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Research paper

# Thiosemicarbazones as *Aedes aegypti* larvicidal

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ABSTRACT

A set of aryl- and phenoxymethyl-(thio)semicarbazones were synthetized, characterized and biologically evaluated against the larvae of *Aedes aegypti* (*A. aegypti*), the vector responsible for diseases like Dengue and Yellow Fever. (Q)SAR studies were useful for predicting the activities of the compounds not included to create the QSAR model as well as to predict the features of a new compound with improved activity. Docking studies corroborated experimental evidence of *AeSCP*-2 as a potential target able to explain the larvicidal properties of its compounds. The trend observed between the *in silico* Docking scores and the *in vitro* pLC50 (equals −log LC50, at molar concentration) data indicated that the highest larvicidal compounds, or the compounds with the highest values for pLC50, are usually those with the higher docking scores (i.e., greater *in silico* affinity for the AeSCP-2 target). Determination of cytotoxicity for these compounds in mammal cells demonstrated that the top larvicide compounds are non-toxic.


## 1. Introduction

A large number of diseases are transmitted by mosquitoe vectors such as filariasis (*Culex quinquefasciatus*) [1], malaria (*Anopheles gambiae*) [2], West Nile fever (*Aedes albopictus*) [3] and dengue, Chikungunya and yellow fever (*Aedes aegypti*) [4]. These four last diseases are transmitted by a virus.

Nowadays, Dengue Fever (DF) is considered one of the most rapidly spreading diseases, being disseminated mainly but not limited to tropical and subtropical regions [5]. Since DF correlates with the vector expansion [6], the possibility of occurrence of DF in non-disease-endemic areas like the continental United State (US) cannot be neglected. The epidemics in Puerto Rico in the 1990's [7] and in Hawaii in 2001 [8] support this possibility. The large international mobility throughout the US territory (the so called imported cases) may partially be responsible but the occurrence of DF was related mainly due to the presence of two principal vectors (*A. albopictus* and *A. aegypti*) in southern and central US [9]. For some of the reasons above mentioned, Australia [10], Spain [11] and Germany [12] may also be considered areas of potential risk for DF infection.

Recent estimates of the World Health Organization (WHO) indicate *c.a.* 50–100 million people worldwide are annually infected with dengue and 2.5 billion people (40% of the word population) are at risk in more than 100 countries [13]. Although many

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efforts have been envisaged for developing a vaccine simultaneously for the four dengue virus serotypes (1–4), none are available at the moment [14]. Recently a fifth serotype was reported [15]. This complicates the situation further. Likewise, there are no specific medicines available for treating Dengue-infected patients. The current standard medical treatment is limited to controlling the symptoms of the disease [16,17]. DF has a strong economic impact. For instance, those affected frequently stay out of the productive and/or educational systems for week(s). Sectors like tourism are extremely impacted as well [18,19]. Besides this, during epidemics both public and privative health systems are frequently exhausted to the extreme. For instance, according to WHO, 500,000 people are hospitalized annually with a mortality rate c.a. 2.5% [13].

To control Dengue transmission, tools for vector control are necessary, including larvicide use, entomological monitoring, biological control as well as public information campaigns [20,21].

Currently, among the most employed larvicides are the organophosphate temephos and the toxins of the *Bacillus thuringiensis (Bti)* var. *israelensis* [22]. Recent studies, have pointed out that the long-term use of themephos is producing *A. aegypti* resistant populations [23–25]. Likewise, new alternatives for low cost production of *Bti* on a large scale [26,27] and the possibility of a mosquito resistant to the Bti toxin [28] are relevant concerns that demand further research to discover better larvicides for *A. aegypti*.

