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Design, synthesis, acetylcholinesterase inhibition and larvicidal activity of girgensohnine analogs on *Aedes aegypti*, vector of dengue fever

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ABSTRACT

Girgensohnine alkaloid was used as a natural model in the design and generation of new alkaloid-like α-aminonitrile series that was completed by the use of SSA-catalyzed Strecker reaction between commercial and inexpensive substituted benzaldehydes, piperidine (pyrrolidine, morpholine and N-methylpiperazine) and acetone cyanohydrin. Calculated ADMETox parameters of the designed analogs revealed their good pharmacokinetic profiles indicating lipophilic characteristics. *In vitro* AChE enzyme test showed that obtained α-aminonitriles could be considered as AChEIs with micromolar IC_{50} values ranging from 42.0 to 478.0 μM (10.3–124.0 μg/mL). Among this series, the best AChE inhibitor was the pyrrolidine α-aminonitrile **3** (IC_{50} = 42 μM), followed by the piperidine α-aminonitriles **2** and **6** (IC_{50} = 45 μM and IC_{50} = 51 μM, respectively), and the compound **7** (IC_{50} = 51 μM). *In vivo* insecticidal activity of more active AChEIs against *Aedes aegypti* larvae was also performed showing a good larvicidal activity at concentrations less than 140 ppm, highlighting products **2** and **7** that could serve as lead compounds to develop new potent and selective insecticides.

1. Introduction

Vector-borne infectious viral diseases, transmitted to human by the bite of arthropod vectors constitute a significant proportion of the global infectious disease burden. Among many arthropods, diverse and numerous insects mosquitoes are a major public health problem as vectors of serious human diseases like malaria, filariasis, dengue fever, dengue hemorrhagic fever and yellow fever. *Aedes aegypti* mosquitoes are the major vector of dengue fever, an endemic disease in America and Asia and represent a serious threat worldwide in terms of public health affecting 40–80 million people every year [1]. Dengue virus belongs to the *Flavivirus genus* of the Flaviviridae family and is primarily transmitted by *Aedes* mosquitoes. In the absence of specific antiviral drugs or efficient vaccine, the *A. aegypti* control methods are therefore critical [2,3]. However, most methods for *A. aegypti* mosquitoes control rely on the use of chemical insecticides, mainly belonging to the organophosphate, carbamate and pyrethroid classes. Subsequently, resistance of mosquito populations to these chemicals is increasing at a dramatic rate, threatening the efficacy of any control methods. According to these facts, there is a continuous need to explore new active molecules with different mechanisms of action [4–8].

Among diverse mechanisms of action, the insect enzyme acetylcholinesterase (AChE) inhibition stands out a promise insecticides control method. Inhibitors of this type of mechanism (for ex., organophosphate and carbamate chemicals) affect the transmission of nerve impulses accumulating acetylcholine in neuromuscular tissue of insects causing paralysis and then death [7]. Therefore, the discovery of insect AChE inhibitors (AChEIs) is an important task [9,10] where insecticide design based on natural molecule findings play a key role.

So, with these facts in mind and as a part of our medicinal research program directed to the development of small molecule

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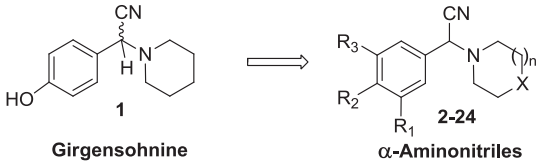


Fig. 1. α-Aminonitriles design inspired by natural molecule, girgensohnine alkaloid.

drug discovery, we started preliminary study on this topic paying attention to an infrequent structure of girgensohnine alkaloid (Fig. 1). The girgensohnine **1**, racemic product is a cyanogenic metabolite extracted with a yield of no more than 0.05% from *Girgensohnia oppositiflora* (Amaranthaceae), shrub that grows in the Russia and Iran deserts [11]. This is one of the unusual cases of cyanogenic compounds with a piperidine ring without a glycosidic linkage in their core structure [12]. Although their structural elucidation and synthesis were reported in 1946, there was not mention in the literature about its bioactivities until our preliminary work [13]. The scarcity of this alkaloid in the natural source, unknown biological properties and at the same time their simple structure, all this was motivated to undertake this study of obtaining new similar molecules with insecticidal activity, which belong to an α-aminonitrile chemical class of compounds (Fig. 1).

According to the accounts described above, our study focused in: *i*) design α-aminonitriles from girgensohnine structure; *ii*) determine and analyze their *in silico* ADME properties, based on the Lipinski's rule; iii) prepare a small library of new girgensohnine alkaloid analogs (α-aminonitriles); *iv*) estimate *in vitro* AChE inhibition of the obtained molecules, and *v*) evaluate *in vivo* insecticidal activity against *A. aegypti* larvae of the most active molecules in AChE inhibition process. All this, are in order to contribute in lead compounds identification to develop new potent and selective insecticides based on this α-aminonitrile class inspired by girgensohnine structure.

2. Results and discussion

2.1. Chemistry

In this study, for new girgensohnine analogs obtaining we chose easily the Strecker reaction that provides diverse α-aminonitriles in an efficient manner using aromatic aldehydes, amines (mainly, cyclic secondary amines) and a cyanide source [14]. A rich set of aromatic aldehydes and cyclic secondary amines is available from different chemical companies and varying them it could be generated numerous α-aminonitriles.

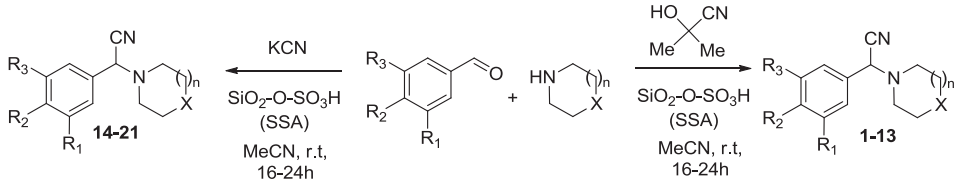
However, we limited our possible reagents list to six benzaldehydes (4-hydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde and piperonal) and four cyclic secondary amines (piperidine, pyrrolidine, morpholine and N-methylpiperazine) based on *in silico* ADME profiles evaluation of new 24 α-aminonitriles, close analogs of girgensohnine alkaloid. So, prior to synthetizing desired molecules, their physico-chemical parameters were evaluated, employing the Lipinski's rule [15–17]. It should be noted that these types of physicochemical parameters and some additional (topological polar surface area, number of rotatable bonds, water solubility, and dissociation constant, etc.) have proven useful for predicting the biological properties not only new pharmacological agents, but also new agrochemical substances [18,19]. The obtained results showed that the proposed α-aminonitriles **1-24** including girgensohnine alkaloid have pharmacokinetic profiles, and fulfill all parameters established by this rule (molecular weight = 202.03–305.37, logP = 0.71–2.22, nON = 2–6, and nOHNH = 0–4) (Table 1). In general, it can be seen that the α-aminonitriles **1-24** are lipophilic molecules showing clogP values below 2.5.

Next, planning their preparation, we had to select cyanide sources and catalysts to perform three-component Strecker reaction. Regarding these aspects, this reaction has been studied

Table 1
Calculated physico-chemical parameters for proposed α-aminonitriles **1**–**24**.

