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Synthesis of new α-amino nitriles with insecticidal action on *Aedes aegypti* (Diptera: Culicidae)

Andrés G. Rueda[a], Aurora L. Carreño Otero[a], Jonny E. Duque[b,*], Vladimir V. Kouznetsov[a,*]

[a] Universidad Industrial de Santander, Escuela de Química, Laboratorio de Química Orgánica y Biomolecular, Santander, Colombia
[b] Universidad Industrial de Santander, Escuela de Medicina, Centro de investigaciones en enfermedades tropicales, Santander, Colombia

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ABSTRACT

Aedes aegypti is the principal vector of arboviral pathogens that may cause diseases as dengue fever, chikungunya and zika. The harmful environmental effects of commercial pesticides coalesced with the development of insecticide-resistant populations encourage the discovery and generation of new alternative products as a tool to reduce the incidence of vector-borne diseases. In this work, through the classic three component Strecker reaction of commercial benzaldehydes, cyclic secondary amines and KCN, a new series of nine α-amino nitriles, girgensohnine analogs, has been synthetized and screened for larvicide and adulticide properties against *A. aegypti*, one of the dominant vectors of dengue, chikungunya and zika in tropical and subtropical areas all over the world. Molecules **3** and **4** were identified as potential larvicidal agents with LC_{50} values of 50.55 and 69.59 ppm, respectively. Molecule **3** showed 100% of mortality after 2 h of treatment when a concentration of 30 ppm in adulticidal assays was evaluated. Additionally, in order to elucidate the mode of action of these molecules, their acetylcholinesterase (AChE) inhibitory properties were evaluated using the Ellman assay. It was found that the molecules possess a weak AChE inhibitory activity with IC_{50} values between 148.80 and 259.40 μM, indicating that AChE could not be a principal target for insecticide activity.

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Introduction

The arboviruses (arthropod-borne viruses) such as dengue (DENV), chikungunya (CHIKV) and zika (ZIKV) viruses are important causes of human diseases that affect public health in tropical and subtropical areas all over the world (Weaver and Reisen, 2010). According to the National Health Institute of Colombia, in 2017 approximately 23,000 cases of dengue, 1000 cases of Chikungunya and 1900 cases of Zika virus have been reported. The increase of the number of cases has positioned these diseases among the most worrisome for public health in different Latin America countries (Instituto Nacional de Salud INS, 2017).

Aedes (*Stegomyia*) *aegypti* (Linnaeus 1762) and *Aedes* (*Stegomyia*) *albopictus* (Skuse 1895) female mosquitoes are hematophagous insects that transmit pathogens to humans which may or may not cause diseases as the described above. The main symptoms of these virus infections are acute fever and polyarthralgia. Dengue infection is a serious disease and it is estimated that approximately 96 million cases are annually reported worldwide (Guzman and Harris, 2015; Simmons et al., 2012). CHIKV virus have an increasingly important impact in humankind morbidity, with potentially life-threatening and a painful arthritis. According to Staples and Fischer other symptoms are headache, myalgia, conjunctivitis, vomiting, and maculopapular rash (Staples and Fischer, 2014). ZIKV virus, like CHIKV virus, is an emerging arbovirus (Musso and Gubler, 2016; Weaver et al., 2016), which is widespread in neotropical regions with recent epidemics outbreaks in Africa, Asia, Europe and recently in America (Hayes, 2009; Ioos et al., 2014; Rodriguez-Morales, 2015; Zanluca et al., 2015). Zika infection is usually asymptomatic but in some cases rash, conjunctivitis and not very high fever can be observed. However, symptomatic ZIKV virus infection has been associated to Guillain-Barré syndrome (Cao-Lormeau et al., 2016) and neonatal microcephaly (Rasmussen et al., 2016).

Currently, there are no specific treatments, effective vaccines, or preventive drugs for these infectious diseases (Rashad et al., 2014; Shan et al., 2016; Stevens et al., 2009). Treatments are only palliative and include rest, hydration, analgesics, and antipyretics. Therefore, these virus infections are best prevented by avoiding the vector bites. Vector control methods involve strategies as the Integrated Vector Management promoted by WHO (2012). These activities

* Corresponding authors.
E-mails: jonedulu@uis.edu.co (J.E. Duque), kouznet@uis.edu.co (V.V. Kouznetsov).