An increasing number of publications have recently reported new natural and synthetic larvicides for *A. aegpti*. For instance, in 2011 Neto and co-workers [29] published a review about natural products as larvicides for *A. aegypti*, covering 21 different plants with $LC_{50}$ values ranging from 0.04 ppm to 100 ppm. Since then, other papers researching chemicals derived from natural products have been published [30–33], but with a range of activity like that of [ref. 29]. On the other hand, studies on a smaller number (in comparison with the number of natural products) of synthetic compounds against *A. aegypti* have appeared in the literature. In this case both Structure-Activity Relationships (SAR) [34] and Quantitative Structure-Activity Relationships (QSAR) [35] based on the ligand approach have been developed. Concerning SAR studies, Cavalcanti and co-workers [36–38] reported on the importance of hydrophobicity for improving larvicide activity for *A. aegypti* for a series of monoterpenic and benzoquinonic derivatives. Similar to this, Cantrell and co-workers observed a clear relationship between the larvicide activity against *A. aegypti* and the number of methylenic units in the linear amine substituents attached to both alantolactone and isoalantolactone [39]. In the 2000's, Eng and co-workers reported studies on four different classes of triorganotin complexes as larvicide activity against *A. aegypti* [40–43]. In 2009, Hansch and Verma [44] revisited the Eng's larvicide activities results for three of these triorganotin complexes [41–43] and proposed QSAR models with the hydrophobic ($\pi_X$) and volume (MR – molar refractivity or $E_S$ – Taft's steric) parameters as the most important for describing larvicide activity.

At this point it is important to stress that it is possible to find in the literature other SAR and QSAR studies on the larvicide activity for other mosquitos (e.g. *C. quinquefasciatus* [45–47]) or about the repellence for adult *A. aegypti* [48–51]. They will not be discussed here, however, since they are out of the scope of the present work.

In 2002, Park and co-workers [52] reported on $LC_{50}$ larvicide activity against *A. aegypti* among four structurally related compounds obtained from the fruits of *Piper nigrum*: pellitorine (0.92 ppm), guineensine (0.89 ppm), pipercide (0.1 ppm), retrofractamide A (0.04 ppm) and the commercial insecticidal piperine (5.10 ppm). The structural comparison of these compounds made it clear that larvicide activity is inversely related to the distance between the 3,4-methylenedioxyphenyl and the isobutylamide groups. Besides, the ability of the isobutylamide moiety to make hydrogen-bond interactions, probably with some polar amino acid residue in a biological target, may explain the expressive decrease in the larvicide activity in piperine when the isobutylamide is changed by a six-member cyclic amide.

In an attempt to synthetize some amidic compounds, our research group discovered that the corresponding carboxylic acid precursors had a higher larvicide activity against *A. aegypti* than the corresponding amides [53]. As consequence, in 2009, our group reported for the first time on the larvicidal activity against *A. aegypti* larvae of 3-(3-aryl-1,2,4-oxadiazol-5-yl) propionic acids (AOPA) where the presence of electron-withdrawing substituents in the *para* position of the phenyl ring was shown to be important for the larvicide activity [54]. Similar observations about the electronic substituent effect on the phenyl ring were explored through QSAR equations on 1-(2,6-halogenbenzoyl)-5-(4-halogen-phenyl) biuret compounds by Bordas and co-workers *for* larvicidal activities ($LC_{50}$) against *A. aegypti* larvae [55].

In this work we report our research on the synthesis, characterization, and evaluation of larvicide activity against *A. aegypti* of aryl-semicarbazones (**1**,**2**), aryl-thiosemicarbazones (**3**–**10**) and phenoxyl thiosemicarbazones (**12**–**18**) (Fig. 1).

The choice of these compounds was based on a compromise among four aspects. The first was to recover through the molecular structure two parameters previously reported in the literature as important for larvicide activity against *A. aegypti*, namely hydrophobicity and the ability to form hydrogen bonds with polar head fragments. The second was to use hydrazones, a well-established class of compounds employed in pest control in both agriculture and horticulture [56,57]. Third was to use (thio)semicarbazones, which are hydrazone structural analogs with broad spectra of biological activities [58–62], easily prepared and can be used as intermediates in the synthesis of further heterocyclic compounds with pharmacological potential [63–66]. Fourth, the presence of the spacer unit $-O-CH_2-CH=$ in the phenoxy-compounds was used to introduce some degree of rotational freedom on the phenyl substituted moiety, improving (as was the case for other biological activities [67]) the chances to modify the larvicide activity.

