Synthesis, molecular docking studies, and larvicidal activity evaluation of new fluorinated neonicotinoids against Anopheles darlingi larvae

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Abstract

Anopheles darlingi is the main vector of malaria in Brazil, characterized by a high level of anthropophilia and endophagy. Imidacloprid, thiacloprid, and acetamiprid are the most widespread insecticides of the neonicotinoid group. However, they produce adverse effects on the non-target insects. Flupyradifurone has been marketed as an alternative to non-fluorinated neonicotinoids. Neonicotinoids containing trifluoroacetyl substituent reveal increased insecticidal activity due to higher hydrophobicity and metabolic stability.

We synthesized novel neonicotinoid insecticides containing fluorinated acceptor groups and their interactions were estimated with the nicotinic acetylcholine receptor (nAChR) binding site by molecular docking studies, to evaluate their larvicidal activity against A. darlingi, and to assess their outdoor photodegradation behavior. New neonicotinoid analogues were prepared and characterized by NMR and mass-spectrometry. The synthesized molecules were modelled by time-dependent density functional theory and analyzed, their interaction with nAChR was investigated by molecular docking. Their insecticide activity was tested on Anopheles larvae collected in suburban area of Manaus, Brazil. Four new fluorinated neonicotinoid analogs were prepared and tested against 3rd instars larvae of A. darlingi showing high larvicidal activity. Docking studies reveal binding modes of the synthesized compounds and suggest that their insecticidal potency is governed by specific interactions with the receptor binding site and enhanced lipophilicity. 2-Chloro-5-(2-trifluoromethyl-pyrrolidin-1-ylmethyl)pyridine 5 showed fast degradation in water maintaining high larvicidal activity. All obtained substances possessed high larvicidal activity in low concentrations in 48 hours of exposure, compared to commercial flupyradifurone. Such activity is connected to a unique binding pattern of the synthesized compounds to insect’s nAChR and to their enhanced bioavailability owing to introduction of fluorinated amino-moieties. Therefore, the compounds in question have a high potential for application as control agents for insects transmitting tropical diseases, and they will be less persistent in the environment.
Introduction

Malaria is a parasitic and endemic disease in several regions of the world with wide distribution mainly in tropical and subtropical areas. Currently, more than 3 billion people are at risk of contracting the disease and about 148 to 304 million of new cases are recorded annually [1].

Anopheles darlingi Root 1926 is the main vector of malaria in Brazil. On one hand, the species is characterized by a high level of anthropophily and endophagy in relation to other mosquito species of the Amazon region. On the other hand, A. darlingi is the malaria transmitting species the most favored by environmental changes resulting from human activities such as occupation of urban and peri-urban spaces in an uncontrolled manner; the construction of hydroelectric power plants, irrigation projects, and fishponds; the exploitation of fossil fuels, minerals, and natural gas; forest fires; deforestation; and road construction [2–5].

Mosquito vector control measures are the most commonly used approach to reduce the number of malaria cases. In relation to entomological data, the above measures include such traditional actions to combat vector-borne diseases as space spraying, indoor residual spraying, long-lasting insecticidal nets, larval source management, biolarvicide application, active search, and appropriate case treatment [6,7].

Management of larval breeding sites plays an important role in the control of malaria. Especially in those specific sites, where the vectors tend to reproduce, which may have characteristics of permanent or semi-permanent breeding sites. The WHO suggests the use of larvicides in those breeding sites located in urban areas, to protect the human population [5,8–10]. Larvicide control actions represent an effective tool for vector control, reducing the transmission of malaria in specific areas. This procedure is particularly important in the Amazonian region, where different breeding sites of malaria vectors are widely distributed [9].

According to the WHO recommendations, only the use of pyrethroid insecticides is indicated for the actions on adult mosquitoes in current vector control program [8,9]. Recently, resistance of the vector to these insecticides were reported in some areas, making research targeted at new classes of insecticides highly relevant [8,11].

The above dependence on the only one class of insecticides can be potentially overcome by implementation of neonicotinoids, a promising class of insecticides that exhibit excellent efficiency and low risk to humans and the environment. They are extensively used throughout the world for crop protection, particularly against sucking insect pests, accounting for one-fourth of the total world insecticide market [12]. Imidacloprid, thiacloprid, and acetamiprid are the most widespread insecticides of the neonicotinoid group. Toxicity of neonicotinoids to mammals is usually much lower than for many insect species. One can find reports on adverse effects of several widely adopted neonicotinoids on the non-target insects (especially honeybees) [13,14]. Prolonged periods of exposure of aquatic insects to neonicotinoids even though at low levels, has given rise to serious risks, since those insects are as sensitive to that class of insecticides, as the bees. Recently, imidacloprid toxic residues have been detected in water surfaces and are associated with the reduction of aquatic insect populations [15,16].

By that reason, the imidacloprid, thiamethoxam, and clothianidin were banned for use on flowering crops in the EU for the two-year term in 2013 [17,18]. After thorough risk assessment, the three pesticides were completely banned in the EU for outdoor use on crops since 2018 [19–21]. At the same time, it was established that several neonicotinoids (e.g. acetamiprid) represent low risk to honeybees [22,23], thus any further restrictions of those substances is neither scientifically nor legally appropriate [24]. Negative effects were mostly caused by improper and excessive use of the insecticides, or laboratory-based experiments that employed greater concentrations of the neonicotinoids than those found in nectar and pollen of the treated plants [17].
Flupyradifurone (FPF, Fig 1) is a new butenolide insecticide chemically similar to neonicotinoids, despite it has been marketed as an alternative to them for commercial reasons [25]. Both FPF and neonicotinoids have the same mode of action being a nicotinic acetylcholine receptor (nAChR) antagonists. The FPF possess low toxicity to non-target insects and mammals, and has recently been registered for use in Canada and the United States [26]. Like neonicotinoids, the FPF is highly water soluble and persistent in the environment [27]. However, it is practically non-toxic to young adult bees when in acute contact [28].

Agricultural and public health (vector control) importance of neonicotinoids have raised the need of their further development, taking into a consideration their mechanism of action. The nAChR is a biochemical target for neonicotinoids, it plays a central role in the mediation of fast excitatory synaptic transmission in the insect central nervous system [12]. Structural differences of nAChRs receptor subunits between vertebrate and in the invertebrate species [29] provides relatively low risk of neonicotinoids to mammals [29]. Interactions of the active compound with the nAChR may be efficiently investigated using the whole gamut of in silico methods, from inexpensive quantitative structure-activity relationships (QSAR) to computer docking and hybrid quantum and molecular mechanics (QM/MM) approaches [18,30–32].

In this study, we perform modeling of nAChR-ligand interactions via molecular docking. By analyzing various binding conformations and quick estimation of binding affinity for each of them, molecular docking allows finding of the best binding mode for virtually any potential ligand. This way we can rank compounds according to the strength of their interaction with the receptor and find specific intermolecular interactions that are important for the activity of a potential insecticide [33].