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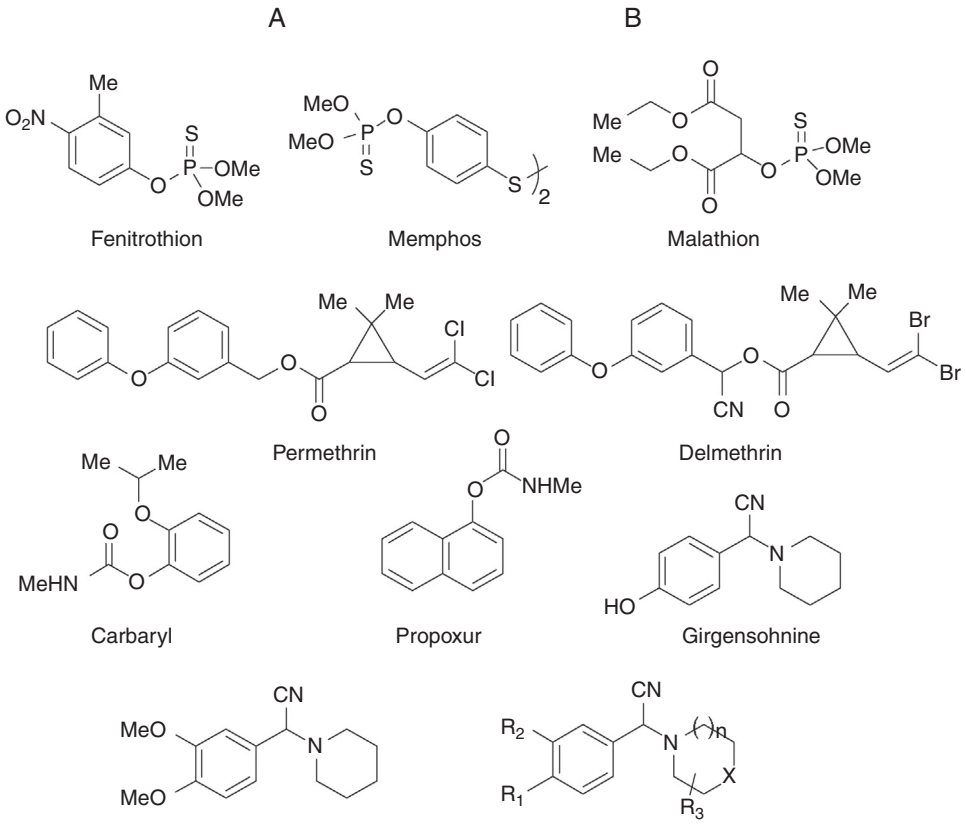


Fig. 1. Structures of insecticide agents and molecules studied in this work.

include the interruption of human-vector contact, environmental management, biological and chemical controls and self-initiative for individual and household protection. The use of repellents, insecticide-treated mosquito nets, aerosol insecticides, mosquito coils and even clothing for minimizing skin exposure and air conditioning to reduce mosquito bite are some of the most popular household strategies used (Kawada et al., 2014; Prajapati et al., 2005). These efforts have been shown not to be sufficient due mainly to the wide range of artificial and natural larval habitats that *A. aegypti* can use for its breeding. Clean water collected in man-made container in rural areas without public water service or for storage in drought periods, provide favorable conditions for the production of a large number of mosquito larvae and adults (Grisales et al., 2013).

Because different reasons, as those explained above, make impossible the elimination of mosquito breeding sites completely, the most common vector control alternative in stages of larvae and adults is the use of synthetic insecticides (larvicides and adulticides). Among them, organophosphorus larvicides (temephos), organophosphorus insecticides (malathion and fenitrothion), carbamates (carbaryl, propoxur) and pyrethroids (permethrin and deltamethrin) (Fig. 1), also toxic to mammals and harmful to the environment, are usually employed against *A. aegypti* mosquitoes and larvae (Braga and Valle, 2007).