Another aspect investigated about these compounds was how safe they are for other living systems. Therefore, in a first approach, toxicological tests were developed for a mammal model.

Electronic structure and lipophilicity calculations were used to create a QSAR model for the ligands with a double objective: to analyze the importance of the parameters used to explain the larvicide activity against *A. aegypti* for a training set of molecules; and to explore the predictability of the model. In fact, from this QSAR study, the larvicide activities of three compounds were predicted and evaluated.

After that, in order to improve understanding of how these compounds act on the *A. aegypti*, the affinity profile of two synthetized compounds were evaluated on the *A. aegypti* Sterol Carrier Protein-2 (*AeSCP-2*, a system believed to be involved in the intracellular transport of cholesterol [68]). These were then compared with the affinity profile of a previous tested inhibitor [69]. Finally, docking studies were performed in order to increase the evidence of this target as the potential receptor for these compounds.

## 2. Results and discussion

### 2.1. Synthesis

Fig. 1 shows the chemical structure of all compounds investigated in this work. The synthetic procedures employed in preparation of compounds (**1**–**18**) are shown in Scheme 1 of the experimental section.

Compounds (**1**–**10**) were prepared by reacting commercially

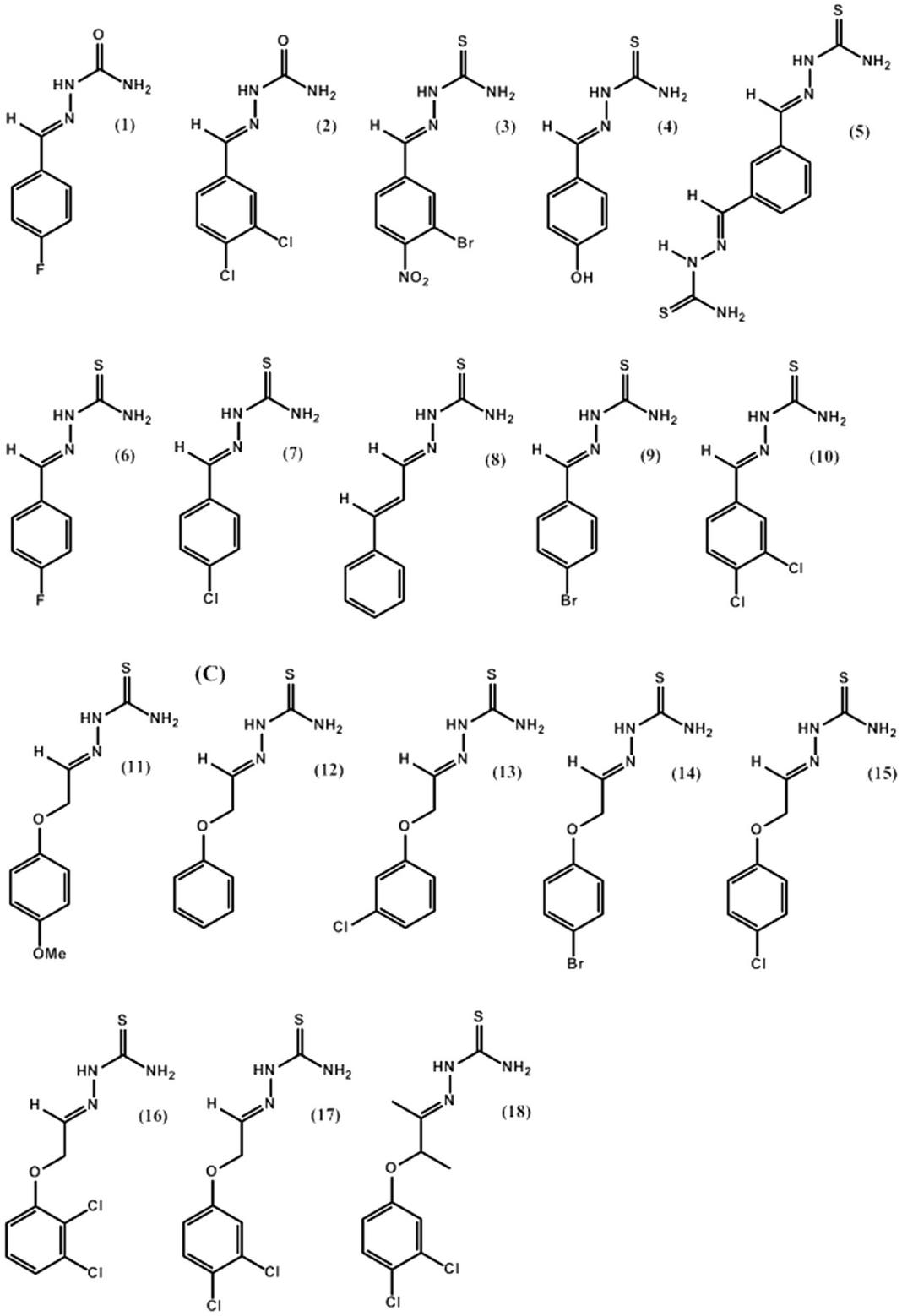
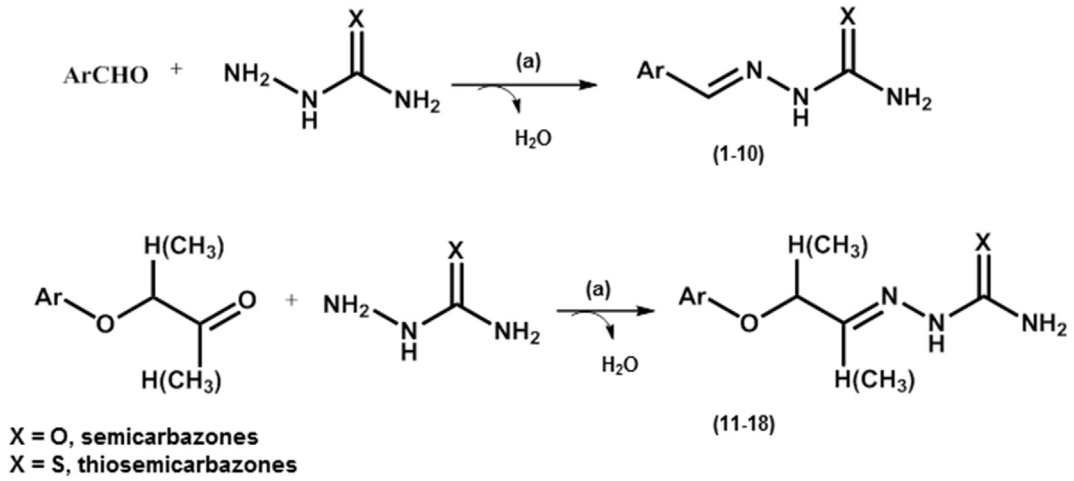



**Fig. 1.** Structure of the compounds (**1**–**18**) tested as larvicide against the L4 stage of *A. aegypti*.

available aryl aldehydes with semicarbazide or thiosemicarbazide. For the synthesis of phenoxymethyl thiosemicarbazones (**11**–**17**), the substituted phenolic compound reacted with bromoacetaldehyde diethyl acetal under basic conditions. After hydrolysis of acetal in aldehyde, the respective aldehyde was then reacted with thiosemicarbazide and catalytic HCl in an ultrasound bath at room temperature. For the synthesis of **18**, the substituted phenolic compound reacted with 3-chloro-2-butanone. The resulting β-ketoether was then reacted with thiosemicarbazide, to produce the compound **18**.