Trifluoromethyl and perfluoroalkyl groups can dramatically affect functional properties of organic molecules, thereby increasing a possibility of their application as agrochemicals, medicines or building blocks to create new organic materials [34–36].

Rationale for the introduction of fluorine atoms is based on the following reasons:

- **Metabolic stability** is one of the key factors in determining the bioavailability of a compound. A frequently employed strategy to circumvent this problem is to block the reactive site by a fluorine atom introduction. There are many examples illustrating an increase of a molecule metabolic stability by replacement of an oxidizable C-H group with a C-F one [37–40].
Fluorine can change basicity of a compound. Presence of highly basic groups may limit bioavailability of a molecule. Introduction of the fluorine atom or fluorinated group in the vicinity of the basic moiety reduces the basicity. Thus, better membrane permeation and improved bioavailability may be expected for the fluorinated compound [41].

Moreover, fluorine substituents are introduced to increase receptor-binding affinity of a compound. For example, most of the neurokinin1 receptor antagonist drugs contain a 3,5-di(trifluoromethyl)phenyl group to increase the binding affinity [42].

Earlier, it was shown that neonicotinoids containing trifluoroacetyl substituent reveal increased insecticidal activity due to higher hydrophobicity and metabolic stability [43–45]. Besides that, the presence of an acceptor substituent (such as a nitro- or a cyano-group) in a terminal moiety is one of the most important factors determining the insecticide activity [29].

Some pesticides have an obvious drawback, because of their possible persistence in the environment. In addition to being environmental contaminants, persistent insecticides develop a resistance to them much more frequently than non-persistent ones. Sunlight photodegradation is one of the most destructive pathways for pesticides after their release into the environment [46]. However, the biocidal activity should be preserved before the photodegradation occurs [47]. Several studies have been devoted to the investigation of the photolysis of the neonicotinoids [48–50]. Half-life of the FPF in water under sunlight was estimated to be as high as 2.5 days [51]. Therefore, reduced photostability becomes an important requirement in the design of new neonicotinoid insecticides.

During last few years we have developed synthetic approaches to generate novel low-molecular weight fluorinated amines and investigated their properties [52–54]. Substances of that kind have never been used as neonicotinoid building blocks. Therefore, the aim of this work was to synthesize new neonicotinoid insecticides containing fluorinated acceptor groups to estimate the interactions of the synthesized compounds with the nAChR binding site by molecular docking studies, to evaluate their larvicidal activity against A. darlingi, and to assess their outdoor photodegradation behavior.

Materials and methods
3-trifluoromethylaniline was purchased from Acros Organics, all other reagents were purchased from Sigma-Aldrich and used as supplied. The solvents (Aldrich) were purified by standard procedures used in synthetic organic chemistry.

Spectroscopy
$^{1}H$ NMR spectra were recorded on a Varian VXR-300 and Mercury 300 spectrometers, $^{19}F$ NMR spectra were obtained on Gemini 200 Varian instrument at 188 MHz. UV- spectra were recorded on an UV-Vis Evolution 220 spectrophotometer (Thermo Fischer Scientific) in the range of 200–500 nm at a bandwidth of 2nm and integration time of 1s.

General synthetic procedures

a) synthesis of fluorosubstituted anilines. 3,5- Bis-trifluoromethylaniline was obtained following the two-step procedure with a total yield of 56% [55,56]. First, the 5-nitroisophthalic acid was fluorinated with SF$_4$ taken in a 1:5 molar ratio at 80–90°C for 16 hours in a presence of 10% of HF as a catalyst. The 3,5-bis(trifluoromethyl)nitrobenzene obtained was then reduced with hydrogen in a methanol solution over 10% Pd/C catalyst. The product was separated by distillation and identified spectroscopically. The spectra correspond to those described in [56].
4-(pentfluoroethoxy)aniline. 4-(pentfluoroethoxy)nitrobenzene was obtained according to the previously described procedure [57]. The fluorination product was then reduced with hydrogen in a methanol solution over 10% Pd/C catalyst, separated by distillation, and identified spectroscopically. The spectra correspond to those described in [58]. The total yield after two steps is 71%.

2-(trifluoromethyl)pyrrolidine was obtained according to our previously described procedure [52].

b) synthesis of neonicotinoids. New neonicotinoids were synthesized as shown in the Fig 2 according to the protocol [http://dx.doi.org/10.17504/protocols.io.9h5h386].

A mixture of 2-chloro-5-(chloromethyl)pyridine 1 (0.324 g, 1.9 mmol), the corresponding substituted aniline 2a-c or 4 (2.0 mmol), and anhydrous potassium carbonate (0.828 g, 6.0 mmol) in 5 ml of anhydrous acetonitrile was vigorously stirred under reflux (Fig 2). Progress of the reaction was monitored by thin layer chromatography (TLC). After the conversion was complete, the reaction mixture was separated from insoluble inorganic salts. The latter were thoroughly washed with dichloromethane. The combined organic solutions were evaporated under reduced pressure and the residue was purified by preparative thin layer chromatography (TLC) on SiO$_2$ (EtOAc/hexane 1:2).

Fig 2. Synthesis of new neonicotinoids containing fluorinated acceptor groups.

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N-[(6-chloropyridin-3-yl)methyl]-4-(pentafluoroethoxy)aniline (3a). Yield 68%. Light-yellow viscous oil. \( ^1H \) NMR (300 MHz, CDCl\(_3\), TMS), \( \delta \): 3.93 (br. s, 1H, NH); 4.26 (s, 2H, CH\(_2\)); 6.49 (d, 2H, C\(_3\)H, C\(_5\)H); 6.79 (d, 2H, C\(_2\)H, C\(_6\)H); 7.23 (d, 1H, C\(_1\)H); 7.58 (m, 1H, C\(_2\)H); 8.31 (s, 1H, C\(_5\)H). \( ^{19}F \) NMR (188 MHz, CDCl\(_3\), CCl\(_3\)F), \( \delta \): -86.46 (s, 3F, CF\(_3\)); -88.41 (s, 2F, CF\(_2\)). ME (EI) m/z 353 (M+1, 100%).

N-[(6-chloropyridin-3-yl)methyl]-3-(trifluoromethyl)aniline (3b). Yield 61%. Light-yellow viscous oil. \( ^1H \) NMR (300 MHz, CDCl\(_3\), TMS), \( \delta \): 4.15 (br. s, 1H, NH); 4.29 (s, 2H, CH\(_2\)); 6.64 (d, 1H, C\(_6\)H); 6.74 (s, 1H, C\(_5\)H); 6.89 (d, 1H, C\(_4\)H); 7.16 (d, 1H, C\(_2\)H); 7.22 (d, 1H, C\(_3\)H); 7.57 (m, 1H, C\(_2\)H); 8.23 (s, 1H, C\(_5\)H). \( ^{19}F \) NMR (188 MHz, CDCl\(_3\), CCl\(_3\)F), \( \delta \): -62.09 (s). ME (EI) m/z 287 (M+1, 100%).