However, resistance to insecticides, documented for more than 500 species of arthropods is one of the main problems in vector control, this phenomenon has been described to all these pesticides (Hemingway et al., 2004; Russell et al., 2004; Smith et al., 2016) and reported in several countries such as Colombia, Brazil and Malaysia (Aguirre-obando et al., 2015; Bona et al., 2016; Low et al., 2015). The increase in doses and frequencies of application are some of the issues to control, leading to preserve the effectiveness of commercial and new pesticides, this is why it is necessary to take into account that resistance is a multifactorial problem that involves environmental, operational and genetic factors (Guedes et al., 2017; Sparks and Nauen, 2015). For these reasons, it is particularly significant to know which are the modes of action of the insecticides and those changes that result in the resistance to the applied product (Lima et al., 2011). Furthermore, the use of new compounds against *A. aegypti* could inhibit various detoxifying enzymes at the same time, leading to not being easily identified by these systems and decreasing the risk of resistance (Carreño Otero et al., 2018). It is well known that action mode of organophosphates and carbamates is through acetylcholinesterase (AChE) inhibition whose decreased sensitivity is attributed to insecticide resistance (Pang, 2014), while pyrethroids are potent disrupters of voltage-sensitive sodium channels (Shafer et al., 2005). This selectivity of insecticides in the action mode is one of the reasons to produce new molecules that affect different targets in the insect.

On the other hand, it was proved that some plant secondary metabolites possess larvicidal and adulticidal activity against *A. aegypti* mosquitoes (Liu et al., 2016) and thus could serve as suitable prototypes for designing new bioactive molecules. We recently reported that alkaloid girgensohnine, a N-cianomethyl piperidine metabolite present in shrub *Girgensohnia oppositiflora* (Amaranthaceae) (Nahrstedt et al., 1993), exhibited moderate *in vitro* anti-AChE properties (IC_{50} = 93 μM), while its closer analog, 2-(3,4-dimethoxyphenyl)-2-(piperidin-1-yl) acetonitrile (α-amino nitrile **A**) (Fig. 1) showed reasonable *in vitro* AChE inhibition activity (IC_{50} = 45 μM) exhibiting *in vivo* larvicidal activity (LC_{50} = 88 ppm) on *A. aegypti* larvae (Carreño et al., 2014; Vargas and Kouznetsov, 2013).

Considering these previous results and the importance of control methods for increasing incidence of vector-borne diseases, this present work aimed to synthetize new series of α-amino nitriles **B** using three component Strecker reaction, assessing first

Table 1
Properties and yields of synthesized α-amino nitriles **3**–**11**.

Comp.	Yield (%)	Mp (°C)	MW (g/mol) ≤500	LogP ≤5	TPSA (Å) ≤90	H-bond donor ≤5	H-bond acceptor ≤10
3	66	78–79	230.31	2.674	36.264	0	3
4	88	95–96	244.33	2.915	36.264	0	3
5	82	87–88	274.36	2.505	45.498	0	4
6	81	88–89	258.32	2.794	45.498	0	4
7	66	57–58	244.33	3.004	36.264	0	3
8	91	94–95	274.36	2.594	45.498	0	4
9	68	80–81	258.32	2.838	45.498	0	4
10	63	Oil	216.28	2.169	36.264	0	3
11	57	79–80	232.28	1.612	45.498	0	4

Mp, melting point; MW, molecular weight (g/mol); LogP, n-octanol–water partition coefficient; TPSA, topological polar surface area; NER, number of rotatable bonds; H-bond donor, hydrogen bond donors (expressed as the sum of OHs and NHs); H-bond acceptor, hydrogen bond acceptors (expressed as the sum of Ns and Os).

their activity on *A. aegypti* larvae, then screening their activity on adults, and evaluating their anti-AChE properties. All this is in order to contribute to the development of new molecules with larvicides and adulticides properties against *A. aegypti* and to elucidate the mode of action of the obtained α-amino nitrile derivatives.

Materials and methods

In silico *evaluation of proposed compounds*

Before the synthesis of the girgensohnine analogs, a theoretical study was performed to predict their bioavailability properties, known as Absorption, Distribution, Metabolism, Excretion and Toxicity properties (ADME-Tox) (Lipinski et al., 2001). The Lipinsky's rules evaluate the physicochemical properties of the proposed structures such as molecular weight, partition coefficient (LogP), solubility, polar surface area (TPSA) and the number of rotatable bonds. For this study, Molinspiration and Osiris online calculation resources were used. Osiris predicts the toxicity of proposed molecules by comparing their structural fragments with those found in toxic compounds reported.