All compounds (**1**–**18**, see Fig. 1) were purified by recrystallization and obtained at an acceptable purity (>95%) in yields




**Scheme 1.** Synthesis of compounds (**1**–**18**). Reagents and conditions: (a) HCl or $H_2SO_4$, ethanol, room temperature, 2–3 h.

ranging from 40 to 97 %. The structures were determined by NMR, infrared spectroscopy, and high-resolution mass spectrometry.

The compounds investigated were represented as (R; SU; X), where (X) stands for the thio- or semicarbazone group, (R) the substituent at the aryl or phenoxy ring, and (SU) the spacer unit –CH= or $-OCH_2CH=$ between the X and R groups.

### 2.2. Larvicide activity

The compounds synthesized **1**–**17** were tested as larvicides against the L4 stage of *A. aegypti*. Tween-80 was selected as the co-solvent because it had the best results for dissolving the compounds in water and because of its low toxicity to larvae, according to the classification of Kramer, Schnell and Nickerson (1983) ($LC_{50}$ > 1%) [70]. The obtained $LC_{50}$ values for **1**–**17** are shown in Table 1.

According Chang and co-workers (2003) essential oils or vegetal extracts showing $LC_{50}$ between 50 and 100 ppm in larvicide bioassays must be considered as active, while those exhibiting $LC_{50}$ lower than 50 ppm may be considered as highly active [71]. If one uses this reference, compounds **1**–**5**, **11** and **12** should be considered inactive, compounds **6** ($LC_{50}$ = 310.8 μmol $L^{-1}$) and **13** ($LC_{50}$ = 287.2 μmol $L^{-1}$) should be considered active and compounds **7**–**10** ($LC_{50}$ = 134.8, 138.3, 122.8 and 69.7 μmol $L^{-1}$) and **14**–**17** ($LC_{50}$ = 92.0, 89.9, 70.1 and 20.9 μmol $L^{-1}$) should be considered highly active.

### 2.3. Structure-active relationship (SAR)

The semicarbazone compounds tested (**1** and **2)** showed low activity ($LC_{50}$ > 861.8 μmol $L^{-1}$). When the carbonyl (C=O) was replaced by the thiocarbonyl (C=S) bond, this led to the corresponding bioisoster compounds (**6** and **10)** to exhibit an improved result $LC_{50}$ = 310.8 μmol $L^{-1}$ and $LC_{50}$ = 69.7 μmol $L^{-1}$, respectively. According to Beraldo, this pattern was previously observed for other pharmacological activities of (thio)semicarbazones [72,73]. This change in larvicide activity indicates a possible change in the magnitude of the intermolecular interactions (e.g. H-bond) with a

**Table 1**
Larvicide activity ($LC_{50}$) and cytotoxicity for the synthetic (thio)semicarbazones derivatives (**1**–**17**).

| Compound (R; SU; X)[a] | Numbering | Conc. range[b] (ppm) | $LC_{50}$ (ppm) | $LC_{50}$ (μmol$L^{-1}$) | Log(1/$LC_{50}$) | Cytotoxicity[c] (μmol$L^{-1}$) |
|---|---|---|---|---|---|---|
| *Aryl-(thio)semicarbazones* | | | | | | |
| (4-F; CH; O) | 1 | >200 | >200 | >1103.9 | | >55.1 |
| (3,4-diCl; CH; O) | 2 | >200 | >200 | >861.8 | | >431 |
| (3-$NO_2$,4-Br; CH; S) | 3 | >200 | >200 | >659.8 | | >3.29 |
| (4-OH; CH; S) | 4 | >200 | >200 | >1024.4 | | 5.12 |
| (5-thiosemicarbazone; CH; S) | 5[d] | >200 | >200 | | | N.T.[e] |
| (4-F; CH; S) | 6 | 50–90 | 61.3 | 310.8 | 3.507 | 25.4 |
| (4-Cl; CH; S) | 7 | 20–60 | 28.8 | 134.8 | 3.873 | 23.3 |
| (H; CH=CH–CH; S) | 8 | 28–50 | 28.4 | 138.3 | 3.860 | <4.87 |
| (4-Br; CH; S) | 9 | 20–50 | 31.7 | 122.8 | 3.910 | <3.87 |
| (3,4-diCl; CH; S) | 10 | 16–20 | 17.3 | 69.7 | 4.157 | 20.2 |
| *Phenoxymethyl-thiosemicarbazones* | | | | | | |
| (4-$OCH_3$;$OCH_2CH$; S) | 11 | 80–160 | 120.4 | 503.1 | 3.298 | 20.9 |
| (H; $OCH_2CH$; S) | 12 | 80–160 | 112.7 | 538.5 | 3.268 | 119 |
| (3-Cl; $OCH_2CH$; S) | 13 | 50–80 | 70.0 | 287.2 | 3.542 | 103 |
| (4-Br; $OCH_2CH$; S) | 14 | 20–40 | 26.5 | 92.0 | 4.036 | >347 |
| (4-Cl; $OCH_2CH$; S) | 15 | 20–30 | 21.9 | 89.9 | 4.046 | 103 |
| (2,3-diCl; $OCH_2CH$; S) | 16 | 10–30 | 19.5 | 70.1 | 4.154 | 17.9 |
| (3,4-diCl; $OCH_2CH$; S) | 17 | 5–9 | 5.8 | 20.9 | 4.680 | 89.8 |