N-[(6-chloropyridin-3-yl)methyl]-3,5-bis(trifluoromethyl)aniline (3c). Yield 73%. Yellowish viscous oil. \( ^1H \) NMR (300 MHz, CDCl\(_3\), TMS), \( \delta \): 3.59 (br. s, 1H, NH); 4.34 (s, 2H, CH\(_2\)); 6.89 (s, 2H, C\(_2\)H, C\(_5\)H); 7.13 (s, 1H, C\(_4\)H); 7.26 (d, 1H, C\(_3\)H); 7.58 (m, 1H, C\(_2\)H); 8.32 (s, 1H, C\(_5\)H). \( ^{19}F \) NMR (188 MHz, CDCl\(_3\), CCl\(_3\)F), \( \delta \), m. d.: -62.55 (s). ME (EI) m/z 355 (M+1, 100%).

2-Chloro-5-(2-trifluoromethyl-pyrrolidin-1-ylmethyl)pyridine (5). Yield 83%. Light-yellow viscous oil. \( ^1H \) NMR (300 MHz, CDCl\(_3\), TMS), \( \delta \): 1.82 (m, 2H, CH\(_2\)C\(_2\)H\(_2\)CH\(_2\)pyrrolidine), 2.01 (m, 2H, C\(_2\)H\(_2\)CHCF\(_3\)), 2.36 (m, 1H, CHCF\(_3\)), 2.93 (m, 1H, CH\(_2\)N pyrrolidine), 3.28 (m, 1H, C\(_2\)H\(_2\)N pyrrolidine), 3.88 (ABX, \( ^3J_{HH} = 153.4 \text{ Hz}, ^4J_{HH} = 13.6 \text{ Hz}, 2\text{H, ArCH}_2\text{N} \)), 7.29 (d, 1H, H-5 pyridine), 7.69 (dd, 1H, H-4 pyridine), 8.29 (d, 1H, H-3 pyridine). \( ^{19}F \) NMR (188 MHz, CDCl\(_3\), CCl\(_3\)F), -77.03 (s broad, 6F, C\(_F\)_3); ME (EI) m/z 265 (M+1, 100%).

Calculation details

Electronic population analysis was conducted within the Gaussian-09 set of programs [59] using BP-86/TZVP approximation [60–62]. Multiwfn program was used for electrostatic potential (ESP) surfaces generation [63,64]. The TD-DFT calculations were carried out at the BP86/6-311G** approximation level using the Gaussian-03 program package. Molecular docking was carried out using AutoDock Vina package [65] and analyzed in AutoDockTools4 [66,67], VMD [68], and BIOVIA Discovery Studio 4.5 [69] programs were used for ESP surfaces and docking results visualization.

Ethical considerations

The larvae used in this study were collected in suburban areas of the city of Manaus, Amazonas, Brazil. We declare that no permission was required for these locations. In addition, the collecting of anopheline larvae did not involve endangered or protected species.

Test organism

Anopheles larvae were collected in suburban areas of the city of Manaus, Amazonas, Brazil and transported to insectary of the Laboratory of Malaria and Dengue at the National Institute of Amazonian Research (INPA). The mosquito larvae were reared according to the protocols described in [70]. Species of the larvae were identified after the realization of the bioassays. All 7,920 larvae used in the bioassays were identified at the species level according to their morphological characteristics following the taxonomic keys of [71–73]. The specimens were placed in Petri dishes containing a few drops of larval fixative prepared according to the protocol adopted at the Laboratory of Malaria and Dengue of the INPA (http://dx.doi.org/10.17504/protocols.io.9qrh5v6) and observed under an optical microscope with magnifications of 100× to 400×. The identification of species was made based on the external morphological aspects, such as spiracular apparatus, head, antenna, apex, abdominal segments, palmate hairs, set of prothoracic hairs, thorax, dorsal view, and ventral integument appearance.
The colony was maintained at a temperature of 26 ± 2°C, a relative humidity around 70–80%, a photoperiod of 12:12 hours [70], and fed with a Tetramin® fish food.

**Larvicidal activity**

The larvicidal activity tests for new substances were performed on the 3rd instar *Anopheles* larvae according to a methodology recommended by the WHO [74,75] https://dx.doi.org/10.17504/protocols.io.9kch4sw. All the experiments were carried out at the temperature of 26 ±2°C, the relative humidity of 70–80% and the photoperiod of 12:12 hours light/dark. For each substance, a stock solution in dimethylsulfoxide (DMSO) was prepared with a concentration of 2000 μg/mL followed by a serial dilution to obtain 3.0, 5.0, 10.0, 20.0, and 30.0 μg/mL for 3a; 2.5, 4.5, 9.5, 14.0, and 19.0 μg/mL for 3b; 2.5, 4.5, 9.0, 14.0, and 18.0 μg/mL for 3c; 6.0, 8.0, 10.0, 12.0, and 14.0 μg/mL for 5; and 2.0, 3.0, 4.0, 5.0, and 6.0 μg/mL for FPF.

The experiment was performed in five replicates of each dosage and two controls: one of them containing 1.0% aqueous solution of DMSO (negative control) and the other one containing flupyradifurone (Sigma Aldrich®) as a positive control. Twenty larvae of the 3rd instar were transferred into each dish of replicas and controls containing 50 mL of distilled water, food, and an aliquot of the stock solution in an appropriate amount for different concentrations. The larval mortalities were observed and registered at 48 hour intervals of exposure. Mortality rate of 10% and a confidence interval of 95% were set as the limits. The tests were repeated 3 times. The larvae were considered dead, when they were immobile and unable to reach the water surface.

**Statistical analysis**

In order to obtain LC50, the mortality data were treated by a statistical software Polo plus [76], with 95% confidence interval and values of p < 0.05 were considered statistically significant. In the case of ≥10% mortality in the control group, the larval mortality was corrected using Abbott’s formula [77].

**Photodegradation test**

40 mg/L solution of 5 was prepared using a deionized milli-Q water. The experiment was performed in Manaus (AM, Brazil; -3.096240°S, -59.986194°W; under an average daily solar irradiation of 4.92 kWh/m², http://www.cresesb.cepel.br/index.php#data). The test solution was placed into a quartz cuvette and tightly stopped there. The cuvette was placed under a direct sunlight, maintaining the temperature at 35°C. Quantity of the compound 5 in the solution was monitored by UV-spectrophotometry, using Thermo Scientific Evolution 220- UV-vis spectrophotometer; Software INSIGHT: 1.3.10; Firmware 3.0.0.109; Scan Speed 120.00 nm/ min; data Interval 1.00 nm; Integration Time 0.500 sec; Bandwidth 2 nm, following the absorption maximum at 268 nm. The spectra were recorded at different time intervals of 1h - 4h, and 1–4 days from 09:00 until 16:00 (i.e. 7 h each day) and analyzed according to [78].

**Results**

New fluorinated neonicotinoids 3a-c and 5 (Fig 3) were synthesized from 2-chloro-(5-chloromethyl)pyridine 1 and the corresponding fluorinated aromatic or alicyclic amines as shown in the Fig 2. The starting amines 2a-c or 4 were refluxed with chloropyridine 1 in acetonitrile in the presence of potassium carbonate. Light-yellow viscous oils of the final compounds were obtained after their purification by preparative TLC.
In order to estimate structure-properties relationship for the substances in question, we performed DFT calculations of the molecules and generated their electrostatic potential surfaces (ESP) (Fig 4).