Equipment and purification of compounds

The melting points (uncorrected) were determined on a Fisher-Johns melting point apparatus (Flores-Conde et al., 2012). Infrared (FT-IR) spectra were recorded on a Lumex Infralum FT-02 spectrometer, ν_{max} in cm^{-1} (Ertürk et al., 2012). Bands are characterized according to the functional group. ^{1}H-NMR spectra were obtained with a Bruker AM-400 spectrometer (400 MHz). Data were reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets, td = triplet of doublets, qd = quartet of doublets, pd = pentet of doublets, m = multiplet), coupling constants (Hz) and proton assignation. ^{13}C-NMR spectra were obtained with a Bruker AM-400 (100 MHz) spectrometer with complete proton decoupling. Chemical shifts were reported in ppm (δ) relative to the solvent peak ($CDCl_3$, 7.24 ppm for ^{1}H and 77.23 ppm for ^{13}C). An Amazon X Bruker Datonics mass spectrophotometer with electrospray nebulization (ESI-MS) was used for MS identification. Elemental analyses were performed on a PerkinElmer 2400 Series II analyzer with theoretical values of ±0.4. The work-up for the reactions, extraction, and purification procedures in column chromatography were carried out using reactants and reagent grade solvents (purchased from Merck, Sigma–Aldrich and J.T. Baker). Thin-layer chromatography (TLC) was performed using Silufol UV254 precoated plates (0.25 mm). UV light of 254 nm was used to observe components and iodine vapor was used for revealing. Column chromatography was performed using neutral aluminum oxide column as solid support (Al_2O_3 neutral active 90, 70–230 Mesh, Merck) using as eluents solvent mixtures of petroleum ether e and ethyl acetate.

General procedure for preparation of the title compounds

Using a 50 mL round-bottom flask, benzaldehydes **1** (10 mmol) and secondary amines **2** (13 mmol) were dissolved with a magnetic stirrer in acetonitrile (10 mL) and kept stirring for 30 min at room temperature. Subsequently, KCN (0.35 g, 15 mmol) and 0.80 g of SSA catalyst were added to the flask. Then, the resulting suspension was stirred for 18 h (TLC control) and filtered. The resulting filtrate was concentrated using a rotary evaporator and the obtained mass was purified with alumina column chromatography (Al_2O_3) eluting with different concentrations of petroleum ether: ethyl acetate to obtain pure α-amino nitrile compounds **3**–**11** (Table 1).

In vivo *evaluation of insecticidal action on* A. aegypti *larvae and adults*

Larvae between third and fourth instar of Rockefeller strain were used, these were reared in CINTROP laboratory, keeping in plastic containers with a temperature of 25 °C ± 5 °C, wet conditions of 80 ± 5% and a photoperiod of 12:12 h. Reaching the third instar, larvae were chosen for the assay beginning with exploratory dosage of three concentrations between 50 and 300 ppm of synthesized molecule dissolved in dimethylsulfoxyde (DMSO) at 1%. For this assay, fifteen larvae were added in each plastic recipient with 50 mL of water using three replications for each concentration and a negative control with DMSO, mortality was registered after 24 and 48 h. For those molecules with highest mortality value, multiple dosage assays were applied, using six concentrations assigned in an asymmetric way and below the dosage when most of the larvae were death in exploratory dosage assay. In these dosages, ten larvae were added in each recipient with 100 mL of water and mortality was registered after 24 and 48 h. Four replicates, a negative control with DMSO and a positive control with the insecticide Temephos were used in this assay (Hemingway, 2005).

For those molecules with the highest values of mortality in multiple dosage larvae assay, adulticidal assays were developed using the CDC bottles protocol of Brogdon and Mcallister. Three replicates for each synthesized molecule concentration (30, 300 and 1000 ppm) and a negative control with acetone were tested. The sides and bottom of the bottles were impregnated with three concentrations of synthesized molecule dissolved in acetone and four replicates for each concentration were made. Solvent used was allowed to dry overnight before the test. Ten adults were added to each bottle and each bottle was read for 2 h started testing each 15 min and a final check within 24 h (Brogdon et al., 2005).

In vitro *AChE inhibition assay*

For AChE inhibition evaluation, a modified protocol of Ellman colorimetric assay with commercial Sigma–Aldrich® *Electrophorus electricus* AChE was used. This method determines the production of thiocholine caused by hydrolysis of acetylthiocholine, followed by its reaction with 5,5-dithiobis-2-nitrobenzoate ion (DTNB), confirming the development of reaction with a yellow color (Ellman et al., 1961).

