the activation of carboxylic acids to form amide bonds.[23] The nitrogenated base was crucial to achieving high yields in the reaction with TBTU and amino acids. In an effort to optimize this synthetic step, triethylamine (Et_3N) and *N*,*N*-diisopropylethylamine (DIPEA) were employed, the latter resulting in significantly higher yields (Table 2, entries 1-12). Such behavior can be associated with the fact that DIPEA is a slightly stronger base than Et_3N (pK_a ca. 11.4 and 10.6, respectively).[25] Furthermore, due to higher steric hindrance, DIPEA reduces the possibility of α-carbonyl hydrogen and α-nitrogen abstraction, maintaining the integrity of the asymmetric center of the amino acid derivatives.[23]

The insertion of the alkyl chain through *N*-acylation was confirmed from ^{1}H NMR spectroscopy. According to the signals in the spectra (see SI section), it is possible to highlight the singlet at 3.75 ppm, attributed to the methoxyl hydrogens, indicating no changes in the ester functional group. Also, it is possible to observe a signal at 4.00-4.60 ppm, approximately, that is attributed to α-carbonyl and α-nitrogen hydrogen, with a relative integral to one hydrogen.

Synthesis of *N*-acylamino amides **20-22**

Initially, the *N*-acylamino esters **12-15** were hydrolyzed to respective *N*-acylamino acids **16-19**. The hydrolysis reactions were monitored by thin-layer chromatography (TLC) using ethyl acetate as eluent. After consumption of the starting material, the solvent was removed under reduced pressure and the remaining aqueous suspension was neutralized with the addition of HCl solution (6 mol L^{-1}). The white precipitate formed was solubilized

Table 2. *N*-Acylamino ester synthesis using fatty acids **11a-11c** with TBTU



7-10 **11a-11c** **12-15**

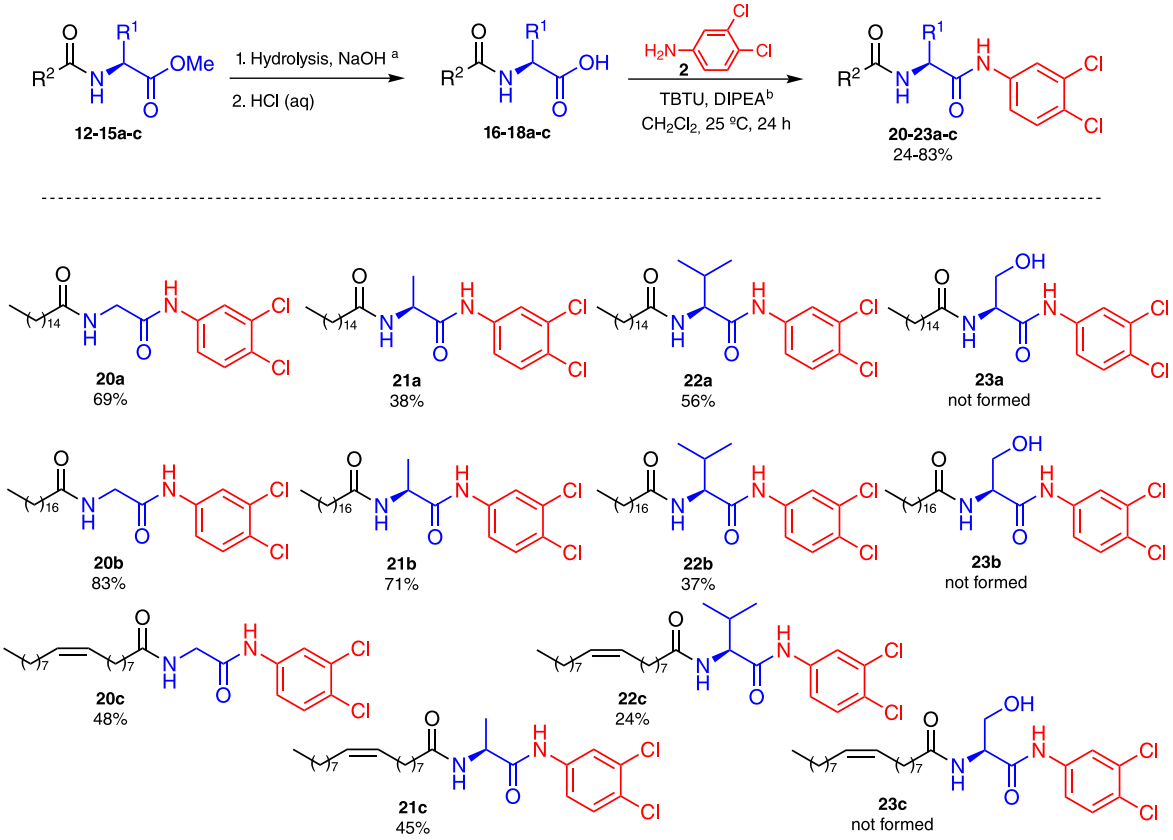
entry	Amino ester	Fatty acid	*N*-Acylamino ester	Yield / %	
				DIPEA	Et_3N
1	**7**, R^1 = H	**11a**, palmitic (C16:0)	**12a**	98	41
2	**8**, R^1 = CH_3		**13a**	89	62
3	**9**, R^1 = $CH(CH_3)_2$		**14a**	89	49
4	**10**, R^1 = CH_2OH		**15a**	70	57
5	**7**, R^1 = H	**11b**, stearic (C18:0)	**12b**	94	59
6	**8**, R^1 = CH_3		**13b**	96	58
7	**9**, R^1 = $CH(CH_3)_2$		**14b**	88	54
8	**10**, R^1 = CH_2OH		**15b**	75	36
9	**7**, R^1 = H	**11c**, oleic (C18:1)	**12c**	95	54
10	**8**, R^1 = CH_3		**13c**	90	44
11	**9**, R^1 = $CH(CH_3)_2$		**14c**	82	41
12	**10**, R^1 = CH_2OH		**15c**	70	36