Molecules	MW	H-bond donor	H-bond acceptor	LogP, average	TPSA Å^2	NER	LogS average	pK_a
	≤500	≤5	≤10	≤5	≤140	≤10	≥ −4.0	
1	216.2	1	3	2.09 (±0.38)	47.26	3	−2.0	4.2 ± 0.4
2	260.3	0	4	2.22 (±0.52)	45.49	5	−2.8	4.0 ± 0.4
3	246.3	0	4	1.82 (±0.47)	45.49	5	−2.3	4.3 ± 0.4
4	262.3	0	5	0.94 (±0.54)	54.72	5	−1.9	2.2 ± 0.5
5	275.3	0	5	1.05 (±0.57)	48.73	5	−1.5	7.6 ± 0.4
6	244.2	0	4	0.97 (±0.46)	45.49	3	−2.6	4.0 ± 0.4
7	230.2	0	4	1.87 (±0.43)	45.49	3	−2.3	4.3 ± 0.4
8	246.2	0	5	0.97 (±0.41)	54.72	3	−1.9	2.2 ± 0.5
9	259.3	0	5	1.09 (±0.46)	48.73	3	−1.6	7.6 ± 0.4
10	290.4	0	5	2.10 (±0.61)	54.72	6	−2.9	3.9 ± 0.4
11	276.3	0	5	1.70 (±0.56)	54.72	6	−2.5	4.2 ± 0.4
12	292.3	0	6	0.83 (±0.62)	63.95	6	−2.0	2.1 ± 0.5
13	305.4	0	6	0.94 (±0.66)	57.96	6	−1.7	7.6 ± 0.4
14	246.3	0	4	1.98 (±0.48)	56.49	4	−2.2	4.0 ± 0.4
15	232.3	1	4	1.60 (±0.44)	56.49	4	−1.8	4.3 ± 0.4
16	248.3	1	5	0.71 (±0.51)	65.72	4	−1.4	2.2 ± 0.5
17	261.3	1	5	0.83 (±0.55)	59.73	4	−1.1	7.7 ± 0.4
18	246.3	1	4	1.99 (±0.48)	56.49	4	−2.2	4.1 ± 0.4
19	232.3	1	4	1.60 (±0.44)	56.49	4	−1.8	4.4 ± 0.4
20	248.3	1	5	0.71 (±0.51)	65.72	4	−1.4	2.3 ± 0.5
21	261.3	1	5	0.83 (±0.55)	59.73	4	−1.1	7.7 ± 0.4
22	202.3	1	3	1.68 (±0.37)	47.26	3	−1.6	4.5 ± 0.4
23	218.3	1	4	0.80 (±0.43)	56.49	3	−1.2	2.4 ± 0.5
24	231.3	1	4	0.94 (±0.50)	50.50	3	−1.0	7.7 ± 0.4

MW: Molecular weight (g/mol); 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); cLogP: n-octanol–water partition coefficient; TPSA: topological polar surface area; NER: number of rotatable bonds; LogS: aqueous solubility; pK_a: dissociation constant.



Scheme 1. Preparation of girgensohnine analogs **1**–**24**.

extensively by using different Lewis acids [20–22] or bases [23,24], and metal–salen complexes [25] as catalysts, and NaCN, KCN, trimethylsilyl cyanide, acetone cyanohydrin (AC) as main cyanide sources. Furthermore, catalyst-free and promoted-sulfuric acid supported on silica gel (SSA) three-component Strecker reactions were reported [26,27], while first syntheses of the girgensohnine were performed using KCN/$NaHCO_3$/H_2O (56% yields) system [11] and NaCN/5% mol $InCl_3$/MeCN (76% yields) system [13]. Additionally, SSA catalyst is recoverable and reusable material with easy handling, low toxicity and wide range of solvent tolerance and acetone cyanohydrin is considered as available, cheap, safe and green organic cyanide source. With this background and in search for the most safe and environmentally friendly protocol to prepare the desired compounds, we considered that AC and SSA should be a good choice of appropriated reaction conditions for planned three-component Strecker reactions. However, to compare the chosen green AC/SSA protocol (method **A**), classical inorganic cyanide source KCN in presence of SSA (KCN/SSA protocol, method **B**) was also used in our investigation. Both synthetic protocols were performed in MeCN at room temperature over 16–24 h.

Thus, by selected and justified reaction conditions, racemic α-aminonitriles **1**–**13** including girgensohnine alkaloid were synthesized following by the method **A** procedure, while α-aminonitriles **14**–**21** with 4-hydroxy-3-methoxy- or 3-hydroxy-4-methoxy-aryl moieties were obtained using reaction conditions of the method **B** (Scheme 1); only three designed molecules **22**–**24** could not be prepared under reaction conditions of both synthetic protocols [28].

In general, all molecules **1**–**21** were prepared with moderate to excellent yields presenting the well-defined melting points (Table 2).

Structural elucidation of the obtained molecules **1**–**21** was made using diverse spectroscopic techniques (FT-IR, GC-EM, ^{1}H NMR and ^{13}C NMR) that identify easily main spectral features (group CN, α cyano group hydrogen, aromatic and N-heterocyclic protons) of these compounds.

2.2. Bioactivity assessments

2.2.1. In vitro AChE inhibition

The limited number of commercially available insecticides that affect a smaller number of enzymes in insects and the resistance to these agrochemicals patronizes and promotes the study of new AChE inhibitors systems. AChE inhibitors (AChEIs) as pesticides, especially against insects and other arthropod vertebrates, are used in control of disease vectors, *e.g.* in dengue fever or malaria control. Insecticidal activity is based on the overstimulation of the cholinergic system in the insect. The original AChEIs used were derived from compounds developed from organophosphorus war gas agents that bind irreversibly to AChE. Although the AChE found in insects are less investigated than mammalian AChE or AChE of the electric eel *Torpedo californica*, insect AChEIs are important targets for some insecticides, which are based on AChE inhibition [29]. In this work, all the synthesized compounds **1**–**21** were screened using Ellman colorimetric method-based AChE inhibition assay [30,31]. Analyzing the biochemical data consigned in Table 3, it is reasonable safe to suggest that AChE inhibitory activity depends considerably on chemical nature of both substituents on aryl moieties and cyclic aminyl fragments in α-aminonitrile structure. It was found that all obtained molecules are considered as AChEIs with micromolar IC_{50} values ranging from 42.0 to 478.0 μM (10.3–124.0 μg/mL) noting that the inclusion of two electron-donating group methoxy or dioxymethylen ring on the aryl fragments in α-aminonitriles **2**–**4** and **6**–**8** (but not in α-aminonitriles **5**,**9**) improved significantly AChE inhibition comparing with the girgensohnine alkaloid **1** activity that showed moderate AChE inhibition (IC_{50} = 93 μM), less than its analogs **2-4**,**6-8** and similar to AChE inhibitory activity of the rest molecules **5**, **9**–**21**. Among this series, the best AChE inhibitor was the pyrrolidine α-aminonitrile **3** (IC_{50} = 42 μM), followed by the piperidine α-aminonitriles **2** and **6** (IC_{50} = 45 μM and IC_{50} = 51 μM, respectively), and the compound **7** (IC_{50} = 51 μM). Surprisingly, it was found that the series of the α-aminonitriles **10**–**21** with 3,4,5-trimethoxy- and 4-hydroxy-3-methoxy- or 3-hydroxy-4-methoxy-aryl moieties did not show appreciable activity and the series of the N-methylpiperazine α-aminonitriles **5**,**9**,**13**,**17** and **21** resulted worse AChEIs (IC_{50} = 340.2–475.0 μM).

Thus, it can conclude that modifications in the size and type of nitrogen heterocycles as well as the inclusion of electron-donating groups (MeO) on the aryl fragments in α-aminonitrile structures influenced considerably *in vitro* AChE inhibition and consequently, could be affected *in vivo* insecticide activity. Finally, we attempted

Table 2
SiO_2–OSO_3H catalyzed the Strecker reaction of heterocyclic amines and aldehydes for synthesis of **1**–**24**.