The ESP maps visualize electrostatic potential energy, i.e. energy of interaction between the molecule and an imaginary positively charged (+1) ion. Color scale in the right side of the figure shows a correspondence between the surface color and the interaction energy. Positive values (red) indicate higher interaction energy and therefore electron-poor positively charged part of the molecule. Negative values (blue) display opposite characteristics. Prevalence of the red and blue colors in the ESP maps evidence a polar character of the molecule, and appropriately colored regions point at regions of the structure that will more likely interact with positively (blue parts of the map) or negatively (red parts) charged groups of the receptor active site. Intermediate values of interaction energy (green) indicate weakly polar hydrophobic parts of the molecule.

Possible binding models of the synthesized neonicotinoids with nAChR were examined by docking studies with AutoDock Vina [65] and shown in the Fig 5. Since it is known that amino acids forming binding pockets are conserved in AChBP (acetylcholine binding proteins), a crystal structure of a Lymnaea stagnalis AChBP co-crystallized with imidacloprid (PDB ID: 2zju) [79] was used as the receptor template.

The calculations also enabled to estimate the protein-ligand binding affinities and partition coefficients P shown in the Table 1.
Approximately 7'920 Anopheles larvae were collected in suburban areas of the city of Manaus (AM), Brazil. After the tests were performed successfully, all larvae were identified to verify the percentage of the species present. *A. darlingi* was the predominant species representing 77.38% of the larvae used in the tests. The remaining percentage was distributed among

Table 1. Estimated binding affinities and calculated logP values for the compounds 3a-c, 5, and FPF.

| Compound | Predicted binding affinity, kJ/mol | logP  |
|----------|------------------------------------|-------|
| 3a       | -33.472                            | 4.42  |
| 3b       | -35.146                            | 3.65  |
| 3c       | -34.727                            | 4.53  |
| 5        | -30.125                            | 2.94  |
| FPF      | -29.288                            | 1.84  |

P = partition coefficient

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the following species: *A. nuneztovari* (16.36%), *A. triannulatus* (5.08%), *A. albitarsis* (0.80%), *A. oswaldoi* (0.20%), *A. evansae* (0.11%), *A. matogrossensis* (0.03%), and *A. nimbus* (0.01%).

The larvicidal activity of all synthesized compounds were evaluated, showing high mortality of the 3rd instar larvae of *A. darlingi* (Table 2). The highest larval mortality rates; i.e. the lowest LC$_{50}$ values were observed for 3a, 3c and 3b with the LC$_{50}$ values of 0.57, 0.90 and 0.97 µg/mL respectively, which are lower than that of the commercially available FPF standard (LC$_{50}$ = 1.18 µg/mL). Within the series under investigation, the highest LC$_{50}$ value of 4.93 µg/mL was found for the compound 5. No mortality was observed in the negative control.

The dose-response graphs (Fig 6) show scatter plots of the experimental data on percentage mortality of *A. darlingi* larvae vs concentration and the trendlines for each of the compounds under investigation and the FPF. The coefficients of determination R$^2$ show perfect fit of the data points by the regression trendlines. According to the graph, all tested compounds showed stable very high larval mortality rates (more than 70%) starting from the lowest concentrations applied. Thus, the data show high efficiency of the new substances against larvae of the main vector of malaria in the Amazonian region. The sample of commercial FPF insecticide was responsible for the highest larvae mortality. This implies that FPF was the compound showing the highest stability of action in the 48-hour interval, because it maintains its high larvicidal efficacy in the first doses tested (Fig 6). The compound 5 revealed a similar behavior, being the most efficient compound within the series under investigation. The compound 5 achieved 100% mortality rates at doses nearly 2 times lower than the compounds 3a-c. By that reason, it was chosen for a photodegradation test under real outdoor conditions in the city of Manaus (AM, Brazil).

The photodegradation was monitored by UV spectra. The UV spectra of 5 in aqueous solution showed absorption band maxima of 210 nm and 267 nm, which correspond well to the values predicted by TD-DFT calculations (Fig 7A). The outdoor photodegradation results are shown in the Fig 7B. As observed in the Fig 7, the compound starts to degrade shortly after 2 hours of exposure. The half-life of the compounds 5 was assessed from 0 h to 4 days of direct exposure to sunlight. The photodegradation half-life time for 5 (DT$_{50}$ = 11.9 h) was derived based on UV/VIS spectral data according to the US Environmental Protection Agency standard operating procedure [78]. Comparison of residual deviances of the first- and second-order kinetic models shows that a single first-order rate model can be considered appropriate.

**Discussion**

Various habitat forms, favorable environmental conditions for the development of mosquitoes, and their distribution in the environment, are the main factors that favor the diversity of species of the genus *Anopheles* in the Amazon region. The anophelines collected in the study region include *A. darlingi*, *A. nuneztovari*, *A. triannulatus*, *A. albitarsis*, *A. oswaldoi*, *A. evansae*, *A. matogrossensis*, and *A. nimbus*; all had previously been reported in the literature [2,3].
However, this diversity may vary according to the hydrological cycle, a phenomenon that influences the seasonality of species; also, a variety of different breeding sites, the intensification of human/vector contact, and the recorded cases of malaria have to be taken into account [5,80]. The dominance of certain anopheline species has also been related to their major ability to adapt to various environmental transformations, especially in the ecosystems altered by anthropic actions and environmental changes, such as increased exposure to sunlight and absence of frequently growing aquatic plants. The above factors affect the development of the mosquito larvae and cause a direct impact on the presence of different species of the same genus [80,81]. This study showed that *A. darlingi* was the dominant species in the collection area. It is considered the main malaria vector in the region, being more susceptible to infection by *Plasmodium spp.* parasites [80,81].

In the present study, we synthesized a series of new fluorinated neonicotinoid analogs starting from some novel amines containing -CF₃ and -OC₂F₅ groups. Introduction of fluorine into a biologically active molecule increases its biological activity by affecting several
parameters, e.g. binding to a target receptor. We suppose the presence of fluorinated amino-moieties will improve hydrophobic interactions with the receptor site.

Introduction of fluorine into a biologically active molecule increases its biological activity by affecting several parameters, e.g. binding to a target receptor. Thus, we synthesized a series of new fluorinated neonicotinoid analogs starting from some novel amines containing -CF$_3$ and -OC$_2$F$_5$ groups. We suppose the presence of fluorinated amino-moieties will improve hydrophobic interactions with the receptor site.

Fig 7. UV spectra. (a) spectra of the compound 5 and FPF. (b) Dependence of the absorption (at $\lambda_{\text{max}} = 268$ nm) from the duration of irradiation.