[a]Reaction conditions: a solution of fatty acid **11a-11c** (0.8 mmol), *O*-(benzotriazole-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetra-fluoroborate (TBTU, 0.85 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 0.85 mmol) or triethylamine (Et_3N, 0.85 mmol), in 10 mL of CH_2Cl_2 were stirred at 25 ºC for 5 min. In a second reaction flask, the amino esters **12-15** (1.76 mmol) were kept under constant stirring at room temperature for 10 min, in the presence of DIPEA (1.8 mmol) and 10 mL of CH_2Cl_2. Afterward, the solution from the second flask is slowly added over the solution containing the fatty acid, keeping the final mixture under agitation for the indicated time and temperature.

in ethyl acetate and the organic phase was separated, with subsequent removal of volatiles under reduced pressure. The *N*-acylamino acids **16-19** were isolated quantitatively and used without further purification. After, the *N*-acylamino acids were subsequently submitted to ammonolysis reaction, in the presence of 3,4-dichloroaniline **2**, resulting in the synthesis of the *N*-acylamino amides **20-22** in 24-83% yields (Scheme 3).[24]

N-Acylamino amides **20-22**, derived from *N*-acylamino acids **16-18**, were isolated in moderate to good yields (24-83%). However, the synthesis of amides **23a-23c** from amino acid **19a-19c** was not successful by the present methodology, recovering the starting materials. One of the possible causes for the non-formation of the amides from serine's derivatives may be associated with the formation of a six-membered ring through hydrogen bonds of the free hydroxyl group, and the carboxylate unit present in the structure, hindering the reaction with coupling agent (Figure 1). Another factor that may also have influenced the reactivity of *N*-acylamino acid, is that when a uronium salt is used as a coupling reagent, a guanidine by-product can arise when the reagent reacts directly with the amine present in the medium. This is often due to the slow activation of the carboxylic acid or the use of excess uronium reagent.[23]

Physicochemical properties of the compounds must be taken into consideration when aiming at technological applications. The synthesis, purification, safe storage, and shelf life reflect these properties.[26,27] Considering this, solubility in different solvents and thermal stability were studied for the synthesized *N*-acylamino amides in this work.



Scheme 3. Fatty *N*-acylamino amides **20-23** synthesized from 3,4-dichloroaniline **2**.

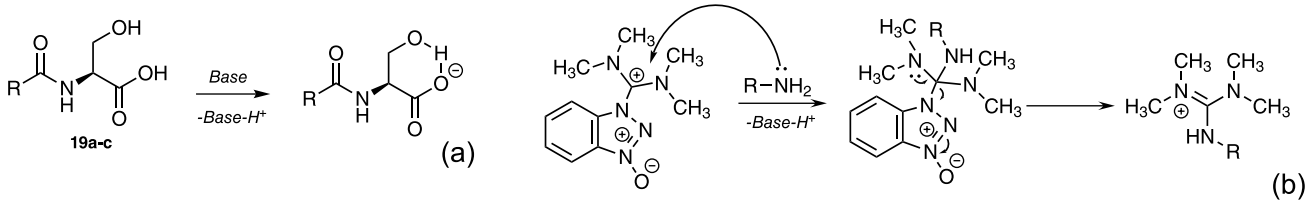


Figure 1. (a) Proposed intramolecular hydrogen bond and six-membered ring formation; (b) proposed side reactions of TBTU and amine leading to the guanidines.

The solubility and thermal profile study of the compounds geared towards technological applications has been constantly described in the works found in the literature.[26] In this sense, the *N*-acylamino amides had their solubility in water and organic solvents investigated, following a standardized procedure. The observed results are presented in Table 3 and are expressed according to the volume of solvent required to completely dissolve the sample, being considered as "good solubility" the compound that is completely dissolved in 1 mL of solvent; "Limited solubility" for compounds that demand the addition of an additional 1 mL of solvent for complete dissolution and the term "low solubility" for compounds that, even after the addition of a total of 3 mL of solvent, remain insoluble or partially soluble in the medium.[26]

It is worth noticing that all the compounds tested were highly soluble in dichloromethane (DCM), except for **20a**, a compound that proved to be poorly soluble in all tested solvents. All of them show low solubility in hexane, as well as in water, acetonitrile, methanol, ethyl acetate, and ethanol. *N*-Oleyl amides **20-22c** (Table 3, entries 7-9) are exceptions, with good solubility in ethanol and ethyl acetate, also presenting limited solubility in methanol.

Thermal profiles of the molecules give insight into their thermal stability for further technological applications, and valuable information regarding their proneness to degradation, polymerization, and other reactions under heating.[27] The thermal profile for the fatty *N*-acylamino amide compounds was analyzed by differential scanning calorimetry (DSC) experiments. The values referring to the observed thermal phenomena were organized and shown in Table 4. The curves obtained for all the fatty amides showed melting phenomena (negative area values represent endothermic phenomena) and in some cases, it was possible to observe glass transition phenomena (see SI section for more details).

Table 3. Solubility of fatty *N*-acylamino amides **20-22** in several solvents

entry	Amide	Solvent						
		Hexane (PI = 1.0)	DCM (PI = 3.1)	EtOH (PI = 4.3)	EtOAc (PI = 4.4)	MeOH (PI = 5.1)	MeCN (PI = 5.8)	H_2O (PI = 10.2)
1	**20a**	–	–	–	–	–	–	–
2	**21a**	–	+	–	–	–	–	–
3	**22a**	–	+	–	–	–	–	–
4	**20b**	–	+	–	–	–	–	–
5	**21b**	–	+	–	–	–	–	–
6	**22b**	–	+	–	–	–	–	–
7	**20c**	–	+	+	+	±	–	–
8	**21c**	–	+	+	+	±	–	–
9	**22c**	–	+	+	+	±	–	–

PI: polarity index; "+": good solubility; "±": solubility limit; "–": low solubility.