Comp.	R_1	R_2	R_3	X	*n*	Mp, °C	Yield, %	
							Method A	Method B
1	H	HO	H	CH_2	1	115–117	73	68
2	H	MeO	MeO	CH_2	1	60–62	75	72
3	H	MeO	MeO	CH_2	0	69–70	58	56
4	H	MeO	MeO	O	1	95–96	78	75
5	H	MeO	MeO	N–Me	1	71–73	73	71
6	H	(OCH_2O)	–	CH_2	1	74–75	72	71
7	H	(OCH_2O)	–	CH_2	0	55–56	54	62
8	H	(OCH_2O)	–	O	1	118–119	79	78
9	H	(OCH_2O)	–	N–Me	1	84–86	71	68
10	MeO	MeO	MeO	CH_2	1	115–117	83	87
11	MeO	MeO	MeO	CH_2	0	91–93	82	86
12	MeO	MeO	MeO	O	1	137–139	77	83
13	MeO	MeO	MeO	N–Me	1	117–118	69	73
14	H	MeO	HO	CH_2	1	93–95	–	70
15	H	MeO	HO	CH_2	0	88–90	–	71
16	H	MeO	HO	O	1	142–143	–	76
17	H	MeO	HO	N–Me	1	167–169	–	63
18	H	HO	MeO	CH_2	1	133–135	–	72
19	H	HO	MeO	CH_2	0	118–119	–	71
20	H	HO	MeO	O	1	107–109	–	72
21	H	HO	MeO	N–Me	1	151–153	–	61
22	H	HO	H	CH_2	0	–	Nil	Nil
23	H	HO	H	O	1	–	Nil	Nil
24	H	HO	H	N–Me	1	–	Nil	Nil

Table 3
In vitro AChE inhibitory activity (IC_{50}) of prepared molecules **1**–**21** and their LogP.

Comp.	R_1	R_2	R_3	X	LogP	IC_{50} (μg/mL)	IC_{50} (μM)
1	H	HO	H	CH_2	2.09	20.1	93.0
2	H	MeO	MeO	CH_2	2.22	11.6	45.0
3	H	MeO	MeO	CH_2	1.82	10.3	42.0
4	H	MeO	MeO	O	0.94	14.1	54.0
5	H	MeO	MeO	N–Me	1.05	104.4	379.0
6	H	(OCH_2O)	–	CH_2	0.97	15.0	61.4
7	H	(OCH_2O)	–	CH_2	1.87	11.6	51.0
8	H	(OCH_2O)	–	O	0.97	14.2	58.0
9	H	(OCH_2O)	–	N–Me	1.09	123.2	275.3
10	MeO	MeO	MeO	CH_2	2.10	110.1	381.0
11	MeO	MeO	MeO	CH_2	1.70	88.7	321.0
12	MeO	MeO	MeO	O	0.83	113.7	389.0
13	MeO	MeO	MeO	N–Me	0.94	124.0	406.0
14	H	MeO	HO	CH_2	1.98	117.7	478.0
15	H	MeO	HO	CH_2	1.60	91.5	394.0
16	H	MeO	HO	O	0.71	90.1	363.0
17	H	MeO	HO	N–Me	0.83	91.5	350.1
18	H	HO	MeO	CH_2	1.99	110.3	448.0
19	H	HO	MeO	CH_2	1.60	83.2	358.2
20	H	HO	MeO	O	0.71	101.6	409.2
21	H	HO	MeO	N–Me	0.83	88.9	340.2
Gal.[a]					1.73	0.30	1.1
Diaz.[b]					3.61	0.57	1.9

[a] Galanthamine, drug used to treat Alzheimer disease.
[b] Diazinon, synthetic insecticide that acts on different soil and foliage pests.

to find a relationship between some physico-chemical parameters of the desired molecules calculated prior to synthesis and enzymatic probes (Table 1) and their AChE inhibition activity. Unfortunately, we could state only that the comparison of clogP values and IC_{50} values against AChE showed that the clogP would not play a role in the AChE inhibition since clogP has similar values for active (comp. **2**–**4** and **6**–**8**) as well as for inactive (for ex., comp. **5**,**9**) molecules.

Having these results in our hands, we wanted to test potential *in vivo* neurotoxic action of the most active inhibitor molecules **2**–**4** and **6**–**8** against the *A. aegypti* larvae.

2.3. In vivo insecticidal activity evaluation

Noteworthy, in the literature there are a few recent studies that evaluating molecules against any form (larvae, pupae, and adult) of the *A. aegypti* mosquitoes [32–35]. Using the biological assays under laboratory on third-instar larvae of *A. aegypti*, larvae were exposed to different concentrations (300, 270, 180, 140, 70, 60, 45 and 25 ppm) of the obtained molecules **2**–**4** and **6**–**8** as first diagnostic test searching higher mortality rates of the larvae. Then, the molecules with higher mortality were again tested determining a mortality of the larvae after 24 and 48 h. The bioassay results of diagnostic tests of compounds **2**–**7** against *A. aegypti* larvae *in vivo* (Table 4), disclosed that of the all molecules tested affected biological development of the species *A. aegypti* in their larval showing larvicidal activity at concentrations less than 140 ppm, highlighting α-aminonitriles **2** and **7**.

For compound **2**, which was obtained amount sufficient to carry out the assay, were evaluated, under specific conditions, five concentrations: 150, 130, 100, 85 and 55 ppm. The results of insecticidal activity bioassays *in vivo* and under laboratory conditions showed that, in the larval stage, *A. aegypti* is susceptible to the use of molecule **2** (resulting in IC_{50} of 11.6 mg/mL in the AChE inhibition evaluation) as insecticide.

The larval mortality due to the action of the molecule **2** was observed after 4 h of application of test pots, during which time morphological changes as thickening, darkening and size reduction were observed, resulting in the death of the larvae. Larvae that were not affected during the experiment emerged as normal adults within the observable parameters within four days. Larvae that could not reach the water surface when touched were considered dead. The results of the bioassay mortality and survival were subjected to Probit analysis (Table 5). It should be note that the organophosphate temephos (Abate) is the most appropriate larvicide to be used in public health programs [36]. Temephos disturbs the central nervous system through inhibition of cholinesterase causing death in larvae before reaching the adult stage. Moreover, the effective dosage recommended by WHO for application of temephos in domestic containers is only1 ppm and recently it was reported that lethal concentration, LC_{50} of *A. aegypti* were 0.006 ppm under natural conditions [37]. However, it may pollute the environment and may have adverse effects upon aquatic insects, such as predators that usually have been controlled in the natural ecological balance. Furthermore in resistant populations of *A. aegyti*, the LC_{50} could be up to 100 times that of susceptible colonies [38]. Although larvicide activity of comp. **2** was less potent than referent agent, studied comp. **2** is considered active ($LC_{50} < 100$ ppm) against *A. aegypti* larvae [39].

Additionally, the theoretical toxicity evaluation of tested compounds was performed using the OSIRIS interactive program, which includes the risk mutagenic, tumorigenic, reproductive and irritating property evaluation [40]. All the synthesized compounds **1**–**21** did not present any toxicity risks (Table 6).

3. Conclusion

In summary, we have designed and synthesized a new series of α-aminonitriles, girgensohnine alkaloid analogs for biochemical studies that include *in silico* ADME properties calculations, *in vitro* AChE inhibition and *in vivo* insecticidal activity. Prior to synthetic and pharmacological studies ADME parameters of this series were calculated analogs revealing their good pharmacokinetic profiles. Twenty one new molecules including girgensohnine alkaloid were tested for enzymatic inhibition as the first filter. It was fond that obtained α-aminonitriles **2**–**4** and **6**–**8** exhibited moderate to good

Table 4
Diagnostic tests of compounds **2**–**7** against *A. aegypti* larvae.

Molecule	Concentration (mg/L, ppm) of comp. and mortality rates of the larvae								48 h. Control
	300	270	180	140	70	60	45	25	
2	zero[a]	nt[b]	zero	nt	nt	nt	nt	5 + [c]	5+
6	nt	zero	zero	zero	zero	3+	zero	zero	5+
3	nt	nt	zero	zero	5+	zero	5+	zero	5+
7	nt	zero	zero	zero	nt	1+	nt	nt	5+
4	nt	nt	nt	zero	5+	5+	5+	nt	5+
8	nt	nt	nt	zero	5+	nt	5+	nt	5+

[a] Zero (0) live larvae.
[b] Not evaluated.
[c] Number (1-3-5+) of live larvae.