Statistical analysis

Evaluation on *A. aegypti* larvae: LC_{50}, LC_{98} and confidence limits values were calculated with Probit analysis (Ashford and Sowden, 1970). For the evaluation on *A. aegypti* adults: Data was tabulated and subjected to a normality test. When the data in each concentration were normal, an ANOVA test was carried out. If the distribution was not normal, non-parametric tests were conducted. The results were analyzed by Kruskal–Wallis and Lilliefors method using Statistica v11 software. Only data with $p < 0.05$ were considered significant.

Results and discussion

In silico *evaluation of the proposed compounds*

Prior to synthetizing desired molecules, their physico-chemical parameters were evaluated, employing the Lipinski's rule (Lipinski, 2001). All products reported molecular weights below 500 g/mol (216.28–274.36 g/mol), a partition coefficient below 5 (LogP 1.612–3.004), hydrogen bond donors (expressed as the sum of OHs and NHs) less than 5 hydrogen bond acceptors (expressed as the sum of Ns and Os) lees than 10 and a polar surface area below 90 Å (36.264–45.498 Å). The obtained results showed that the proposed α-amino nitriles have pharmacokinetic profiles, and fulfill all parameters established. As shown in Supporting information (Table S1); no risk of toxicity was predicted for all compounds. With these results, synthesis of all proposed compounds was performed.

Obtention of title compounds

One of most common and simplest methods for the preparation of α-amino nitriles is the direct three component reaction of aldehyde, amine and potassium cyanide known as Strecker reaction (Wang et al., 2011; Strecker, 1850; Shafran et al., 1989; Otto and Opatz, 2014). As α-amino nitriles are important for synthesizing useful α-amino acids in both laboratory and industrial scale, Strecker-type reactions have been studied during the last few years looking mainly for new and better catalyst systems and cyanide sources. Looking for literature survey in Strecker synthesis, we could note that methods reported for this reaction often require not only the use of expensive reagents, and hard work-up as high catalyst loadings, high temperatures and pressures, but also involve long reaction times and tedious post-reaction procedures (Brahmachari, 2016). Moreover, all these reactions are carried out for small-scale α-amino nitrile preparation. Taking into consideration the latter comment and that the reported *in vivo* insecticidal assays need a large amount of substances, our attention addressed to a simple protocol, which involves the use of classical inorganic cyanide source KCN in the presence of sulfuric acid supported on silica gel (SSA, SiO_2–O–SO_3H), as a robust procedure proceeding under mild reaction conditions. In order to obtain the desired **3**–**11** α-amino nitriles (series **B**), commercial aldehyde components **1** (*p*-anisaldehyde, piperonal and 3,4-dimethoxybenzaldehyde) and amine components **2** (piperidine, pyrrolidine, morpholine, 2-methylpiperidine and 4-methylpiperidine) were chosen. Thus, a mixture of benzaldehydes **1**, secondary amines **2**, and KCN was stirred in the presence of SSA in acetonitrile at room temperature for 18 h (Scheme 1).

These mild reaction conditions allowed the large-scale preparation of the needed products as stable white crystal solids purified by flash column chromatography on alumina, using petroleum ether/ethyl acetate as eluent (Table 1). Structure of the obtained compounds **3**–**11** was confirmed by common spectral methods. The obtained α-amino nitriles showed in the IR spectra the characteristic CN bands appearing in the region of 2190–2222 cm^{-1}. Their structures were also confirmed with their ^1H-, ^{13}C-NMR and bidimensional experiments (COSY, HSQC), and supported by the mass spectrometric data (Supporting information, General Methods). Compounds **4**–**9** are diastereoisomeric mixtures, which resulted to be inseparable by conventional column chromatography, and were proceed to be tested as an enantiomeric mixture. All the α-amino nitriles obtained are moderate lipophilic compounds ($1.61 < LogP < 3.00$) with favorable values of TPSA (36.26–45.49) for agrochemical substances (Table 1). Having pure nine α-amino nitriles **3**–**11** with suitable pharmacokinetic profiles, we began their screening on *A. aegypti* mosquitoes and larvae.