Table 4. Thermal behavior of *N*-acylamino amides by differential scanning calorimetry experiments

entry	Amide	T OnSet / ºC	Peak / ºC	T EndSet / ºC	Area / (J g^{-1})	Glass transition (Tg) / ºC
1	**20a**	96.6 109.1	100.2 114.7	104.5 116.2	–15.9 –49.8	–
2	**21a**	90.3	94.6	97.6	–120	162.7
3	**22a**	72.6 95.3	74.2 99.9	79.8 105.1	–72.7 –12.31	–
4	**20b**	94.2 98.5	95.6 103.2	103.3 102.1	–93.42 14.2	168.3
5	**21b**	100.4	103.4	105.4	–102.8	–
6	**22b**	–	–	–	–	–
7	**20c**	54.0 84.8	58.9 89.6	63.7 94.7	–33.9 –28.7	– –
8	**21c**	–	–	–	–	–
9	**22c**	62.4	67.9	74.9	–108.9	146.7

T: temperature.

Moving on with the application-geared experiment for this study, it was demonstrated that new fatty *N*-acylamino amides interfered in the biological responses to economically important target insects in the strawberry crop, by bioassays conducted in different arthropod species (Figure 2). After 120 h of exposure, the level of toxicity of compounds **20c**, **21a**, and **22a** in *C. fragaefolli* nymphs was higher than the other treatments, with a mortality of ≅ 53%. These values were significantly higher than those obtained with dimethyl carbonate (DMC), **20a**, **20b**, and **21b** (F = 2.4627; degrees of fredom (DOF) = 6; coefficient of variation (CV) = 70.68%), which caused mortality between 8 and 32% in topical application. When compound **20c** was applied to the target insects *D. suzukii* and *D. fovealis*, higher mortality rates of 46 and 80% were observed, respectively, in relation to the other compounds (F = 12.7725; GL = 6; CV = 53.55% for *D. suzukii* and F = 32.3116; GL = 6; CV = 35.83% for *D. fovealis*). Furthermore, compounds **21a**, **22a**, and **12c** caused dehydration in *C. fragaefolli*. Whereas compound **20c** caused macroscopic abnormalities on the surface of the cuticles of the 3rd instar caterpillars of *D. fovealis*, including darkening and peeling of the cuticle, as well as tremors and disordered movements in *D. suzukii*. These results suggest that the effects of **20c** can be attributed to the length of the carbon chain (C18:1) combined with the presence of chemical bonds, saturated and unsaturated.[28] Furthermore, complex interactions between *N*-acylamino amides and fatty acids, have been reported to have a synergistic action against pests, promoting increased effectiveness of the formulations.[29]

Conclusions

A series of *N*-acylamino amides with potential insecticidal properties were synthesized through a four-step process, using readily available amino acids and fatty acids from oil seeds, and incorporating the 3,4-dichlorophenylamine pharmacophoric group present in commercial herbicides. The solubility in several organic solvents was evaluated and the thermal profiles of products were determined by DSC analysis. Preliminary bioassays conducted against arthropod strawberry pests, such as *C. fragaefolii*, *D. fovealis*, and *D. suzukii*, offered some evidence of the insecticidal potential of these compounds, particularly the oleylglycine **19c** derivative. The observed mortality percentages, ranging from 20-80%, could be attributed to the recently reported synergy between the fatty chain and amino acid components of these compounds. However, more extensive testing is required to substantiate these findings. Future research will focus on exploring the structure-activity relationships of these compounds, their mode of action, and evaluating their safety, environmental impact, and effects on non-target organisms. By extending this research to encompass other agricultural settings and a broader range of pests, it is hoped that additional insights into the potential of these compounds as effective and sustainable insecticides will be uncovered.

Experimental

The reagents and solvents used for the syntheses described in this work were obtained from Sigma-Aldrich, São Paulo, Brazil, and used as received. When necessary, the reagents were submitted to the purification or drying habitual procedures reported in the literature.[30] The NMR spectroscopic analyses were performed at the Multiuser NMR Laboratory, located in the Department of Chemistry at the Federal University of Paraná. About 20 mg of the samples were weighed in Eppendorf, dissolved in 600 µL of deuterated chloroform ($CDCl_3$), and transferred to 5 mm NMR tubes. The ^{1}H and ^{13}C NMR spectra were acquired at 25 ºC in a Bruker Avance 400 spectrometer (Bruker, Karlsruhe,Germany), operating at 9.4 Tesla, equipped with a direct detection probe (BBO), observing the ^{1}H and ^{13}C nuclei at 400 and 100 MHz, respectively. In some exceptional cases, ^{13}C experiments were acquired on a Bruker DPX200 spectrometer, operating at 4.7 T, observing the ^{13}C core at 50 MHz.