Table 5
Lethal concentration test of comp. **2** against *A. aegypti* larvae.

Time exposure	Lethal concentrations (LC) causing mortality in 50% (LC_{50}, ppm) and 99% (LC_{99}, ppm)					
	LC_{50}	CI[a]	LC_{99}	CI	x^2[b]	AI[c]
24 h.	88.12	83.43–94.74	147.54	125.30–210.54	0.58	10.39 ± 2.01
48 h.	87.47	82.38–94.82	155.57	128.63–243.93	7.14	9.30 ± 1.96

[a] Confidence intervals.
[b] Chi-square.
[c] Slope ± standard deviation.

AChE inhibition (IC_{50} = 42–58 μM) that was enough argument to test them in *in vivo* insecticidal activity. Using the biological assays under laboratory on third-instar larvae of *A. aegypti*, it was disclosed that the molecules **2**–**4** and **6**–**8** affected biological development of the species *A. aegypti* in their larval showing larvicidal activity at concentrations less than 140 ppm, highlighting α-aminonitriles **2** and **7** (with IC_{50} = 45 and 51 μM, respectively) that makes them possible lead compounds as insecticides. Additionally, green reaction conditions, simplicity and efficacy of the proposed synthetic procedure of the α-aminonitriles allow for their large-scale preparation. Further investigations on detailed biochemical mechanism of action are now under way in our laboratories and their results will be published elsewhere.

4. Experimental

4.1. Rational design: virtual screenings and molecular properties calculations

A computational study of all compounds was performed for the prediction of ADME, toxicological, and physicochemical property endpoints such as absorption (%ABS), polar surface area (TPSA), miLog P, number of rotatable bonds, and violations of Lipinski's rule of five by using ACD/Lab/Molinspiration/VCCLAB/Osiris online calculation resources (Tables 1 and 6) [41–44].

Table 6
Osiris calculations of compounds **1**–**21**.

Compd.	Prediction of toxicity risks[a]				Molecular properties' calculations[b]				
	Mut	Tumo	Irri	Rep	MW	CLP	LogS	DL	D-S
1	–[c]	–	–	–	216	2.40	−2.01	−2.74	0.49
2	–	–	–	–	260	2.49	−2.34	−1.48	0.54
3	–	–	–	–	246	2.17	−2.07	0.95	0.80
4	–	–	–	–	262	1.28	−1.45	−0.61	0.64
5	–	–	–	–	275	1.4	0.95	4.33	0.94
6	–	–	–	–	244	2.8	−3.02	−3.28	0.45
7	–	–	–	–	230	2.48	−2.75	−0.82	0.59
8	–	–	–	–	246	1.59	−2.13	−2.34	0.51
9	–	–	–	–	259	1.71	−1.63	2.69	0.91
10	–	–	–	–	290	2.38	−2.36	−0.12	0.66
11	–	–	–	–	276	2.07	−2.09	2.28	0.88
12	–	–	–	–	292	1.18	−1.47	0.69	0.78
13	–	–	–	–	305	1.3	−0.97	5.62	0.93
14	–	–	–	–	246	2.3	−2.03	−2.35	0.50
15	–	–	–	–	232	1.98	−1.76	0.12	0.72
16	–	–	–	–	248	1.09	−1.14	−1.54	0.56
17	–	–	–	–	261	1.21	−0.64	3.68	0.94
18	–	–	–	–	246	2.3	−2.03	−2.97	0.48
19	–	–	–	–	232	1.98	−1.76	−0.48	0.65
20	–	–	–	–	248	1.09	−1.14	−2.14	0.53
21	–	–	–	–	261	1.21	−0.64	3.11	0.94
22	–	–	–	–	202	2.08	−1.74	−0.25	0.68
23	–	–	–	–	218	1.19	−1.12	−2.02	0.54
24	–	–	–	–	231	1.31	−0.62	3.43	0.95

[a] Mut – mutagenic; Tumo – tumorigenic; Irri – irritant; Rep – reproductive effective.
[b] MW – molecular weight; CLP – cLogP: n-octanol–water partition coefficient; DL – Druglikeness; D-S – Drug-score.
[c] No any toxicity risks.

4.2. General

IR spectra were recorded on a Lumex Infralum FT-02 spectrophotometer and the values are expressed as υ_{max} cm^{-1}. ^1H and ^{13}C NMR spectra were measured on a Bruker AM-400 spectrometer (400 MHz ^1H NMR and 100 MHz ^{13}C NMR), using $CDCl_3$ or DMSO-d6 as solvents. TMS was used as an internal standard. Chemical shifts (δ) and *J* values are reported in ppm and Hz, respectively. On DEPT-135 spectra, the signals of CH_3, CH_2, and CH carbons are shown as positive (+) and negative (−), respectively. Quaternary carbons are not shown. A Hewlett Packard 5890a Series II Gas Chromatograph interfaced to an HP 5972 Mass Selective Detector with an HP MS ChemStation Data system was used for MS identification at 70 eV using a 60 m capillary column coated with HP-5 [5% phenylpoly(dimethylsiloxane)]. Melting points were measured on a Fisher Johns melting point apparatus. Elemental analyses were performed on a Perkin–Elmer 2400 Series II analyzer and were within ± 0.4 of theoretical values. The reaction progress was monitored using thin layer chromatography on a Silufol UV 254 TLC aluminum sheets. Column chromatography was carried out using alumina gel (100–200 mesh) and appropriate mixture of petroleum ether/ethyl acetate for elution. All reagents were purchased from Sigma, Sigma–Aldrich and J.T. Baker. The aldehydes were used as received and the amines were distillated before use. All anhydrous solvents were dried and purified by standard techniques just before use.

4.3. General procedure for the synthesis of α-aminonitrile derivatives

The analogs were synthesized using a modified Strecker reaction as follows: For one-pot reaction of an equimolar mixture of benzaldehyde (1 mmol) and cyclic amine (1.1 mmol), a round bottom flask 100 mL capacity was employed and stirred for 30 min. The source of cyanide was added selecting between KCN and acetone cyanohydrin (1.5 mmol) and the catalyst SSA (1:1 by weight) in acetonitrile (15–20 mL) at room temperature (16–24 h). The progress of the reaction was monitored by TLC (ether:ethyl acetate, 10:1). Then, the reaction mixture was filtered and evaporated of the solvent, the residue was purified by column chromatography on alumina (100–200 mesh) eluting with petroleum ether:ethyl acetate (15:1) to furnish title compounds as white solids with defined melting points (Table 2).

4.3.1. 2-(4-Hydroxyphenyl)-2-(piperidin-1-yl)acetonitrile (**1**)

IR (KBr): 2232 $\nu_{(C\equiv N)}$. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 9.61 (1H, s, OH), 7.22 (2H, d, J = 8.5 Hz, 2-H_{Ar} y 6-H_{Ar}), 6.80 (2H, d, J = 8.6 Hz, 3-H_{Ar} and 5-H_{Ar}), 5.11 (1H, s, 1′-H), 2.40 (4H, m, 2-H_{Pip} and 6-H_{Pip}), 1.51 (3-H_{Pip} and 5-H_{Pip}), 1.39 (2H, m, 4-H_{Pip}). ^{13}C NMR (100 MHz) δ (ppm): 157.5 (4-C_{Ar}), 128.9 (2-C_{Ar} and 6-C_{Ar}), 123.6 (1-

C_{Ar}), 116.2 (CN), 115.2 (3-C_{Ar} and 5-C_{Ar}), 60.9 (CH), 50.0 (2-C_{Pip} and 6-C_{Pip}), 25.2 (3-C_{Pip} and5-C_{Pip}), 23.6 (4-C_{Pip}). GC–MS (70 eV): t_R = 18.9 min, m/z = (216, $M^{+\bullet}$, 17), 132 (48), 85 (15), 84 (100), 77 (12). Anal. Calcd for $C_{13}H_{16}N_2O$: C, 72.19; H, 7.46; N, 12.95; Found: 72.25; H, 7.54; N, 12.87.