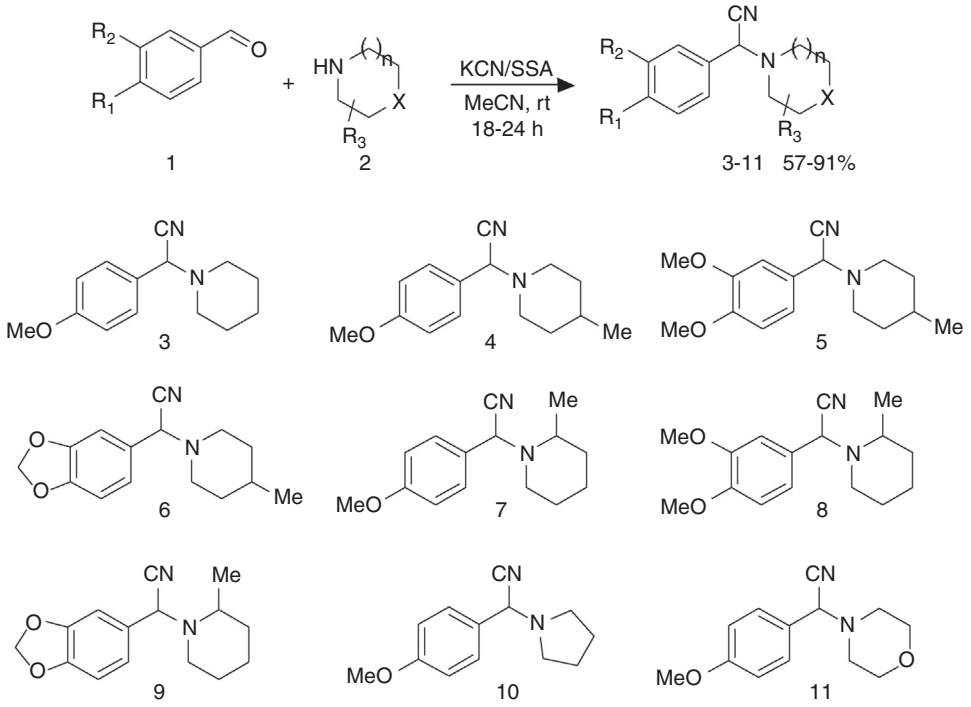
In vivo *evaluation of insecticidal activity on* A. aegypti *larvae and adults*

Following by published protocols (Carreño et al., 2014), larvae between third and fourth instar of *A. aegypti* were exposed to different concentrations of the obtained girgensohnine analogs **3**–**11** (range of 300–50 ppm). These diagnostic tests looked for the higher mortality rates. Larvae mortality produced by comp. **3**–**11**, dissolved in DMSO at 1%, was determined after 24 and 48 h, using fifteen larvae in each triplicated experiment. Analyzing results obtained (Table 2), it could be concluded that: (1) all compounds tested were active at the highest concentration (300 ppm) registering between 93 and 100% of larvae mortality, (2) no mortality was observed in control evaluation, (3) only three α-amino nitriles (comp. **3**, **4** and **7**) conversed high larvicidal activity (100% of larvae mortality) at concentration of 120 ppm, (4) at a concentration of 70 ppm compound **4** still killed all fifteen larvae, and (5) in contrast of compounds **3** and **10** that reported high mortality at the lowest dose, all molecules reported low mortality at a concentration of 50 ppm). Having these preliminary biological data, it could discuss on α-amino nitrile structure – larvicidal activity relationship. Noteworthy, all three active comp. **3**, **4** and **7** possess *para*-methoxy group on aryl ring and piperidine or methyl-piperidine skeleton.

Incorporation of another methoxy substituent or dioxymethylen fragment into aryl ring of α-amino nitrile derivatives (comp. **5**, **6**, **8** and **9**) decreased insecticide activity. α-Amino nitriles containing *p*-methoxyphenyl moiety (comp. **10** and **11**) were inactive, thus chemical nature of cyclic amine is also important factor (piperidine skeleton *vs* pyrrolidine and morpholine rings).

Thus, the multiple dosage assays were applied for molecules **3** and **4**, which showed the highest larvicidal activity, calculating their lethal concentrations (LC_{50} and LC_{98} values) with Probit analysis (Table 3). The chi-square values were significant at $p < 0.05$ level. Possessing respective LC_{50} values of 50.55 and 69.59 ppm, these two compounds resulted to be better than reported early α-amino nitrile **A** (Fig. 1) and could be considered as suitable models for developing new agents against *A. aegypti* larvae (Dias and Moraes, 2013).

It should be commented that larvae mortality and their morphologic changes (darkness of the body and decreasing of their size) were observed 2 h after starting the bioassay. Although their LC_{50} values are higher than those reported for commercial insecticides (temephos, $LC_{50} = 0.0059$ ppm) (Harris et al., 2010),



Scheme 1. Preparation of new α-amino nitriles **3**–**11** by modified Strecker reaction.