[a] SU means spacer unit, see the text.
[b] The concentration values in ppm are shown just for comparison with previous results published in the literature.
[c] The highest non-toxic concentration on spleen cell of BALB/c mice, saponin (<1.0 μg $mL^{-1}$).
[d] See Fig. 1.
[e] N.T. means not tested.

biological target owing to the chemical change C=O for C=S in the above-mentioned compound. Another plausible hypothesis for explaining this change in the larvicide activity may be related to the higher stability of the thiopeptidic compared to the peptidic environment under attack by proteolytic enzymes [74].

Interesting results come from the comparison of the larvicide activities in compounds containing different spacer units (SU) between the aryl-substituted and the thiosemicarbazone moieties. For instance, the phenoxymethyl-thiosemicarbazone derivatives **15**, **14** and **17** ($LC_{50}$ = 89.9, 92.0 and 20.9 μmol $L^{-1}$, respectively) where SU = −O−$CH_2$−CH= have a higher activity than the corresponding aryl-thiosemicarbazone derivatives **7**, **9**, and **10** ($LC_{50}$ = 134.8, 122.8 and 69.7 μmol $L^{-1}$, respectively) where SU = −CH−. These results demonstrate the importance of the spacer unit −O−$CH_2$−CH= for the compounds with larvicide activity for *A. aegypti*.

The satisfactory results for the activity of the thiosemicarbazones **7**, **9**, **14**, and **15** ($LC_{50}$ = 134.8, 122.8, 92.0 and 89.9 μmol $L^{-1}$, respectively) points out the importance of polarizable halogen substituents at the *para* position for the larvicide activity. On the other hand the thiosemicarbazones with substituents 4-F (**6**, $LC_{50}$ = 310.8 μmol $L^{-1}$) and 3-Cl (**13**, $LC_{50}$ = 287.2 μmol $L^{-1}$) performed slightly worse. According Hernandes and co-workers [75], bulk and polarizable halogen atoms both may occupy available pockets and interact via a halogen bond at the binding site of biological targets. Thus, the relative smaller activity of **6** may be explained in terms of the smaller atomic radius and polarizability of the substituent fluorine. Introduction of bulk halogen atoms in the *orto* and *meta* positions tends to create steric hindrances and conformational changes. Perhaps, this also may explain the relatively low activity of **13**. It is interesting to observe that the order of activity for the *para*-substituents in **6**, **7** and **9**, i.e., F < Cl < Br, was the same observed for AOPA by Neves Filho and co-workers [54].