*4.3.2. 2-(3,4-Dimethoxyphenyl)-2-(piperidin-1-yl)acetonitrile (**2**)*

IR (KBr): 2229 $\nu_{(C\equiv N)}$, 1250 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.21–6.12 (3H, m, 2,5,6-H_{Ar}), 4.38 (1H, s, 1′-H), 2.95 (6H, s, 3,4-OCH_3), 1.72–1.54 (4H, m, 2,6-H_{Pip}), 0.79–0.64 (4H, m, 3,5-H_{Pip}), 0.59 (2H, d, J = 5.2 Hz, 4-H_{Pip}). ^{13}C NMR (100 MHz) δ (ppm): 149.9 (4-C_{Ar}), 149.0 (3-C_{Ar}), 126.8 (1-C_{Ar}), 120. 8 (6-C_{Ar}), 117.1 (–CN), 112.4 (2-C_{Ar}), 112.0 (5-C_{Ar}), 62.1 (1′-C), 56. 4 (3,4-OCH_3), 51.1 (2,6-C_{Pip}), 26.2 (3,5-C_{Pip}), 24.6 (4-C_{Pip}). GC–MS (70 eV): t_R = 20.7 min, m/z = (260, $M^{+\bullet}$, 6), 177 (30), 176 (100), 131 (5), 84 (59). Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76; Found: C, 69.05; H, 7.89; N, 10.54.

*4.3.3. 2-(3,4-Dimethoxyphenyl)-2-(pyrrolidin-1-yl)acetonitrile (**3**)*

IR (KBr): 2229 $\nu_{(C\equiv N)}$, 1273 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.28–6.26 (3H, m, 2,5,6-H_{Ar}), 4.62 (1H, s, 1′-H), 3.06 (6H, s, 3,4-OCH_3), 1.88–1.77 (4H, m, 2,5-H_{Pyr}), 1.04–1.01 (4H, m, 3,4-H_{Pyr}). ^{13}C NMR (100 MHz) δ (ppm): 150.2 (4-C_{Ar}), 150.1 (3-C_{Ar}), 128.1 (1-C_{Ar}), 120.8 (6-C_{Ar}), 118.0 (–CN), 112.8 (2-C_{Ar}), 112.4 (5-C_{Ar}), 58.9 (1′-C), 56.8 (3,4-OCH_3), 50.9 (2,5-C_{Pyr}), 24.1 (3,4-C_{Pyr}). GC–MS (70 eV): t_R = 19.4 min, m/z = 246 ($M^{+\bullet}$), 177 (29), 176 (100), 151 (6), 146 (6), 70 (34). Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37; Found: C, 68.36; H, 7.55; N, 11.16.

*4.3.4. 2-(3,4-Dimethoxyphenyl)-2-(morpholin-1-yl)acetonitrile (**4**)*

IR (KBr): 2229 $\nu_{(C\equiv N)}$, 1281 $\nu_{(N\text{-}C)}$, 1111 $\nu_{(C\text{-}O\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6,23–6.13 (3H, m, 2,5,6-H_{Ar}), 4.43 (1H, s, 1′-H), 2.95 (6H, s, 3,4-OCH_3), 2.79 (4H, sa, 3,5-H_{Morph}), 1.68 (2H, m, 6-H_{Morph}), 1.63–1.52 (2H, m, 2-H_{Morph}). ^{13}C NMR (100 MHz) δ (ppm): 150.1 (4-C_{Ar}), 149.8 (3-C_{Ar}), 125.9 (1-C_{Ar}), 121.1 (6-C_{Ar}), 117.0 (–CN), 112.4 (2-C_{Ar}), 112.2 (5-C_{Ar}), 66.9 (1′-C), 61.6 (3,5-C_{Morph}), 56.5 (3,4-OCH_3), 50.4 (2,6-C_{Morph}). GC–MS (70 eV): t_R = 21.0 min, m/z = (262, $M^{+\bullet}$, 7), 177 (23), 176 (100), 86 (38), 56 (29). Anal. Calcd for $C_{14}H_{18}N_2O_3$: C, 64.10; H, 6.92; N, 10.68; Found: C, 64.28; H, 6.87; N, 10.59.

*4.3.5. 2-(3,4-Dimethoxyphenyl)-2-(4-methylpiperazin-1-yl) acetonitrile (**5**)*

IR (KBr): 2220 $\nu_{(C\equiv N)}$, 1247 $\nu_{(N\text{-}C)}$, cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.06 (^1H, ddd, J = 8.3, 2.1, 0.7 Hz, 6-H_{Ar}), 6.98 (1H, d, J = 2.0 Hz, 2-H_{Ar}), 6.83 (1H, d, J = 8.3 Hz, 5-H_{Ar}), 4.76 (1H, s, 1′-H), 3.87 (6H, s, 3,4-OCH_3), 2.74–2.16 (11H, m, 2-6-HN_{pip}, -NCH_3). ^{13}C NMR (100 MHz) δ (ppm): 149.6 (3-C_{Ar}), 149.4 (4-C_{Ar}), 125.6 (1-C_{Ar}), 120.6 (+, 6-C_{Ar}), 115.8 (–CN), 111.0 (+, 5-C_{Ar}), 110.9 (+, 2-C_{Ar}), 62.0 (+, 1′-C), 56.2 (+, 3-OCH_3), 56.2 (+, 4-OCH_3), 55.1 (–, 2-6-CN-$_{pip}$), 46.2 (+, –NCH_3). GC–MS (70 eV): t_R = 21.0 min, m/z = (275, $M^{+\bullet}$, 28), 176 (10), 99 (100), 70 (14), 56 (34). Anal. Calcd for $C_{15}H_{21}N_3O_2$: C, 65.43; H, 7.69; N, 15.26; Found: C, 65.57; H, 7.81; N, 15.08.

*4.3.6. 2-(3,4-Dioxymethylenphenyl)-2-(piperidin-1-yl)acetonitrile (**6**)*

IR (KBr): 2229 $\nu_{(C\equiv N)}$, 1257 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.16–6.08 (3H, m, 2,5,6-H_{Ar}), 5.24 (2H, s, 3-OCH_2O), 4.36 (1H, s, 1′-H), 1.70–1.52 (4H, m, 2,6-H_{Pip}), 0.77–0.62 (4H, m, 3,5-H_{Pip}), 0.58 (2H, d, J = 5.1 Hz, 4-H_{Pip}). ^{13}C NMR (100 MHz) δ (ppm): 148.6 (3-C_{Ar}), 148.4 (4-C_{Ar}), 128.4 (1-C_{Ar}), 121.9 (6-C_{Ar}), 117.0 (–CN), 108.9 (2-C_{Ar}), 108.8 (5-C_{Ar}), 102.4 (3-OCH_2O), 62.0 (1′-C), 51.1 (2,6-C_{Pip}), 26.2 (3′,5′-C_{Pip}), 24.6 (4′-C_{Pip}). GC–MS (70 eV): t_R = 20.0 min, m/z = (244, $M^{+\bullet}$, 10), 161 (24), 160 (100), 84 (85). Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47; Found: C, 68.66; H, 6.49; N, 11.32.