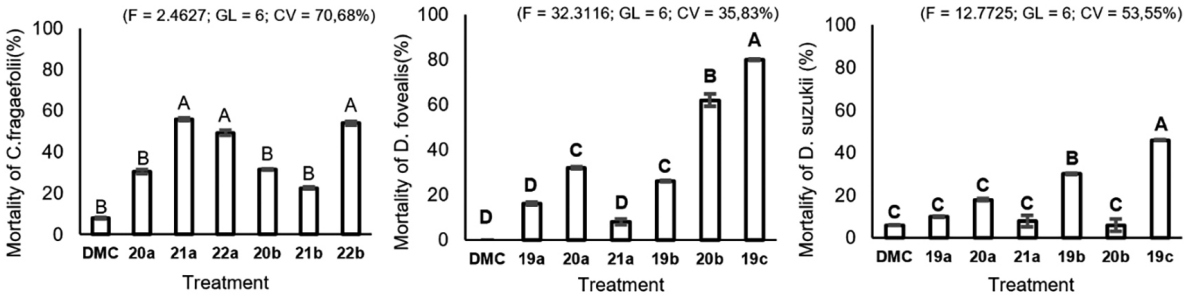


Figure 2. *Chaetosiphon fragaefolii*, *Drosophila suzukii* and *Duponchelia fovealis* mortality when submitted to various treatments in topical application (DMC: dimethyl carbonate). Means followed by different letters on the columns indicate significant differences between treatments (GLM with *quasi*-binomial distribution followed by post hoc Tukey test, P<0.05).

General procedure for the synthesis of amino esters monohydrochloride **7-10**

In an oven-dried reaction flask, the amino acid (**3-6**, 20 mmol, 1 equiv.) was added to 20 mL of methanol. The suspension was cooled to 0 °C, followed by the slow addition of freshly distilled thionyl chloride ($SOCl_2$, 30 mmol, 1.5 equiv.). After completing the addition, the mixture was stirred and refluxed for 3 h. The volatiles were then evaporated under reduced pressure and the crude reaction was purified by recrystallization.

Methyl glycinate monohydrochloride (**7**)[24]

Molar mass (MM) 125.55 g mol^{-1}; mp 158-165 °C; white solid; yield: 96%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.73 (s, 3H), 3.76 (s, 2H), 8.67 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.4, 167.9.

Methyl L-alaninate monohydrochloride (**8**)[24]

MM 139.58 g mol^{-1}; mp 109-111 °C; white solid; yield: 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (d, 3H, *J* 7.2 Hz), 3.74 (s, 3H), 4.01 (q, 1H, *J* 7.2 Hz), 8.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.0, 16.1, 48.2, 53.1, 170.7.

Methyl L-valinate monohydrochloride (**9**)[24]

MM: 167.63 g mol^{-1}; mp 171-173 °C; white solid; yield: 81%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (d, 3H, *J* 6.9 Hz), 0.95 (d, 3H, *J* 6.9 Hz), 2.15 (m, 1H), 3.71 (s, 3H), 3.76 (d, 1H, *J* 4.7 Hz), 8.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.5, 18.5, 52.4, 57.3, 169.1.

Methyl L-serinate monohydrochloride (**10**)[24]

MM: 155.58 g mol^{-1}; mp 157-161 °C; white solid; yield: 92%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.74 (s, 3H), 3.84 (d, 2H, *J* 3.6 Hz), 4.06 (t, 1H, *J* 3.6 Hz), 5.62 (s, 1H), 8.65 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.7, 54.4, 59.4, 168.4.

General procedure for the synthesis of fatty *N*-acylamino esters **12-15**

In a reaction flask, the fatty acid (**11a-11c**, 0.8 mmol, 1 equiv.), TBTU (0.85 mmol, 1.05 equiv.) and DIPEA (0.85 mmol, 1.05 equiv.) were added in order to 10 mL of dichloromethane (CH_2Cl_2). The solution was stirred at room temperature for 5 min. In a second reaction flask, the amino ester (**7-10**, 1.76 mmol, 2.2 equiv.) was kept under constant stirring at room temperature for 10 min, in the presence of DIPEA (1.8 mmol, 2.25 equiv.) and 10 mL of CH_2Cl_2. Subsequently, the amino ester solution is slowly added over the first prepared solution of fatty acid, keeping the final mixture under agitation for 24 h at 25 °C. After completion of the reaction, volatiles were removed under reduced pressure, and the reaction crude was then subjected to purification by column chromatography, using a mixture of hexane and ethyl acetate as eluents.

Methyl *N*-palmitylglycinate (**12a**)[24]

MM: 327.50 g mol^{-1}; white crystal; yield: 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3H, *J* 6.8 Hz), 1.28 (s, 24H), 1.64 (q, 2H, *J* 7.5 Hz), 2.24 (t, 2H, *J* 7.7 Hz), 3.76 (s, 3H), 4.05 (d, 2H, *J* 5.1 Hz), 6.06 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.6, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9, 36.4, 41.2, 52.3, 170.6, 173.4.

Methyl *N*-stearylglycinate (**12b**)[24]

MM 355.56 g mol^{-1}; white crystal; yield: 94%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* 7.0 Hz), 1.25 (s, 28H), 1.64 (q, 2H, *J* 7.5 Hz), 2.24 (t, 2H, *J* 7.5 Hz), 3.76 (s, 3H), 4.05 (d, 2H, *J* 5.2 Hz), 5.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.9, 25.8, 29.5, 29.5, 29.7, 29.9, 32.1, 36.6, 41.4, 52.5, 170.8, 173.5.

Methyl *N*-oleylglycinate (**12c**)[24]

MM: 353.54 g mol^{-1}; white crystal; yield: 77%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* 7.0 Hz), 1.28 (d, 20H, *J* 14.9 Hz), 1.64 (m, 2H), 2.00 (m, 4H), 2.23 (t, 2H, *J* 7.7 Hz), 3.76 (s, 3H), 4.05 (d, 2H, *J* 5.0 Hz), 5.34 (m, 2H), 5.96 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.6, 25.5, 27.1, 27.2, 29.1, 29.2, 29.3, 29.5, 29.6, 29.7, 31.8, 36.3, 41.1, 52.3, 129.7, 129.9, 170.6, 173.2.