The presence of two atoms of chlorine at the *meta* and *para* positions of the aromatic ring led to a significant increase in the larvicide activity (compounds **10** and **17** with $LC_{50}$ = 69.7 and 20.9 μmol $L^{-1}$, respectively) in comparison to their corresponding mono-chlorine compounds (**7** and **15** with $LC_{50}$ = 134.8 and 89.9 μmol $L^{-1}$, respectively). In fact, these double-chlorine compounds are the most active thiosemicarbazone derivatives synthesized in this work. Corroborating our results, Bordas and co-worker, studying larvicide activity in a series of biurets, also observed the highest activities for the compound chlorine di-substituted in the *meta*- and -*para* positions [55].

On the other hand, the presence of the electron donor groups *para*-OH (**4**, $LC_{50}$ > 1024.4 μmol $L^{-1}$) and *para*-$OCH_3$ (**11**, $LC_{50}$ = 503.1 μmol $L^{-1}$) decreased larvicide activity. This effect is in agreement with results of Simas and co-workers who reported less active phenylpropanoide containing hydroxyl and methoxyl groups in the *para* position on the aromatic ring [76]. The presence of a methoxyl group attached to the *para* position on the aromatic ring did not seem to contribute to the larvicide activity in the works of Neves Filho and co-workers [54] and Bordas and co-workers [55].

It has been reported that the presence of a phenylic ring conjugated to the α and β unsaturated carbonyl groups in a cynanic aldehyde improves larvicide activity [76]. Because of this, a closely related system **8** was synthesized. In fact, this activity was among the most successful ($LC_{50}$ = 138.3 μmol $L^{-1}$). However, it is important to note that in **8** the aromatic ring is conjugated not to a carbonyl group, but to an imine function through an allylic system. Since this allylic system favors electron delocalization, an increase in the electronic density is expected to occur on the nitrogen and sulfur atoms with implications for the ability of these atoms to bind with enzyme metallic ions or to interact via H-bond with residues in a biological target [72]. Nitro-compounds are well established bioactive compounds used as antimicrobial, antiparasitic and antitumor agents [77]. The presence of the nitro group (-$NO_2$), however, seems not have contributed to the mortality of *A. aegypti*'s larvae since compound **3** showed a $LC_{50}$ > 659.8 μmol $L^{-1}$, whereas compound **9** (that does not contain the nitro group) showed a high activity with $LC_{50}$ = 122.8 μmol $L^{-1}$. One possiblility that cannot be neglected is that the nitro group could be bio-reduced to one amino group [78] which, as an electron-donar group, is thought to lessen larvicide activity.

### 2.4. Cytotoxicity analysis

The evaluation of eventual side effects on non-target organisms from a newly synthesized compound developed for larvicide purposes is essential, even in the beginning steps of the research. In this work the synthesized thiosemicarbazone derivatives were submitted to cytotoxicity assays using BALB/c mice splenocytes (as described in the experimental section) as the model mammal. In the last column of Table 1, the highest non-toxic concentrations against the tested cells are shown. Fortunately, when compared with saponin (the positive control), the 4-Cl-, 4-Br- and 3,4-diCl-phenoxymethyl-thiosemicarbazones (top larvicide compounds) are non-toxic.

### 2.5. QSAR

B3LYP/6-311++G(d,p) calculations to acess geometric, energetic and electronic properties for compounds (**1**–**18**) have been reported by us elsewhere [79]. Table 2 shows the data matrix used to obtain the quantitative structure-activity larvicide relationship (QSAR).

The QSAR model obtained (multiple linear regression) is shown in Eq. (1).

$$Log\left(\frac{1}{LC_{50}}\right) = 2.490(\pm 0.335) - 0.112(\pm 0.0356)\mu + 0.671(\pm 0.0912)\log P \quad (1)$$

$$n = 10;\ R = 0.97;\ R^2 = 0.94;\ F = 54.61;\ s = 0.12;\ p = 0.00005$$

Using this equation, Fig. 2a shows a comparison between predicted and observed larvicide activities. The quality of the adjustment (Eq. (1)) can be visualized in the residue plot as shown in Fig. 2b. As one can see, the predicted and the observed activity values are highly correlated. The quality of this regression can be appreciated, considering the equation parameter deviation, the statistics parameters $R^2$ and F as well as the distribution of the points around the zero in the residue plot.