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In order to find structural features that may contribute to activity of the synthesized compounds compared to the FPF, the calculations of the electrostatic potential surfaces and molecular docking were carried out. Comparison of the molecular surface maps (Fig 4) shows a wide diversity of configurations for the compounds under investigation. The molecules of 3a-b are bulkier than that of the FPF, a commercially available insecticide. Earlier, it was shown that bulky neonicotinoid analogs often have insecticidal potencies comparable to or even higher than popular commercial insecticides [31,43,82–84]. Such bulky substances can engage more regions of the ligand-binding domain of the nAChRs than conventional ones, or even dock to entirely different locations of those receptors [85,86].

Comparison of the ESP maps for the compounds 3a-c and 5 with that of FPF (Fig 4) reveals two main distinctive features of the synthesized neonicotinoid analogues. First, the electronic density distribution and shape of the molecular surfaces vary substantially within the series and considerably differ from those of the FPF. Second, all the substances 3a-c and 5 have enlarged weakly polar (green) lipophilic areas, capable to hydrophobic interactions with the receptor active site.

Binding modes of the synthesized neonicotinoids 3a-c and 5 to nAChR are shown in the Fig 5. General feature of fluoroalkyl-phenyl substituted compounds 3a-c is represented by orientation of the fluorinated groups and phenyl moiety to the hydrophobic region of the receptor binding pocket consisting of five aromatic amino acid residues—TRP53, TRP143, TYR164, TYR185 and TYR192. They contribute to the π-stacking interactions with phenyl rings at the distances of ca. 3.6 Å (that is within a usual range for parallel displaced π-stacking mode) [87] and to hydrophobic interactions with fluorinated groups. The stacking seems to have a large contribution to binding affinities, since the molecule of pyrrolydine derivative 5 is turned over, when compared to 3a-c ones. Thus, the pyridine moiety can participate in stacking interaction with TYR185 phenyl ring. Three out of four compounds under discussion also take part in S–π interactions with MET114 sulfur atom at a distance of ca. 3.5 Å. The compound 3a falls out of the row, evidently, because of the large volume of the -OC\textsubscript{2}F\textsubscript{5} group. However, it is oriented similarly to the 3b and 3c allowing the hydrophobic interactions between the fluorine atoms of the neonicotinoid and aromatic amino acid residues.

Besides the non-covalent interactions described above, a hydrogen bonding between the NH-moiety of the neonicotinoid and a hydroxyl group of the TYR192 is present for 3c and 3b compounds. That bonding enhances stability of the receptor-ligand complex that is immediately seen in the results of molecular docking (compounds 3a,b have the highest affinities). Measured larvicidal activities confirm the docking results, both compounds have low LC\textsubscript{50} values.

According to the docking calculation results, the best predicted binding affinities do not vary significantly, being in the range of -30 ÷ -35 kJ/mol (Table 1). The LC\textsubscript{50} values determined generally decrease along with a growing interaction strength. Fluorine substitution greatly enhances the lipophilicity of the amines employed for the synthesis. A common measure of lipophilicity of a compound is the partition coefficient P, i.e. ratio of its equilibrium concentrations in two immiscible liquids, usually octanol and water. Calculated logP values for the compounds under investigation and the FPF are shown in the Table 1. Comparison of the logP values for the compound 3b having one CF\textsubscript{3} group and the 3c one with two CF\textsubscript{3} groups shows that fluorine substitution has a substantial influence on the lipophilicity of the compound. Lethal concentrations of the substances 3a-c, 5 decrease with the increase of the calculated logP. The above results lead to an additional explanation of high larvicidal activity of the neonicotinoids in question. In addition to the binding strength to the insect’s nAChR, the enhanced activity of compounds 3a-c, and 5 could also result from their high bioavailability due to facile blood-brain barrier crossing [29].
Position of the compound 5 at the receptor binding site is also very similar to that of flupyr-adifurone (Fig 8), which indicates similar receptor-ligand interactions of these compounds. CF$_3$-group in 5 is a substitution of electronegative lactone pharmacophore of the FPF, while conformational mobility of 5 allows it to achieve simultaneous hydrogen bonding, aromatic interactions, and halogen bond.

Very high larvicidal activity of the new substances was confirmed in the test using *A. darlingi* larvae. The compound 3a showed the best larvicidal potential ($LC_{50} = 0.57 \mu g/mL$) among the compounds under investigation, considering higher mortality of the larvae during the 48 h interval, even when compared to the FPF positive control $LC_{50} = 1.18 \mu g/mL$.

Since the compounds under investigation promoted a high mortality in contact with the larvae, they may be considered as potential larvicides. However, more specific tests are necessary to evaluate the mechanism of action and to classify them as possible insecticides to control the vectors of malaria in the Amazonian region.

Larvae of *Anopheles* mosquitos prefer to live in clean, unpolluted water. Despite a high insecticidal potential of neonicotinoids for vector control applications outdoors, quite a little is known about direct sunlight photodegradation in water of neonicotinoid insecticides containing fluorinated acceptor groups.

Since the habitat of *Anopheles* larvae is water, the eventual use of the compounds under real outdoor conditions may be modelled in an aqueous solution under intense natural sunlight and elevated environmental temperature, typical for the region. In this work we studied experimentally a photodegradation of 5 under real outdoor conditions (sunlight, environment, temperature) (Fig 7B) and compared those data to similar data for the FPF [25,88]. A reported DT$_{50}$ value for the FPF (13.8 h) [88] is slightly higher than for the compound 5 (11.9 h). Hence, the 2-chloro-5-(2-trifluoromethyl-pyrrolidin-1-ylmethyl)pyridine would be less persistent in the environment.

Rather often, continuous and indiscriminate use of persistent in the environment insecticides in inadequate concentrations has led to the development of insect resistance to agents...
currently used in vector control. In the areas of Amazon region with high incidence of malaria, the application of insecticides is restricted to round the house places and some mosquito breeding site places. The former method is not very effective, since the houses are scattered in rural areas, and thus, the impact is minimal and should be monitored regularly. The latter is only permitted on a non-continuous base, when malaria outbreak happens and it is not possible to permanently eliminate the breeding site [9,89]. In such a way, environmental contamination may be avoided.

The fluorinated neonicotinoids under investigation showed high efficiency and rapid photodegradation; hence, they may be regarded as a viable option for insect vector control in places, where the resistance was observed. Another important safety factor is related to higher selectivity of neonicotinoids to the mosquito nAChRs [12,29] with respect to the mammalian ones.

The above would require further testing of the proposed compounds for their action on non-target organisms and persistence in the environment to ensure a more selective spectrum of action.

**Conclusion**

Our results showed that the *A. darlingi* larvae were highly susceptible to new neonicotinoid analogs 3a-c and 5 containing fluorinated acceptor groups. The substances 3a-c revealed higher larvicidal activity at low concentrations in 48 hours of exposure, when compared to flupyradifurone. The reason of such activity may be connected to the unique binding pattern of the synthesized compounds to insect’s nAChR and to their enhanced bioavailability owing to introduction of fluorinated amino-moieties. The larvicidal activity tests showed the LC$_{50}$ values that are in a good agreement with the theoretically predicted binding of the active compound to the nAChR. The photodegradation of 5 was studied under real outdoor conditions (sunlight, environment, temperature) and compared to the FPF data. The compound 5 degrades rapidly in water under direct sunlight, the DT$_{50}$ value found was slightly lower than that of the FPF. Thus, in view of all the data reported in this study, the compound 5 has a lower persistence time in water without reducing its efficacy as a larvicide. However, further testing is required to evaluate those and other factors in real field conditions.