Methyl *N*-palmityl-L-alaninate (**13a**)[24]

MM: 341.53 g mol^{-1}; white crystal; yield: 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* 7.0 Hz), 1.25 (s, 24H), 1.39 (d, 3H, *J* 7.1 Hz), 1.62 (q, 2H, *J* 7.0 Hz), 2.19 (q, 2H, *J* 7.4 Hz), 3.75 (s, 3H), 4.61 (q, 1H, *J* 7.2 Hz), 6.01 (d, 1H, *J* 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 18.6, 22.7, 25.5, 29.2, 29.3, 29.4, 29.6, 29.6, 31.9, 36.6, 47.8, 52.4, 172.6, 173.7.

Methyl *N*-stearyl-L-alaninate (**13b**)[24]

MM: 369.59 g mol^{-1}; white crystal; yield: 96%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* 6.9 Hz), 1.25 (s, 28H), 1.40 (d, 3H, *J* 7.2 Hz), 1.63 (q, 2H, *J* 7.7 Hz), 2.20 (t, 2H, *J* 7.8 Hz), 3.75 (s, 3H), 4.61 (q, 1H, *J* 7.3 Hz), 6.01 (d, 1H, *J* 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 18.6, 22.7, 25.6, 29.2, 29.3, 29.5, 29.7, 31.9, 36.6, 47.9, 52.4, 172.7, 173.7.

Methyl *N*-oleyl-L-alaninate (**13c**)[24]

MM: 367.57 g mol^{-1}; oil; yield: 66%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* 7.0 Hz), 1.28 (d, 20H, *J* 13.5 Hz), 1.40 (d, 2H, *J* 7.1 Hz), 1.63 (q, 2H, *J* 7.3 Hz), 2.01 (m, 4H), 2.20 (t, 2H, *J* 7.7 Hz), 3.75 (s, 3H), 4.60 (q, 1H, *J* 7.2 Hz), 5.34 (m, 2H), 6.04 (d, 1H, *J* 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 18.6, 22.7, 25.5, 27.2, 27.2, 29.1, 29.2, 29.2, 29.3, 29.5, 29.7, 29.8, 31.9, 36.5, 47.9, 52.4, 129.7, 130.0, 172.6, 173.7.

Methyl *N*-palmityl-L-valinate (**14a**)[24]

MM: 369.58 g mol^{-1}; white crystal; yield: 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (m, 6H), 0.94 (d, 3H, *J* 6.7 Hz), 1.25 (s, 25H), 1.64 (q, 2H, *J* 7.5 Hz), 2.23 (t, 2H, *J* 7.8 Hz), 3.73 (s, 3H), 4.58 (dd, 1H, *J* 8.8, 4.8 Hz), 5.34 (m, 2H), 5.94 (d, 1H, *J* 8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 17.8, 18.9, 22.7, 25.7, 29.1, 29.2, 29.3, 29.5, 29.6, 29.6, 29.7, 31.3, 31.9, 36.7, 52.1, 56.8, 172.8, 173.1.

Methyl *N*-stearyl-L-valinate (**14b**)[24]

MM: 397.64 g mol^{-1}; white crystal; yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (m, 6H), 0.94 (d, 3H, *J* 6.7 Hz), 1.25 (s, 28H), 1.64 (q, 2H, *J* 7.3 Hz), 2.23 (t, 2H, *J* 7.3 Hz), 3.74 (s, 3H), 4.59 (dd, 1H, *J* 8.8, 4.9 Hz), 5.34 (m, 2H), 5.98 (d, 1H, *J* 8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 17.8, 18.9, 22.7, 24.7, 25.7, 29.1, 29.2, 29.4, 29.4, 29.5, 29.7, 31.3, 31.9, 34.0, 36.7, 52.1, 56.8, 172.9, 173.3, 179.2.

Methyl *N*-oleyl-L-valinate (**14c**)[24]

MM: 395.63 g mol^{-1}; oil; yield: 41.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (m, 6H), 0.93 (d, 3H, *J* 6.8 Hz), 1.28 (d, 20H, *J* 15.0 Hz), 1.64 (m, 2H), 2.00 (m, 4H), 2.24 (t, 2H, *J* 7.7 Hz), 3.75 (s, 3H), 4.59 (dd, 1H, *J* 8.8, 5.0 Hz), 5.34 (m, 2H), 5.98 (d, 1H, *J* 8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 17.8, 18.9, 22.7, 24.8, 25.7, 27.2, 27.2, 29.1, 29.2, 29.3, 29.5, 29.7, 29.8, 30.9, 31.3, 31.9, 33.8, 52.1, 56.8, 129.7, 130.0, 172.8, 173.1.

Methyl *N*-palmityl-L-serinate (**15a**)[24]

MM: 357.53 g mol^{-1}; white crystal; yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3H, *J* 6.9 Hz), 1.24 (s, 24H), 1.64 (q, 2H, *J* 7.3 Hz), 2.26 (t, 2H, *J* 8.0 Hz), 3.78 (s, 3H), 3.91 (dd, 1H, *J* 11.2, 3.2 Hz), 3.96 (dd, 1H, *J* 11.2 Hz, 4.0 Hz), 4.67 (m, 1H), 6.57 (d, 1H, *J* 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.6, 29.1, 29.2, 29.3, 29.5, 29.7, 29.7, 31.9, 36.5, 52.7, 54.6, 63.5, 171.1, 173.9.