*4.3.7. 2-(3,4-Dioxymethylenphenyl)-2-(pyrrolidin-1-yl)acetonitrile (**7**)*

IR (KBr): 2229 $\nu_{(C\equiv N)}$, 1257 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.16–6.08 (3H, m, 2,5,6-H_{Ar}), 5.23 (2H, s, 3-OCH_2O), 4.51 (1H, s, 1′-H), 1.78–1.71 (2H, m, 2-H_{Pyr}), 1.69–1.63 (2H, m, 5-H_{Pyr}), 0.92 (4H, m, 3,4-H_{Pyr}). ^{13}C NMR (100 MHz) δ (ppm): 148.6 (3-C_{Ar}), 148.4 (4-C_{Ar}), 129.3 (1-C_{Ar}), 121.8 (6-C_{Ar}), 117.6 (–CN), 109.0 (2-C_{Ar}), 108.8 (5-C_{Ar}), 102.3 (3-OCH_2O), 58.5 (1′-C), 50.6 (2,5-C_{Pyr}), 23.8 (3,4-C_{Pyr}). GC–MS (70 eV): t_R = 18.3 min, m/z = (230, $M^{+\bullet}$, 10), 161 (23), 160 (100), 70 (20). Anal. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.96; H, 6.32; N, 12.31.

*4.3.8. 2-(3,4-Dioxymethylenphenyl)-2-(morpholin-1-yl)acetonitrile (**8**)*

IR (KBr): 1250$\nu_{(N\text{-}C)}$, 1119 $\nu_{(C\text{-}O\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.18–6.09 (3H, m, 2,5,6-H_{Ar}), 5.24 (2H, s, 3-OCH_2O), 4.43 (1H, s, 1′-H), 2.83–2.72 (4H, m, 3,5-H_{Morph}), 1.74–1.64 (2H, m, 6-H_{Morph}), 1.61–1.53 (2H, m, 2-H_{Morph}). ^{13}C NMR (100 MHz) δ (ppm): 148.7 (3-C_{Ar}), 148.6 (4-C_{Ar}), 127.5 (1-C_{Ar}), 122.3 (6-C_{Ar}), 116.8 (–CN), 109.0 (2-C_{Ar}), 109.0 (5-C_{Ar}), 102.4 (3-OCH_2O), 66.8 (1′-C), 61.5 (3,5-C_{Morph}), 50.4 (2,6-C_{Morph}). GC–MS (70 eV): t_R = 20.3 min, m/z = (246, $M^{+\bullet}$, 12), 161 (21), 160 (100), 102 (14), 86 (69), 56 (64). Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38; Found: C, 63.22; H, 5.85; N, 11.27.

*4.3.9. 2-(3,4-Dioxymethylenphenyl)-2-(4-methylpiperazin-1-yl) acetonitrile (**9**)*

IR (KBr): 2227 $\nu_{(C\equiv N)}$,1247 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.00 (1H, ddd, J = 8.0, 1.8, 0.9 Hz, 6-H_{Ar}), 6.97 (1H, dd, J = 1.3, 0.5 Hz, 2-H_{Ar}), 6.79 (1H, d, J = 8.0 Hz, 5-H_{Ar}), 5.98 (2H, s, 3-OCH_3O), 4.73 (1H, s, 1′-H), 2.72–2.19 (11H, m, 2-6-H_{Pip}, N–CH_3). ^{13}C NMR (100 MHz) δ (ppm): 148.5 (3-C_{Ar}), 148.4 (4-C_{Ar}), 127.1 (1-C_{Ar}), 121.8 (+, 6-C_{Ar}), 115.7 (–CN), 108.5 (+, 2-C_{Ar}), 108.4 (+, 5-C_{Ar}), 101.8 (+, 3-OCH_2O), 61.9 (+, 1′-C), 55.1 (–, 2-6-$C_{N\text{-}pip}$), 46.2 (+, N–CH_3). GC–MS (70 eV): t_R = 20.2 min, m/z = (259, $M^{+\bullet}$, 31), 160 (19), 99 (100), 70 (14), 56 (35). Anal. Calcd for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.20; Found: C, 64.62; H, 6.39; N, 16.43.

*4.3.10. 2-(3,4,5-Trimethoxyphenyl)-2-(piperidin-1-yl) acetonitrile (**10**)*

IR (KBr): 2221$\nu_{(C\equiv N)}$,1231 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.76 (2H, s, 2,6-H_{Ar}), 4.75 (1H, s, 1′-H), 3.87 (6H, s, 3,5-OCH_3), 3.84 (3H, s, 4-OCH_3), 2.59–1.68 (4H, m, 2,6-H_{Pip}), 1.68–1.53 (4H, m, 3-5-H_{Pip}), 1.48 (2H, dd, J = 10.8, 5.2 Hz 4-H_{Pip}). ^{13}C NMR (100 MHz) δ (ppm): 153.6 (3,5-C_{Ar}), 138.2 (4-C_{Ar}), 129.4 (1-C_{Ar}), 116.0 (–CN), 104.8 (+, 2,6-C_{Ar}), 63.3 (+, 1′-C), 61.2 (+, 4-OCH_3), 56.5 (+, 3,5-OCH_3), 51.2 (–, 2,6-C_{Pip}), 26.1 (–, 3,5-C_{Pip}), 24.2 (–, 4-C_{Pip}). GC–MS (70 eV): t_R = 21.2 min, m/z = (290, $M^{+\bullet}$), 207 (63), 206 (100), 192 (12), 176 (32), 84 (50). Anal. Calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65; Found: C, 66.25; H, 7.86; N, 9.47.

*4.3.11. 2-(3,4,5-Trimethoxyphenyl)-2-(pyrrolidin-1-yl) acetonitrile (**11**)*

IR (KBr): 2220$\nu_{(C\equiv N)}$,1235 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.73 (2H, s, 2,6-H_{Ar}), 4.97 (1H, s, 1′-H), 3.87 (6H, s, 3,5-OCH_3), 3.84 (3H, s, 4-OCH_3), 2.71–2.59 (4H, m, 2,5-H_{Pir}), 1.89–1.78 (4H, m, 3,4-H_{Pir}). ^{13}C NMR (100 MHz) δ (ppm): 153.7 (3,5-C_{Ar}), 138.3 (4-C_{Ar}), 130.1 (1-C_{Ar}), 116.5 (–CN), 104.8 (+, 2,6-C_{Ar}), 61.2 (+, 1′-C), 59.8 (+, 4-OCH_3), 56.5 (+, 3,5-OCH_3), 50.6 (–, 2,5-C_{Pyr}), 23.8 (–, 3,4-C_{Pyr}). GC–MS (70 eV): t_R = 20.0 min, m/z = (276, $M^{+\bullet}$), 207 (68), 206 (100), 192 (15), 176 (39), 70 (21). Anal. Calcd for

$C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14; Found: C, 65.42; H, 7.51; N, 10.27.

4.3.12. 2-(3,4,5-Trimethoxyphenyl)-2-(morpholin-1-yl)acetonitrile (**12**)

IR (KBr): 2224$\nu_{(C\equiv N)}$,1248 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.74 (2H, s, 2,6-H_{Ar}), 4.74 (1H, s, 1′-H), 3.87 (6H, s, 3,5-OCH_3), 3.83 (3H, s, 4-OCH_3), 3.79–3.66 (4H, m, 3,5-H_{Morf}), 2.68–2.43 (4H, m, 2,6-H_{Morf}). ^{13}C NMR (100 MHz) δ (ppm): 153.7 (3,5-C_{Ar}), 138.5 (4-C_{Ar}), 128.2 (1-C_{Ar}), 115.5 (−CN), 105.1 (+, 2,6-C_{Ar}), 66.9 (−, 3,5-C_{Morf}), 62.8 (+, 1′-C), 61.2 (+, 4-OCH_3), 56.5 (+, 3-5-OCH_3), 50.3 (−, 2,6-C_{Morf}). GC–MS (70 eV): t_R = 21.3 min, m/z = (292, $M^{+\bullet}$), 207 (52), 206 (100), 176 (14), 86 (20), 66 (14). Anal. Calcd for $C_{15}H_{20}N_2O_4$: C, 61.63; H, 6.90; N, 9.58; Found: C, 61.47; H, 6.63; N, 9.45.