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References
1. World malaria report 2016 [Internet]. Geneva; 2016. Available from: http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1
2. Tadei WP, Santos JMM dos, Scarpassa VM, Rodrigues IB. Incidência, distribuição e aspectos ecológicos de espécies de *Anopheles* (Diptera: Culicidae) em regiões naturais e sob impacto ambiental da Amazônia brasileira. In: Ferreira EJG, Santos GM, Leão ELM, Oliveira LA, editors. Bases Científicas para Estratégias Preserv e Desenvolv da Amaz. Secretaria de Ciência e Tecnologia, Instituto Nacional de Pesquisas da Amazônia; 1993. p. 167–96.
3. Deane LM. Malaria vectors in Brazil. Mem. Inst. Oswaldo Cruz. 1986. p. 5–14.
4. Tadei WP, Santos JMM, Rodrigues IB, Rafael MS. Laboratório de Vetores da Malária e Dengue. Ministério da Ciência e Tecnologia–MCT Pesquisa e Ações em Saúde nos Institutos Pesquisa do Ministério da Ciência e Tecnol. Brasília, Distrito Federal; 2010.
5. Tadei WP, Rodrigues IB, Rafael MS, Sampaio RTM, Mesquita HG, Pinheiro VCS, et al. Adaptive processes, control measures, genetic background, and resilience of malaria vectors and environmental changes in the Amazon region. Hydrobiologia. 2017; 789:179–96.
6. Global malaria control and elimination—Report of a technical review. World Health Organization; 2008.
7. Guia prático de tratamento da malária no Brasil. Brasília: Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica.; 2010.
8. World Health Organization. Division of Malaria and Other Parasitic Diseases. From Malaria Control to Malaria Elimination: A Manual for Elimination Scenario Planning. World Health Organization; 2014.
9. World Health Organization. Larval source management: a supplementary measure for malaria vector control: an operational manual. Geneva: World Health Organization; 2013. p. 116. Available from: www.who.int/malaria/publications/atoz/9789241505604/en/
10. Maheu-Giroux M, Castro MC. Impact of Community-Based Larviciding on the Prevalence of Malaria Infection in Dar es Salaam, Tanzania. Beeson JG, editor. PLoS One. 2013; 8:e71638. https://doi.org/10.1371/journal.pone.0071638 PMID: 23977099
11. Rose RI. Pesticides and Public Health: Integrated Methods of Mosquito Management. Emerg Infect Dis J. 2001; 7:17.
12. Jeschke P, Nauen R, Schindler M, Elbert A. Overview of the status and global strategy for neonicotinoids. J. Agric. Food Chem. 2011; 59:2897–908. https://doi.org/10.1021/jf103499x PMID: 20565065
13. Decourtye A, Devillers J. Ecotoxicity of Neonicotinoid Insecticides to Bees. Insect Nicotinic Acetylcholine Recept. 2010. p. 85–95.
14. Tsvetkov N, Samson-Robert O, Sood K, Patel HS, Malena DA, Gaiwala PH, et al. Chronic exposure to neonicotinoids reduces honey bee health near corn crops. Science (80-). 2017; 356:1395–7.
15. Morrissey CA, Mineau P, Devries JH, Sanchez-Bayo F, Liess M, Cavallaro MC, et al. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. Environ Int. 2015; 74:291–303. https://doi.org/10.1016/j.envint.2014.10.024 PMID: 25454246
16. Van Dijk TC, Van Staalduinen MA, Van der Sluijs JP. Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid. Desneux N, editor. PLoS One. 2013; 8:e62374.
17. Kessler SC, Tiedeken EJ, Simcock KL, Derveau S, Mitchell J, Softley S, et al. Bees prefer foods containing neonicotinoid pesticides. Nature. 2015; 521:74–6. https://doi.org/10.1038/nature14414 PMID: 25901684
18. Shao X, Lee PW, Liu Z, Xu X, Li Z, Qian X. cis-Configuration: a new tactic/rationale for neonicotinoid molecular design. J Agric Food Chem. 2011; 59:2943–9. https://doi.org/10.1021/jf103499x PMID: 21043520
19. The European Commission. Commission Implementing Regulation (EU) 2018/783 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance imidaclopridText with EEA relevance. Off J Eur Union. 2018; 132:31–4. Available
New fluorinated neonicotinoids against Anopheles darlingi larvae

from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2018.132.01.0031.01.ENG&toc=OJ:L:2018:132:TOC

20. The European Commission. Commission Implementing Regulation (EU) 2018/785 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance thiamethoxam Text with EEA relevance. Off J Eur Union. 2018; 61:40–4. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2018.132.01.0040.01.ENG&toc=OJ:L:2018:132:TOC

21. The European Commission. Commission Implementing Regulation (EU) 2018/784 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance clothianidin Text with EEA relevance. Off J Eur Union. 2018; 61:35–9. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2018.132.01.0035.01.ENG&toc=OJ:L:2018:132:TOC

22. Peer review of the pesticide risk assessment of the active substance acetamiprid. EFSA J. 2016; 14(11):1–26.

23. The European Commission. Commission Implementing Regulation (EU) 2018/113 of 24 January 2018 renewing the approval of the active substanc e acetamiprid in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant pro. Off J Eur Union. 2018; 61:7–10. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32018R0113&from=EN

24. European Commi. Current status of the neonicotinoids in the EU. EU Pestic. Database. 2019. Available from: https://ec.europa.eu/food/plant/pesticides/approval_active_substances/approval_renewal/neonicotinoids_en

25. Giorio C, Safer A, Sánchez-Bayo F, Tapparo A, Lentola A, Girolami V, et al. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 1: new molecules, metabolism, fate, and transport. Environ Sci Pollut Res. 2017;1–33.