Methyl *N*-stearyl-L-serinate (**15b**)[24]

MM: 385.59 g mol^{-1}; white crystal; yield: 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* 6.9 Hz), 1.25 (s, 28H), 1.64 (q, 2H, *J* 7.6 Hz), 2.26 (t, 2H, *J* 7.8 Hz), 3.78 (s, 3H), 3.91 (dd, 1H, *J* 11.2, 3.2 Hz), 3.96 (dd, 1H, *J* 11.2 Hz, 4.0 Hz), 4.67 (m, 1H), 6.49 (d, 1H, *J* 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.5, 29.2, 29.3, 29.5, 29.7, 31.9, 36.5, 38.6, 52.7, 54.7, 63.5, 171.1, 173.8.

Methyl *N*-oleyl-L-serinate (**15c**)[24]

MM: 383.57 g mol^{-1}; white crystal; yield: 35.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3H, *J* 6.8 Hz), 1.28 (d, 20H, *J* 15.8 Hz), 1.64 (q, 2H, *J* 7.4 Hz), 2.00 (m, 4H), 2.26 (t, 2H, *J* 7.4 Hz), 3.78 (s, 3H), 3.90 (d, 1H, *J* 10.8 Hz), 3.97 (d, 1H, *J* 8.0 Hz), 4.67 (m, 1H), 5.34 (m, 2H), 6.49 (d, 1H, *J* 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.6, 25.5, 27.1, 27.2, 29.1, 29.2, 29.2, 29.3, 29.5, 29.7, 29.7, 31.8, 36.4, 38.6, 52.6, 54.6, 63.4, 129.7, 130.0, 171.0, 173.7.

General procedure for the synthesis of fatty *N*-acylamino amides **20-22**

In a reaction flask, the fatty *N*-acylamino esters (**12-15**, 1.2 mmol, 1 equiv.) were added to a mixture of methanol/tetrahydrofuran (THF) 1:1 v/v cooled to 0 ºC. The aqueous solution of NaOH (0.4 mol L^{-1}) was added and stirred for 4 h. After, the solution was neutralized with the addition of an HCl solution (6 mol L^{-1}), and the white precipitate formed was solubilized and extracted with ethyl acetate.[24] Following, in the crude *N*-acylamino acids (**16-18**, 1.0 mmol, 1 equiv.) were added 10 mL of dichloromethane (CH_2Cl_2), TBTU (1.5 mmol, 1.5 equiv.) and DIPEA (1.5 mmol, 1.5 equiv.) and the solution was stirred at room temperature for 5 min. Then, 3,4-dichloroaniline (**2**, 2 mmol, 2 equiv.) was added, and the mixture was kept under constant stirring for 24 h at 25 °C. After completion of the reaction, the solvents were removed under reduced pressure, and the reaction crude was then subjected to purification by column chromatography, using silica gel as the stationary phase and mixtures of hexane and ethyl acetate as eluents.

N-(3,4-Dichlorophenyl), *N*-palmitylglycinamide (**20a**)

MM: 457.48 g mol^{-1}; white solid; yield: 69%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3H, *J* 7.0 Hz), 1.24 (s, 24H), 1.67 (q, 2H, *J* 7.5 Hz), 2.32 (t, 2H, *J* 7.5 Hz), 4.18 (d, 2H, *J* 5.0 Hz), 6.77 (t, 1H, *J* 4.6 Hz), 7.34 (d, 1H, *J* 8.7 Hz), 7.44 (dd, 1H, *J* 8.7 Hz, 2.3 Hz), 7.80 (d, 1H, *J* 2.3 Hz), 9.60 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.6, 27.1, 27.2, 29.1, 29.2, 29.2, 29.3, 29.5, 26.7, 29.8, 31.9, 36.3, 44.5, 118.9, 121.4, 127.4, 129.6, 130.0, 132.7, 137.5, 167.2, 174.6; HRMS (ESI) *m/z*, calcd. for $C_{24}H_{38}Cl_2N_2O_2$ $[M - 1]^-$: 455.2232; found: 455.2240.

N-(3,4-Dichlorophenyl), *N*-stearylglycinamide (**20b**)

MM: 485.53 g mol^{-1}; white solid; yield: 83%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3H, *J* 7.0 Hz), 1.25 (s, 26H), 1.67 (q, 2H, *J* 7.5 Hz), 2.31 (t, 2H, *J* 7.5 Hz), 4.17 (d, 2H, *J* 5.0 Hz), 6.78 (t, 1H, *J* 4.6 Hz), 7.34 (d, 1H, *J* 8.7 Hz), 7.44 (dd, 1H, *J* 8.7 Hz, 2.3 Hz), 7.80 (d, 1H, *J* 2.3 Hz), 9.60 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.6, 29.2, 29,3, 29.5, 29.7, 31.9, 36.3, 38.6, 44.5, 118.9, 121.4, 127.4, 130.4, 132.7, 137.5, 167.3, 174.6; HRMS (ESI) *m/z*, calcd. for $C_{26}H_{42}Cl_2N_2O_2$ [M – 1]$^-$: 483.2545; found: 483.2553.

N-(3,4-Dichlorophenyl), *N*-oleylglycinamide (**20c**)

MM: 483.52 g mol^{-1}; yellowish solid; yield: 48%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3H, *J* 7.0 Hz), 1.27 (d, 20H, *J* 14.0 Hz), 1.67 (q, 2H, *J* 7.2 Hz), 2.00 (q, 4H, *J* 6.1 Hz), 2.32 (t, 2H, *J* 7.5 Hz), 4.18 (d, 2H, *J* 5.0 Hz), 5.34 (m, 2H), 6.74 (t, 1H, *J* 4.6 Hz), 7.34 (d, 1H, *J* 8.7 Hz), 7.44 (dd, 1H, *J* 8.7, 2.3 Hz), 7.80 (d, 1H, *J* 2.3 Hz), 9.56 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.6, 27.1, 27.2, 29.1, 29.2, 29.2, 29.3, 29.5, 26.7, 29.8, 31.9, 36.3, 44.5, 118.9, 121.4, 127.4, 129.6, 130.0, 132.7, 137.5, 167.2, 174.6; HRMS (ESI) *m/z*, calcd. for $C_{26}H_{40}Cl_2N_2O_2$ [M – 1]$^-$: 481.2389; found: 481.2380.