4.3.13. 2-(3,4,5-Trimethoxyphenyl)-2-(4-methylpiperazin-1-yl) acetonitrile (**13**)

IR (KBr): 2221$\nu_{(C\equiv N)}$,1247 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.73 (2H, d, J = 0.5 Hz, 2,6-H_{Ar}), 4.76 (1H, s, 1′-H), 3.86 (6H, s, 3,5-OCH_3), 3.83 (3H, s, 4-OCH_3), 2.78–2.14 (11H, m, 2-6-H_{Pip}, N–CH_3). ^{13}C NMR (100 MHz) δ (ppm): 153.7 (3,5-C_{Ar}), 138.4 (4-C_{Ar}), 128.8 (1-C_{Ar}), 115.6 (−CN), 104.9 (+, 2,6-C_{Ar}), 62.4 (+, 1′-C), 61.2 (+, 4-OCH_3), 56.5 (+, 3,5-OCH_3), 55.1 (−, 2-6-$C_{N\text{-}pip}$), 46.2 (+, N–CH_3). GC–MS (70 eV): t_R = 22.1 min, m/z = (305, $M^{+\bullet}$), 305 (26), 206 (16), 99 (100), 70 (14), 56 (31). Anal. Calcd for $C_{16}H_{23}N_3O_3$: C, 62.93; H, 7.59; N, 13.76; Found: C, 62.80; H, 7.82; N, 13.49.

4.3.14. 2-(3-Hydroxy-4-methoxyphenyl)-2-(piperidin-1-yl) acetonitrile (**14**)

IR (KBr): 2232$\nu_{(C\equiv N)}$,1248 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.09 (1H, dd, J = 2.2, 0.6 Hz, 2-H_{Ar}), 7.02 (1H, ddd, J = 8.3, 2.2, 0.8 Hz, 6-H_{Ar}), 6.83 (1H, d, J = 8.3 Hz, 5 H_{Ar}), 4.72 (1H, s, 1′-H), 3.89 (3H, s, 4-OCH_3), 2.58–2.34 (4H, m, 2,6-H_{Pip}), 1.66–1.51 (4H, m, 3,5-H_{Pip}), 1.51–1.41 (2H, dd, J = 10.8, 5.2 Hz 4-H_{Pip}). ^{13}C NMR (100 MHz) δ (ppm): 147.1 (4-C_{Ar}), 146.1 (3-C_{Ar}), 126.9 (1-C_{Ar}), 119.8 (+, 6-H), 116.1 (−CN), 114.4 (+, 5-C_{Ar}), 110.5 (+, 2-C_{Ar}), 62.8 (+, 1′-C), 56.3 (+, 4-OCH_3), 51.1 (−, 2,6-C_{Pip}), 26.1 (−, 3,5-C_{Pip}), 24.3 (−, 4-C_{Pip}). GC–MS (70 eV): t_R = 21.5 min, m/z = (246, $M^{+\bullet}$), 221 (24), 137 (100), 98 (20), 84 (77). Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37; Found: C, 68.45; H, 7.59; N, 11.52.

4.3.15. 2-(3-Hydroxy-4-methoxyphenyl)-2-(pyrrolidin-1-yl) acetonitrile (**15**)

IR (KBr): 2220 $\nu_{(C\equiv N)}$,1218 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.06 (1H, d, J = 2.2 Hz, 2-H_{Ar}), 7.00 (1H, ddd, J = 8.3, 2.2, 0.7 Hz, 6-H_{Ar}), 6.82 (1H, d, J = 8.3 Hz, 5-H_{Ar}), 4.94 (1H, s, 1′-H), 3.88 (3H, s, 4-OCH_3), 2.69–2.57 (4H, m, 2,5-H_{pir}), 1.85–1.77 (4H, m, 3,4-H_{pir}). ^{13}C NMR (100 MHz) δ (ppm): 147.2 (4-C_{Ar}), 146.1 (3-C_{Ar}), 127.6 (1-C_{Ar}), 119.5 (+, 6-H), 116. 6 (−CN), 114.3 (+, 5-C_{Ar}), 110. 7 (+, 2-C_{Ar}), 59.0 (+, 1′-C), 56.3 (+, 4-OCH_3), 50.5 (−, 2,5-C_{pir}), 23.6 (−, 3,4-C_{pir}). GC–MS (70 eV): t_R = 20.7 min, m/z = (232, $M^{+\bullet}$), 207 (20), 137 (100), 84 (22), 70 (50). Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06; Found: C, 67.01; H, 7.02; N, 11.91.

4.3.16. 2-(3-Hydroxy-4-methoxyphenyl)- 2-(morpholin-1-yl) acetonitrile (**16**)

IR (KBr): 2238$\nu_{(C\equiv N)}$,1249 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.07 (1H, d, J = 2.2, 2-H_{Ar}), 7.01 (1H, ddd, J = 8.3, 2.2, 0.7 Hz, 6-H_{Ar}), 6.84 (1H, d, J = 8.3 Hz, 5-H_{Ar}), 5.90 (1H, sa, 3-OH), 4.72 (1H, s, 1′-H), 3.89 (3H, s, 4-OCH_3), 3.78–3.62 (4H, m, 3,5-H_{Morf}), 2.66–2.45 (4H, m, 2,6-H_{Morf}). ^{13}C NMR (100 MHz) δ (ppm): 147.4 (4-C_{Ar}), 146.2 (3-C_{Ar}), 125.7 (1-C_{Ar}), 120.0 (+, 6-H), 115.7 (−CN), 114.5 (+, 5-C_{Ar}), 110.6 (+, 2-C_{Ar}), 67.0 (−, 3,5-C_{Morph}), 62.2 (+, 1′-C), 56.3 (+, 4-OCH_3), 50.1 (−, 2,6-C_{Morph}). GC–MS (70 eV): t_R = 21.9 min, m/z = (248, $M^{+\bullet}$, 16), 162 (100) 163 (23), 86 (57), 56 (37). Anal. Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28; Found: C, 62.74; H, 6.78; N, 11.11.

4.3.17. 2-(3-Hydroxy-4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)acetonitrile (**17**)

IR (KBr): 2232$\nu_{(C\equiv N)}$,1236 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.03 (1H, dd, J = 2.2, 2-H_{Ar}), 6.98 (1H, ddd, J = 8.3, 2.2, 0.7 Hz, 6-H_{Ar}), 6.82 (1H, d, J = 8.3 Hz, 5-H_{Ar}), 4.72 (1H, s, 1′-H), 3.88 (3H, s, 4-OCH_3), 2.74–2.30 (8H, m, 2-6-H_{Pip}), 2.28 (3H, s, N–CH_3). ^{13}C NMR (100 MHz) δ (ppm): 147.6 (4-C_{Ar}), 146.4 (3-C_{Ar}), 126.2 (1-C_{Ar}), 119.8 (+, 6-C_{Ar}), 115.8 (−CN), 114.7 (+, 5-C_{Ar}), 110.7 (+, 2-C_{Ar}), 61.7 (+, 1′-C), 56.3 (+, 4-OCH_3), 55.0 (−, 2,6-$C_{N\text{-}pip}$), 46.1 (+, -NCH_3). GC–MS (70 eV): t_R = 22.6 min, m/z = (261, $M^{+\bullet}$), 236 (67), 165 (47), 137 (100), 99 (62), 56 (47). Anal. Calcd for $C_{14}H_{19}N_3O_2$: C, 64.35; H, 7.33; N, 16.08; Found: C, 64.57; H, 7.46; N, 16.17.