Table 2
Larvicidal activity of compounds (Comp.) **3**–**11** on *A. aegypti* larvae after 24 and 48 h.

Comp.	Concentrations, ppm				Control (DMSO)	Time of exposure
	300	120	70	50	L/D	
	L/D[a]	L/D	L/D	L/D		
3	0/15	0/15	7/8	8/7	15/0	
4	0/15	0/15	0/15	14/1	15/0	
5	0/15	11/4	13/2	14/1	15/0	
6	0/15	14/1	12/3	13/2	15/0	24 h
7	0/15	0/15	10/5	12/3	15/0	
8	0/15	7/8	14/1	15/0	15/0	
9	0/15	5/10	14/1	14/1	15/0	
10	1/14	8/7	nt[b]	10/5	15/0	
11	0/15	5/10	nt	13/2	15/0	
3	0/15	0/15	6/9	7/8	15/0	
4	0/15	0/15	0/15	12/3	15/0	
5	0/15	10/5	12/3	12/3	15/0	
6	0/15	12/3	11/4	13/2	15/0	48 h
7	0/15	0/15	10/5	12/3	15/0	
8	0/15	4/11	13/2	14/1	15/0	
9	0/15	5/10	15/0	14/1	15/0	
10	1/14	7/8	nt	10/5	15/0	
11	0/15	4/11	nt	14/1	15/0	

[a] ·L/D, live/death.
[b] Not tested.

Table 3
LC_{50} values for compounds (Comp.) **3**, **4** and temephos®.

Comp.	**3**		**4**		Temephos
	24 h (confidence limits)	48 h (confidence limits)	24 h (confidence limits)	48 h (confidence limits)	24 h (confidence limits)
LC_{50}[a] (ppm)	50.55 (48.13–52.98)	48.53 (46.18–50.94)	69.59 (66.01–73.32)	64.51 (61.32–67.79)	0.0021 (0.0019–0.0022)
LC_{98} (ppm)	86.39 (79.02–97.77)	82.18 (74.21–95.81)	139.54 (125.51–160.76)	122.07 (111.09–138.24)	0.0057 (0.0046–0.0080)
χ^2[b]	3.77	2.63	4.50	3.78	8.01
AI[c]	8.82 ± 0.82	8.98 ± 0.99	6.79 ± 0.55	7.42 ± 0.58	9.21 ± 0.56

[a] LC, lethal concentration.
[b] Chi-square.
[c] Slope standard deviation.

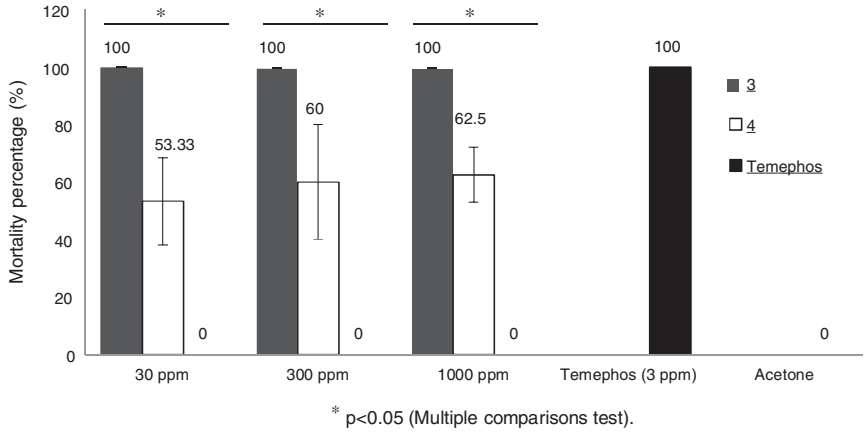


Fig. 2. Mortality percentage on *A. aegypti* adults of α-amino nitriles 3,4 after 2 h started the assay.