N-(3,4-Dichlorophenyl), *N*-palmityl-L-alaninamide (**21a**)

MM: 471.51 g mol^{-1}; white solid; yield: 38%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 7H, *J* 7.0 Hz), 1.25 (s, 58H), 1.43 (d, 5H, *J* 7.0 Hz), 1.65 (m, 4H), 2.26 (m, 4H), 4.70 (q, 1H, *J* 7.0 Hz), 4.79 (m, 1H), 6.29 (d, 1H, *J* 7.3 Hz), 6.65 (s, 1H), 7.34 (m, 2H), 7.41 (m, 2H), 7.80 (d, 2H, *J* 2.3 Hz), 9.36 (s, 1H), 9.68 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 17.5, 18.5, 22.7, 25.6, 25.7, 29.2, 29.3, 29.5, 29.7, 31.9, 36.6, 40.6, 119.0, 121.5, 127.3, 130.4, 132.6, 137.6, 137.7, 170.6, 171.0, 174.0, 174.3; HRMS (ESI) *m/z*, calcd. for $C_{25}H_{40}Cl_2N_2O_2$ [M – 1]$^-$: 469.2389; found: 469.2397.

N-(3,4-Dichlorophenyl), *N*-stearyl-L-alaninamide (**21b**)

MM: 499.56 g mol^{-1}; white solid; yield: 71%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 7H, *J* 7.0 Hz), 1.25 (s, 26H), 1.43 (d, 3H, *J* 7.0 Hz), 1.65 (q, 2H, *J* 7.0 Hz), 2.28 (t, 2H, *J* 7.5 Hz), 4.78 (q, 1H, *J* 7.0 Hz), 6.55 (d, 1H, *J* 7.3 Hz), 7.33 (d, H, *J* 8.7 Hz), 7.43 (dd, 1H, *J* 8.6, 2.3 Hz), 7.81 (d, 2H, *J* 2.3 Hz), 9.62 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 18.3, 22.7, 25.7, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 36.6, 49.6, 119.0, 121.5, 127.3, 130.4, 132.6, 137.7, 170.9, 174.0; HRMS (ESI) *m/z*, calcd. for $C_{27}H_{44}Cl_2N_2O_2$ [M – 1]$^-$: 497.2702; found: 497.2772.

N-(3,4-Dichlorophenyl), *N*-palmityl-L-valinamide (**22a**)

MM: 499.56 g mol^{-1}; yellowish solid; yield: 56%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 6H, *J* 7.0 Hz), 0.94 (m, 9H), 1.24 (s, 50H), 1.64 (s, 3H, *J* 7.0 Hz), 1.83 (s, 1H), 2.12 (m, 1H), 2.23 (t, 1H, *J* 7.7 Hz), 2.29 (t, 2H, *J* 7.5 Hz), 4.59 (m, 1H), 6.59 (d, 1H, *J* 8.7 Hz), 7.32 (d, 1H, *J* 8.6Hz), 7.42 (dd, 1H, *J* 8.6, 2.3 Hz), 7.80 (d, 1H, *J* 2.3 Hz), 9.64 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 17,8, 18.4, 18.9, 19.2, 22.7, 25.7, 25.9, 29.2, 29.3, 29.5, 29.7, 31.3, 31.4, 31.9, 36.8, 52.1, 56.4, 59.1, 119.2, 121.6, 127.4, 130.3, 132.6, 137.5, 170.3, 174.0; HRMS (ESI) *m/z*, calcd. for $C_{27}H_{44}Cl_2N_2O_2$ [M – 1]$^-$: 497.2702, found: 497.2788.

N-(3,4-Dichlorophenyl), *N*-stearyl-L-valinamide (**22b**)

MM: 527.62 g mol^{-1}; yellowish solid; yield: 37%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (m, 29H), 1.2 (s, 97H), 1.63 (q, 7H, *J* 7.4 Hz), 2.14 (m, 3H), 2.23 (m, 6H), 4.58 (m, 3H), 5.97 (d, 2H, *J* 8.7 Hz), 6.51 (d, 1H, *J* 8.8Hz), 7.32 (d, 1H, *J* 8.6Hz), 7.42 (dd, 1H, *J* 8.6, 2.3 Hz), 7.81 (d, 1H, *J* 2.3 Hz), 9.53 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 17.8, 18.4, 18.9, 19.3, 22.7, 25.7, 25.8, 29.2, 29.3, 29.5, 29.7, 31.3, 31.9, 36.7, 52.1, 56.8, 59.1, 119.1, 121.6, 127.4, 130.3, 132.6, 137.5, 170.7, 172.8, 173.1, 174.0; HRMS (ESI) *m/z*, calcd. for $C_{29}H_{48}Cl_2N_2O_2$ [M – 1]$^-$: 525.3015, found: 525.3085.

N-(3,4-Dichlorophenyl), *N*-oleyl-L-valinate (**22c**)

MM: 525.60 g mol^{-1}; wax; yield: 24%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (t, 6H, *J* 7.0 Hz), 0.90 (t, 6H, *J* 6.5 Hz), 1.18 (s, 36H), 1.58 (m, 3H), 1.92 (m, 6H), 2.22 (t, 2H, *J* 7.4 Hz), 4.50 (m, 1H), 5.26 (m, 2H), 7.25 (d, 1H, *J* 8.6Hz), 7.35 (dd, 1H, *J* 8.6 Hz, 2.3 Hz), 7.73 (d, 1H, *J* 2.3 Hz), 9.54 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 18.4, 19.3, 22.7, 25.9, 27.2, 27.2, 29.1, 29.2, 29.3, 29.5, 29.7, 29.8, 31.4, 31.9, 36.8, 59.1, 119.1, 121.6, 127.4, 129.6, 130.0, 130.3, 132.6, 137.5, 170.3, 174.0; HRMS (ESI) *m/z*, calcd. for $C_{29}H_{46}Cl_2N_2O_2$ [M – 1]$^-$: 523.2858, found: 523.2851.