4.3.18. 2-(4-Hydroxy-3-methoxyphenyl)-2-(piperidin-1-yl) acetonitrile (**18**)

IR (KBr): 2230$\nu_{(C\equiv N)}$,1248 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.04 (1H, ddd, J = 8.2, 2.1, 0.8 Hz, 6-H_{Ar}), 7.00 (1H, d, J = 2.0 Hz, 2-H_{Ar}), 6.91 (1H, d, J = 8.2 Hz, 5-H_{Ar}), 4.75 (1H, s, 1′-H), 3.91 (3H, s, 3-OCH_3), 2.58–2.41 (4H, m, 2,6-H_{Pip}), 1.68–1.45 (6H, m, 3-5-H_{Pip}). ^{13}C NMR (100 MHz) δ (ppm): 146.9 (3-C_{Ar}), 146.2 (4-C_{Ar}), 125.6 (1-C_{Ar}), 121.2 (+, 6-C_{Ar}), 116.2 (−CN), 114.6 (+, 5-C_{Ar}), 110.4 (+, 2-C_{Ar}), 63.0 (+, 1′-C), 56.4 (+, 3-OCH_3), 51.3 (+, 2,6-C_{pip}), 26.1 (−, 3,5-C_{pip}), 24.3 (−, 4-C_{pip}). GC–MS (70 eV): t_R = 23.6 min, m/z = (246, $M^{+\bullet}$, 19), 162 (80), 163 (19), 85 (19), 84 (100). Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37; Found: C, 68.11; H, 7.23; N, 11.45.

4.3.19. 2-(4-Hydroxy-3-methoxyphenyl)-2-(pyrrolidin-1-yl) acetonitrile (**19**)

IR (KBr): 2217$\nu_{(C\equiv N)}$,1232 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.01 (1H, dd, J = 8.2, 2.0 Hz, 6-H_{Ar}), 6.98 (1H, d, J = 1.8 Hz, 2-H_{Ar}), 6.89 (1H, d, J = 8.1 Hz, 5-H_{Ar}), 4.95 (1H, s, 1′-H), 3.88 (3H, s, 3-OCH_3), 2.70–2.58 (4H, m, 2,5-H_{Pyr}), 1.89–1.76 (4H, m, 3,4-H_{Pyr}). ^{13}C NMR (100 MHz) δ (ppm): 147.0 (3-C_{Ar}), 146.3 (4-C_{Ar}), 126.3 (1-C_{Ar}), 121.0 (+, 6-C_{Ar}), 117.0 (−CN), 114.7 (+, 5-C_{Ar}), 110.3 (+, 2-C_{Ar}), 59.4 (+, 1′-C), 56.3 (+, 3-OCH_3), 50.6 (−, 2,5-C_{Pyr}), 23.7 (−, 3,4-C_{Pyr}). GC–MS (70 eV): t_R = 20.1 min, m/z = (232, $M^{+\bullet}$), 207 (38), 206 (33), 138 (48), 137 (100), 70 (73). Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06; Found: C, 67.45; H, 6.83; N, 12.27.

4.3.20. 2-(4-Hydroxy-3-methoxyphenyl)-2-morpholinoacetonitrile (**20**)

IR (KBr): 2236$\nu_{(C\equiv N)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.02 (1H, ddd, J = 8.2, 2.1, 0.7 Hz, 6-H_{Ar}), 6.98 (1H, d, J = 2.0 Hz, 2-H_{Ar}), 6.90 (1H, d, J = 8.2 Hz, 5-H_{Ar}), 4.73 (1H, s, 1′-H), 3.90 (3H, s, 3-OCH_3), 3.79–3.64 (4H, m, 3,5-H_{Morf}), 2.63–2.49 (4H, m, 2,6-H_{Morf}). ^{13}C NMR (100 MHz) δ (ppm): 147.1 (3-C_{Ar}), 146.5 (4-C_{Ar}), 124.4 (1-C_{Ar}), 121.5 (+, 6-C_{Ar}), 115.7 (−CN), 114.8 (+, 5-C_{Ar}), 110.6 (+, 2-C_{Ar}), 66.9 (−, 3,5-C_{Morf}), 62.4 (+, 1′-C), 56.3 (+, 3-OCH_3), 50.2 (−, 2,6-C_{Morf}). GC–MS (70 eV): t_R = 23.9 min, m/z = (248, $M^{+\bullet}$, 29), 162 (100), 87 (24), 86 (55), 56 (39). Anal. Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28; Found: C, 62.67; H, 6.78; N, 11.45.

4.3.21. 2-(4-Hydroxy-3-methoxyphenyl)-2-(4-methylpiperazin-1-yl)acetonitrile (**21**)

IR (KBr): 2247$\nu_{(C\equiv N)}$,1219 $\nu_{(N\text{-}C)}$ cm^{-1}. ^1H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.02 (1H, d, J = 1.7 Hz, 2-H_{Ar}), 5.95 (1H, dd, J = 8.2, 1.7 Hz, 6-H_{Ar}), 5.91 (1H, d, J = 8.1 Hz, 5-H_{Ar}), 4.28 (1H, s, 1′-H), 2.87 (3H, s, 3-OCH_3), 1.73–1.21 (11H, m, 2-6-H_{Pip}, N–CH_3). ^{13}C NMR (100 MHz) δ (ppm): 148.7 (3-C), 148.0 (4-C), 124.9 (1-C), 121.3 (+, 6-H), 117.3 (−CN), 116.3 (+, 5-C), 112.6 (+, 2-C), 61.4 (+, 1′-C),

56.6 (+, 3-OCH_3), 55.4 (−, 2-6-$C_{N\text{-}Pip}$), 46.6 (−, N−CH_3). GC−MS (70 eV): t_R = 24.6 min, m/z = (261, $M^{+\cdot}$,37), 162 (46), 99 (20), 56 (36), 44 (21), 42 (16). Anal. Calcd for $C_{14}H_{19}N_3O_2$: C, 64.35; H, 7.33; N, 16.08; Found: C, 64.57; H, 7.21; N, 16.23.

4.4. In vitro AChE inhibition

The Ellman assay was used to test AChE inhibition activity [30,31]. This colorimetric assay is based on chromophores generated *in situ* after enzymatic cleavage of acetylthiocholine and the resulting thiocholine with Ellman's reagent, DTNB (5,5′-dithio-bis-2-nitrobenzoic acid). The plates were incubated at room temperature and the enzymatic activity was determined by spectrophotometric reading at 412 nm using a microplate reader Molecular Devices VERSAMAX. Galantamine and diazinon were used as chemical agent references.

4.5. In vivo insecticidal activity

The biological assay was conducted against third-instar larvae of *A. aegypti* (7 ± 1 day old). Rockefeller larvae used in the bioassays were maintained on plastic trays (35.5 cm × 21.5 cm × 6.5 cm) with approx. 3000 mL of dechlorinated water at room temperature (25 °C ± 5), humidity (80 ± 5%) and photoperiod (12:12). After reaching the larval stage between third and fourth instar were counted and separated with Pasteur pipettes and taken to disposable plastic cups with a capacity of 100 mL containing 20 mL of dechlorinated water itself and a total of 10 larvae per pot. A diagnostic test with six products obtained was performed. Larvae were exposed to different concentrations (1000, 300, 270, 180, 140, 70, 60, 45 and 25 ppm) during the first diagnostic evaluation. Five larvae were placed per pot, four replicates per concentration and a control using only 1% DMSO. Finally, a second bioassay with the molecule with higher mortality rates in the previous trial was performed. Mortality of the larvae exposed to treatment was determined after 24 and 48 h. Larvae that could not reach the water surface when touched were considered dead. The lethal concentrations (LC_{50} and LC_{99}) and their respective confidence intervals were calculated through Probit analyses, using the Statistics Analyses System (SAEG, version 9.1 − 2007).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.067.

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