26. Bartlett AJ, Hedges AM, Intini KD, Brown LR, Maisonneuve FJ, Robinson SA, et al. Lethal and sublethal toxicity of neonicotinoid and butenolide insecticides to the mayfly, Hexagenia spp. Environ Pollut. 2018; 238:63–75. https://doi.org/10.1016/j.envpol.2018.03.004 PMID: 29544197

27. Glaberman, S., White K. Environmental Fate and Ecological Risk Assessment for Foliar, Soil Drench, and Seed Treatment Uses of the New Insecticide Flupyradifurone (BYI 02960). Washington, DC; 2014. Available from: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=2ahUKEwjIvJu589beAhVMCCwKHZBCA4wQFjAAegQICBAC&url=https%3A%2F%2Fwww.farmlandbirds.net%2Fsites%2Fdefault%2Ffiles%2F2017-07%2FFluspyradifurone%2520New%2520Insecticide.pdf&usg=

28. Hesselbach H, Scheiner R. Effects of the novel pesticide flupyradifurone (Sivanto) on honeybee taste and cognition. Sci Rep. 2018; 8:4954. https://doi.org/10.1038/s41598-018-23200-0 PMID: 29563522

29. Tomizawa M, Casida JE. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu Rev Entomol. 2003; 48:339–64. https://doi.org/10.1146/annurev.ento.48.091801.112731 PMID: 12208819

30. Shao X, Zhang W, Peng Y, Li Z, Tian Z, Qian X. cis-Nitromethylene neonicotinoids as new nicotinic family: synthesis, structural diversity, and insecticidal evaluation of hexahydropyrimidazo[1,2-alpha]pyridine. Bioorg Med Chem Lett. 2008; 18:6513–6. https://doi.org/10.1016/j.bmcl.2008.10.048 PMID: 18951786

31. Xu R, Xia R, Luo M, Xu X, Cheng J, Shao X, et al. Design, synthesis, crystal structures, and insecticidal activities of eight-membered azabridge neonicotinoid analogues. J Agric Food Chem. 2014; 62:381–90. https://doi.org/10.1021/jf4046683 PMID: 24364696

32. Wang B, Cheng J, Xu Z, Xu X, Shao X, Li Z. Synthesis and biological activity evaluation of novel β-substituted nitromethylene neonicotinoid analogues. Molecules. 2012; 17:10014–25. https://doi.org/10.3390/molecules170910014 PMID: 22922273

33. Morris GM, Lim-Wilby M. Molecular Docking. Methods Mol Biol [Internet]. Clifton, NJ: Springer; 2008. p. 365–82

34. Tressaud A, editor. Fluorine and Health. Molecular Imaging, Biomedical Materials and Pharmaceuticals. Amsterdam: Elsevier B.V; 2008.

35. Kirsch P. Modern Fluoroorganic Chemistry. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA; 2004. Available from: http://doi.wiley.com/10.1002/352760393X

36. O’Hagan D, Rzepa HS. Some influences of fluorine in bioorganic chemistry. Chem Commun. 1997;645–6.

37. Böhm H-J, Banner D, Bendels S, Kansy M, Kuhn B, Müller K, et al. Fluorine in Medicinal Chemistry. ChemBioChem. 2004; 5:637–43. https://doi.org/10.1002/cbc.200301023 PMID: 15122635
38. Smith DA, van de Waterbeem d H, Walker DK, editors. Pharmacokinetics and Metabolism in Drug Design. Weinheim, FRG: Wiley-VCH Verlag GmbH; 2001. Available from: http://doi.wiley.com/10.1002/3527600213

39. Barnette WE, Nicolau KC. The Synthesis and Biology of Fluorinated Prostacyclins. Crit Rev Biochem. 1984; 15:201–35.

40. Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, et al. Synthesis and Biological Evaluation of the 1,5-Diarylpyrazole Class of Cyclooxygenase-2 Inhibitors: Identification of 4-[5-(4-Methylpheno)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, Celecoxib). J Med Chem. 1997; 40:1347–65. https://doi.org/10.1021/jm960803q PMID: 9135032

41. Shah P, Westwell AD. The role of fluorine in medicinal chemistry. J Enzyme Inhib Med Chem. 2007; 22:527–40. https://doi.org/10.1080/14756360701425014 PMID: 18035820

42. Swain C, Rupniak NMJ. Chapter 6. Progress in the Development of Neurokinin Antagonists. 1999. p. 51–60. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0065774308605680

43. Tomizawa M, Kagabu S, Ohno I, Durkin KA, Casida JE. Potency and selectivity of trifluoroacetylimino and pyrazinoyl imino nicotinic insecticides and their fit at a unique binding site niche. J Med Chem. 2008; 51:4213–8. https://doi.org/10.1021/jm800191a PMID: 18570364

44. Ohno I, Tomizawa M, Durkin KA, Casida JE, Kagabu S. Neonicotinoid substituents forming a water bridge at the nicotinic acetylcholine receptor. J Agric. Food Chem. 2009; 57:4236–40. https://doi.org/10.1021/ja903985r PMID: 19253973

45. Ohno I, Tomizawa M, Aoshima A, Kumazawa S, Kagabu S. Trifluoroacetamid neonicotinoid insecticides with enhanced hydrophobicity and effectiveness. J Agric. Food Chem. 2010; 58:4999–5003. https://doi.org/10.1021/jf100272a PMID: 20369802

46. Katag i T. Photodegradation of pesticides on plant and soil surfaces. Rev Environ Contam Toxicol. 2004; 182:1–189. https://doi.org/10.1007/978-1-4419-9098-3_1 PMID: 15217019

47. Burrows HD, Canle LM, Santaballa JA, Steenken S. Reaction pathways and mechanisms of photodegradation of pesticides. J Photochem Photobiol B Biol. 2002; 67:71–108.

48. Banić ND, Šojić D V., KrtićJB, Abramović BF. Photodegradation of neonicotinoid active ingredients and their commercial formulations in water by different advanced oxidation processes. Water Air Soil Pollut. 2014; 225.

49. Kurwadkars S, Evans A, DeWinne D, White P, Mitchell F. Modeling Photodegradation Kinetics of Three Systemic Neonicotinoids—Dinotefuran, Imidacloprid, and Thiamethoxam—in Aqueous and Soil Environment. Environ Toxicol Chem. 2016; 35:1718–26. https://doi.org/10.1002/etc.3335 PMID: 26660507

50. Lu Z, Challis JK, Wong CS. Quantum Yields for Direct Photolysis of Neonicotinoid Insecticides in Water: Implications for Exposure to Nontarget Aquatic Organisms. Environ Sci Technol Lett. 2015; 2:188–92.

51. Glaberman S, White K. Environmental Fate and Ecological Risk Assessment for Foliar, Soil Drench, and Seed Treatment Uses of the New Insecticide Fluopyridafurone (BYI 02960). Washington, DC; 2014.

52. Bezdudny A V., Alekseenko AN, Mykhailiuk PK, Manolenko O V., Shishkin O V., Pustovit YM. (5S)-5-(trifluoromethyl)pyrrolidin-2-one as a promising building block for fluoroorganic chemistry. European J Org Chem. 2011; 1782–5.

53. Yarmolchuk VS, Bezdudny A V., Tolmacheva NA, Lukin O, Boyko AN, Chekotylo A, et al. An efficient and safe method for the multigram synthesis of trans-2-(trifluoromethyl)cyclopropylamine. Synthesis (Stuttg). 2012; 44:1152–4.