Solubility

The solubilities in water and organic solvents, hexane, dichloromethane, ethanol, ethyl acetate, methanol, and acetonitrile, compounds were performed according to the literature.[26] Approximately 0.1 g of sample was added to an Eppendorf, plus the addition of 1 to 3 mL of the respective solvent. After, stirring in a sonicator for 30 s the behavior of the samples was observed and recorded. A 0.1 g sample completely dissolved in 1 mL of solvent was considered to have "good solubility", and the term "limited solubility" indicated that 0.1 g of sample was completely dissolved in 3 mL of solvent. The term "low solubility" indicated that the sample could not be dissolved in 3 mL of solvent.

Differential exploratory calorimetry (DSC)

For thermal profile analyses, the compounds were submitted to differential scanning calorimetry. About 6 to 14 mg of samples were properly weighed in aluminum crucibles and later sealed in the recipients with the aid of a press. The metallic supports containing the sample were submitted to DSC analysis in a Netzsch DSC-200F3 oven, in a nitrogen (N_2) atmosphere, configured for cooling the samples to –40 °C, kept for 5 min, and followed by subsequent heating until reaching a temperature of 300 °C, at a temperature increase rate of 10 K min^{-1}, and also maintained at this temperature for a period of 5 min.

Toxicity of *N*-acylamino amides about strawberry pest arthropods

Breeding and maintenance of arthropods

The arthropods used for the bioassays came from the creations established by the Ângelo Moreira da Costa Lima laboratory (Department of Basic Pathology, UFPR), under controlled conditions (25 ± 2 °C, 70 ± 10% relative humidity and 14light: 10dark).

Specimens of *C. fragaefolii* were collected from 'San Andreas' strawberry crops in the municipality of Curitiba, State of Paraná, southern Brazil (31°38'20"S, 52°30'43"W; altitude 930 m) in August 2022. In a greenhouse, *C. fragaefolii* were multiplied in San Andreas strawberry plants protected by a rearing cage (2 m × 2 m × 2 m) lined with an anti aphid screen (0.64 mm × 0.20 mm). *Duponchelia fovealis* was established from larvae and adults collected in organic cultivation of 'Camino Real' strawberry in São José do Pinhais, Paraná, Brazil (25°37'05.32" S; 49°04'46" W, altitude 900 m) in 2010, with the introduction of insects collected from the field, at least once a year. The creation methodology proposed by Zawadneak *et al.*,[31] with the larvae fed an artificial diet and the adults a nutrient solution based on honey and beer. Breeding of *D. suzukii* was started with insects collected from 'Albion' strawberry fields in January 2018 in Curitiba, Paraná, Brazil (31°38'20"S, 52°30'43"W). In the laboratory, *D. suzukii* individuals were raised in glass containers (300 mL) with 12 mL of an artificial diet based on corn flour, yeast, and sugar, following the methodology proposed by Schlesener *et al.*[32]

Bioassays

The insecticidal toxicity of the synthesized substances was evaluated by the contact method on the insect (knock-down). For this, trefoils containing 2nd instar nymphs of *C. fragaefolii* were chosen and with the aid of a fine brush (No. 02), only ten 2nd instar nymphs were maintained. These leaflets were placed in glass containers (4 cm × 2.5 cm) containing distilled water to avoid loss of turgidity. For *D. fovealis*, 2nd instar caterpillars were selected and transferred to Petri dishes closed with Parafilm M® paper, which contained a 'Camino Real' strawberry leaflet for feeding. Meanwhile, adults of *D. suzukii* were separated and placed in transparent glass tubes (1.3 cm in diameter × 10 cm in length), closed at the top with hydrophilic cotton. Subsequently, the flies were transferred to a Petri dish (9 cm in diameter) lined with filter paper and sedated in ethyl ether for 40 to 60 s to apply the treatments. After spraying, the insects were placed in transparent plastic cages (1 L) and fed an artificial diet and distilled water throughout the evaluation period.

In each treatment, solutions (2 mL) diluted in dichloromethane were applied using a manual sprayer on the dorsal region of the insects, homogeneously. These treatments were also compared with the negative control, dichloromethane. All bioassays were performed under laboratory conditions of 25 ± 2 °C, 70% ± 10% RH, photophase 14:10 h (light:dark), maintained until the end of the evaluation, in a completely randomized design with 5 replications, with 10 insects for each repetition (n = 50). Insect mortality was evaluated every 24 h for 5 days. In all bioassays, insects that did not show motor movements were considered dead.

Generalized linear models of the exponential family of distributions were used to analyze studied variables.[33] The data were submitted to the Shapiro-Wilk test and, when necessary, data transformation (arcsine) was applied, and normality was verified, analysis of variance was used, using the *F* test. When a significant difference was observed between the treatments, means were compared by Tukey test, $P<0.05$).

Supplementary Information

Supplementary information (NMR selected spectra, DSC curves) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

All authors contributed to conceptualization, methodology, investigation, data curation, visualization, formal analysis, validation,

writing of original draft, and writing-reviewing and editing. Caroline Da Ros Montes D'Oca, Paulo Zarbin, and Maria Aparecida Cassilha Zawadneak also contributed with resources, supervision, project administration, and funding acquisition.

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