these α-amino nitriles showed in the *in silico* analysis good pharmacokinetic properties and low toxicity compared to temephos (Supporting information, Table S1). Evaluation of larvicidal activity using products extracted from plants have been studied by several authors. Low LC_{50} values such as those found for *Croton zehntneri* (LC_{50} = 26.2 ppm) and *Croton nepetaefolius* (LC_{50} = 66.4 ppm) essential oils have been reported (Pacelli et al., 2013), furthermore, Seo et al. (2015) evaluated the constituents of Apiaceae essential oils against *Aedes albopictus*, revealing for carvacrol a larvicidal activity of 80% at a concentration of 50 ppm and an IC_{50} of 57 ppm, showing a correlation between larvae mortality and acetylcholinesterase activity for this constituent of ajowan plant (*Trachyspermum ammi*), in spite of this activity, carvacrol is found in ajowan plant just in 0.55%. Although the active compounds found in these works showed larvicidal activity in a concentration range comparable to those previously reported for essential oils, it should be noted that these synthetic compounds can be produced in considerable quantities with high purity and that also their *in silico* analysis indicates a low toxicity in mammals.

Molecules **3** and **4**, with the highest mortality values in larvae assay, were evaluated according to Brogdon and Mcallister method (Brogdon et al., 2005). Results showed that 2-(4-methoxyphenyl)-2-(piperidin-1-yl) acetonitrile **3** had the highest adulticidal activity with 100% of mortality at 300 ppm. In contrast, 2-(4-methoxyphenyl)-2-(4-methylpiperidin-1-yl) acetonitrile (**4**) presented more than 50% of mortality at the same concentration (Fig. 2).

Calculated probability values in Kruskal–Wallis test confirmed significant differences between the concentrations used in this experiment with probability values below 0.05 at the concentrations of 300 ppm and 1000 ppm. After that, AChE inhibition properties of the obtained α-amino nitriles **3**–**11** were evaluated looking for some relationship between enzymatic and larvicidal activities.

In vitro *AChE inhibition assay*

Evaluation of enzyme inhibitory capacity of these compounds was performed by Ellman assay (Ellman et al., 1961), in which acetylcholinesterase from *Electrophorus electricus* (EC 3.1.1.7, Type VI-S) was used (Carreño et al., 2014). According to these results (Supporting information, Table S2), the synthesized compounds possess weak anti-AChE activity with IC_{50} values between 36.31 and 60.25 ppm (148.80–259.40 μM). It could be noted that 2-(4-methoxyphenyl)-2-(4-methylpiperidin-1-yl) acetonitrile **4** resulted to be the more active compound, followed by 2-(4-methoxyphenyl)-2-(2-methylpiperidin-1-yl)acetonitrile **7**. It was also observed that pyrrolidine or morpholine rings (comp. **10** and **11**) did not contribute in AChE inhibition activity. Taking into consideration that agrochemical Propoxur, insecticide and acetylcholinesterase inhibitor, exhibited IC_{50} 0.0150 ppm and comparing results of enzyme and insecticide activities, it was observed that more active comp. **3** against *A. aegypti* larvae (LC_{50} 50.55 ppm) showed IC_{50} 60.25 μg/mL, while another active comp. **4** (LC_{50} 69.59 ppm) exhibited an IC_{50} value of 36.31 ppm that means there is not relationship between AChE inhibition activity and larvicidal activity of the tested compounds.

Conclusion

New α-amino nitriles analogs of alkaloid girgensohnine were designed and synthetized. These compounds were obtained in good yields under mild conditions as stable white powders with defined melting points. Prior to synthetic and enzymatic studies ADME parameters of these molecules were calculated revealing their acceptable pharmacokinetic profiles showing *in silico* favorable physicochemical properties and a low risk of toxicity for humans. Their insecticide action tested on mosquito larvae reported mortality at concentrations below 120 ppm, highlighting compounds **3** and **4** with LC_{50} values of 50.55 and 69.59 ppm, respectively. Insecticide action test on mosquitoes showed a mortality of 100% for compound **3** and a 53.33% of mortality for compound **4** when a concentration of 30 ppm was evaluated. Additionally, it was found that these molecules possess weak anti-AChE activity with values of IC_{50} = 36.31–60.25 ppm (148.80–259.40 μM) indicating that AChE enzyme could not be a principal target responsible for their insecticide activity and that the evaluated molecules would present another mechanism or mode of action on insects. With these biological results, α-amino nitriles **3** and **4** could be considered as suitable models for developing new agents against *A. aegypti* larvae as insecticide candidates. Further investigations on detailed biochemical mechanism of action, as detoxifying enzyme evaluations, are now under way in our laboratories and their results will be published elsewhere.

Conflicts of interest

The authors declare no conflict of interest concerning this work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rbe.2018.01.004.

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