54. Gerus II, Mironetz RX, Kondratov IS, Bezdudny A V., Dmytriv Y V., Shishkin O V., et al. “Reported, but still unknown.” A closer look into 3,4-bis- and 3,4,5-tris(trifluoromethyl)pyrazoles. J Org Chem. 2012; 77:47–56. https://doi.org/10.1021/jo202305c PMID: 22171748

55. Maginnity PM, Gaulin CA. Derivatives of o-, m- and p-Aminobenzotrifluoride 1. J Am Chem Soc. 1951; 73:3579–80.

56. Zalesskaya IM, Blakitnyi AN, Saenko EP, Fialkov YA YL. No Title. J Org Chem USSR (English Transl). 1980; 16:1031–8.

57. Jaunzems Janis; Claassen Uta; Braun MJ. Fluorination process. 2015.

58. Sheppard WA. α-Fluorinated Ethers. I. Aryl Fluoroalkyl Ethers 1. J Org Chem. 1964; 29:1–11.
60. Schäfer A, Huber C, Ahlrichs R. Fully optimized contracted Gaussian basis sets of triple zeta valence quality for atoms Li to Kr. J Chem Phys. 1994; 100:5829–35.
61. Becke AD. Density-functional exchange-energy approximation with correct asymptotic behavior. Phys Rev A. 1988; 38:3098–100.
62. Perdew JP. Density-functional approximation for the correlation energy of the inhomogeneous electron gas. Phys Rev B. American Physical Society; 1986; 33:8822–4.
63. Lu T, Chen F. Multiflwn: A multifunctional wavefunction analyzer. J Comput Chem. 2012; 33:580–92. https://doi.org/10.1002/jcc.22885 PMID: 22162017
64. Lu T, Chen F. Quantitative analysis of molecular surface based on improved Marching Tetrahedra algorithm. J Mol Graph Model. 2012; 38:314–23. https://doi.org/10.1016/j.jmgm.2012.07.004 PMID: 23085170
65. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. Wiley Subscription Services, Inc., A Wiley Company; 2010; 31:455–61. https://doi.org/10.1002/jcc.21334 PMID: 19499576
66. Michel F. Sanner. Python: A Programming Language for Software Integration and Development. J Mol Graph Model. 1999; 17:57–61. PMID: 8744570
67. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009; 30:2785–91. https://doi.org/10.1002/jcc.21256 PMID: 19399780
68. Humphrey W, Dalke A, Schulten K. VMD: Visual molecular dynamics. J Mol Graph. 1996; 14:33–8. https://doi.org/10.1016/0263-7855(96)00018-5 PMID: 8744570
69. Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 4.5. San Diego: Dassault Systèmes; 2015.
70. Santos JMM, Contel EPB, Kerr WE. Biologia de anofelinos amazonicos. 1. Ciclo biológico, postura e estádios larvais de Anopheles darlingi Root, 1926 (Diptera: Culicidae) da rodovia Manaus/Boa Vista. Acta Amaz. 1981; 11:789–97.
71. Gorham J. Richard, Stojanovich Chester J, Scott Harold George. Clave Ilustrada para los Mosquitos Anofelinos de Sudamerica Oriental. Atlanta: United States Department of Health; 1967.
72. Rotraut A. Consoli G. B., de Oliveira Ricardo Lourenço. Principais Mosquitos de Importância Sanitária no Brasil. 1a ed. Rio de Janeiro: Fiocruz; 1994.
73. Oswaldo Paulo Forattini. Culicidologia Médica: Identificação, Biologia, Epidemiologia. 1st ed. USP, editor. São Paulo; 2002.
74. da Silva Mesquita R, Tadei WP, Mena Barreto Bastos A. Determination of the larvicidal activity of benzoyl thiosemicarbazone and its Ni(II) complex against Aedes aegypti and Anopheles darlingi larvae in Amazonas, Brazil. J Entomol Nematol. 2018; 10:37–42.
75. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization. Dept. of Communicable Disease Prevention, Control and Eradication WHO Pesticide Evaluation Scheme; 2005.
76. Robertson J, Preisler H, Russel R. Poloplus probit and logit analysis. Parma (MO), USA: LeOra Software LLC; 2003. p. 1–36.
77. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925; 18:265–7.
78. Bohaty R, Eckel W, Shamim M, Spatz D, White K, Young D. Standard Operating Procedure for Using the NAFTA Guidance to Calculate Representative Half-Life Values and Characterizing Pesticide Degradation 1. eBook. 2015.
79. Ihara M, Okajima T, Yamashita A, Oda T, Hirata K, Nishiwaki H, et al. Crystal structures of *Lymnaea stagnalis* AChBP in complex with neonicotinoid insecticides imidacloprid and clothianidin. Invertebr Neurosci. 2008; 8:71–81.
80. Martins-Campos KM, Pinheiro WD, Vitor-Silva S, Siqueira AM, Melo GC, Rodrigues IC, et al. Integrated vector management targeting *Anopheles darlingi* populations decreases malaria incidence in an unstable transmission area, in the rural Brazilian Amazon. Malar. J. 2012. Available from: http://www.malarialjournal.com/content/11/1/351
81. Moulinho PR, Gil LHS, Cruz RB, Ribolla PEM. Population dynamics, structure and behavior of Anopheles darlingi in a rural settlement in the Amazon rainforest of Acre, Brazil. Malar J. 2011; 10:174. https://doi.org/10.1186/1475-2875-10-174 PMID: 21702964
82. Kagabu S, Takagi M, Ohno I, Mikawa T, Miyamoto T. Crown-capped imidacloprid: a novel design and insecticidal activity. Bioorg Med Chem Lett. 2009; 19:2947–8. https://doi.org/10.1016/j.bmcl.2009.04.053 PMID: 19409782
83. Clark DA, Lahm GP, Smith BK, Barry JD, Clagg DG. Synthesis of insecticidal fluorinated anthranilic diamides. Bioorg Med Chem. 2008; 16:3163–70. https://doi.org/10.1016/j.bmc.2007.12.017 PMID: 18207749

84. Moriya K, Shibuya K, Hattori Y, Tsuboi S, Shiokawa K, Kagabu S. 1-(6-Chloronicotinyl)-2-nitroimino-imidazolidines and Related Compounds as Potential New Insecticides. Biosci Biotechnol Biochem. 1992; 56:364–5.

85. Kagabu S. Discovery of imidacloprid and further developments from strategic molecular designs. J Agric Food Chem. 2011; 59:2887–96. https://doi.org/10.1021/jf101824y PMID: 20718435

86. Chen Y, Sun C, Wen X, Zhang W. Design, synthesis, insecticidal evaluation and molecular docking studies of cis-nitenpyram analogues bearing diglycine esters. Sci China Chem. 2012; 56:159–68.

87. Bissantz C, Kuhn B, Stahl M. A medicinal chemist’s guide to molecular interactions. J Med Chem. 2010; 53:5061–84. https://doi.org/10.1021/jm100112j PMID: 20345171

88. APVMA. Evaluation of the New Product Sivanto Prime 200 SL Insecticide. Kingston ACT; 2018. Available from: https://apvma.gov.au/sites/default/files/publication/31501-prs-84727-sivanto-prime-200sl-insecticide.pdf

89. Ministério da Saúde Brasil, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. Ações de controle da malária: manual para profissionais de saúde na atenção básica. Brasília; 2006. Available from: http://bvsms.saude.gov.br/bvs/publicacoes/acoes_controle_malaria_manual.pdf