From Equation-1, one can see that the electric dipole moment (μ) and the decimal logarithm of the octanol–water partition coefficient (log P), with negative and positive coefficients,

**Table 2**
Experimental larvicide activity, gas phase B3LYP/6-311++G(d,p) electronic and lipophilic descriptors for the aryl- and phenoxymethyl-thiosemicarbazone derivatives.

| Numbering | log(1/$LC_{50}$) | μ[a] (D) | $\varepsilon_{HOMO}$[a] (eV) | $\varepsilon_{LUMO}$[a] (eV) | Δε[a] (eV) | $\Sigma q_{benzeno}$[a] (e) | $qC_1$[a] (e) | $qN_1$[a] (e) | $qN_2$[a] (e) | logP[b] | $(logP)^2$[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 3.507 | 3.702 | −6.045 | −2.224 | 3.821 | −0.370 | 0.778 | 0.192 | −0.033 | 2.180 | 4.752 |
| 7 | 3.873 | 3.503 | −6.078 | −2.336 | 3.742 | −1.050 | 0.522 | 0.179 | −0.035 | 2.630 | 6.917 |
| 9 | 3.910 | 3.502 | −6.084 | −2.357 | 3.727 | 0.043 | 1.054 | 0.227 | −0.047 | 2.870 | 8.237 |
| 10 | 4.157 | 3.133 | −6.185 | −2.507 | 3.678 | −1.681 | −0.006 | 0.179 | −0.035 | 3.270 | 10.693 |
| 11 | 3.298 | 5.915 | −5.968 | −1.589 | 4.379 | −0.565 | −0.187 | 0.082 | −0.020 | 2.090 | 4.368 |
| 12 | 3.268 | 4.816 | −6.088 | −1.634 | 4.454 | −0.675 | 0.037 | 0.078 | −0.018 | 2.010 | 4.040 |
| 13 | 3.542 | 4.665 | −6.159 | −1.746 | 4.413 | −0.989 | −0.534 | 0.082 | −0.023 | 2.650 | 7.023 |
| 14 | 4.036 | 2.932 | −6.168 | −1.753 | 4.414 | −0.495 | −0.049 | 0.082 | −0.019 | 2.900 | 8.410 |
| 15 | 4.046 | 2.936 | −6.164 | −1.748 | 4.417 | −1.000 | −0.349 | 0.081 | −0.019 | 2.650 | 7.023 |
| 16 | 4.154 | 5.516 | −6.108 | −1.713 | 4.395 | −1.377 | −1.465 | 0.090 | −0.011 | 3.300 | 10.890 |
| 17 | 4.680 | 1.774 | −6.212 | −1.825 | 4.387 | −1.571 | −0.849 | 0.088 | −0.023 | 3.300 | 10.890 |

[a] [Ref.79].
[b] This work.

respectively, are the most important descriptors related to larvicide activity. This linear model indicates that substituents that decrease μ (since it is always ≥0) and/or increase logP will improve the larvicide activity. These equation features match the importance, previously in the literature, of both hydrophobic and electronic parameters for describing larvicide activity against *A. aegypti*.

In order to check the QSAR model (Eq. (1)) a compound exhibiting a better activity was predicted. Besides, some compounds showing intermediate activity and also one of the worst activities (the model must work inside and outside the training set as well as in both directions) were analyzed. The compound with the higher activity, **18**, was purchased from our particular collection of compounds and tested after Equation-1 was obtained. The compound with intermediate activity, **13**, was synthesized and tested at the same time as those used in the training set. However this compound was not used to build the model because it is the only one mono-substituted at the *meta* position. Finally, compound **4**, showing one of the worst results among the compounds shown in Table 1, was analyzed in light of that QSAR equation. The calculated electric dipole moment, logP, and the predicted and observed $LC_{50}$ values of these compounds are shown in Table 3.

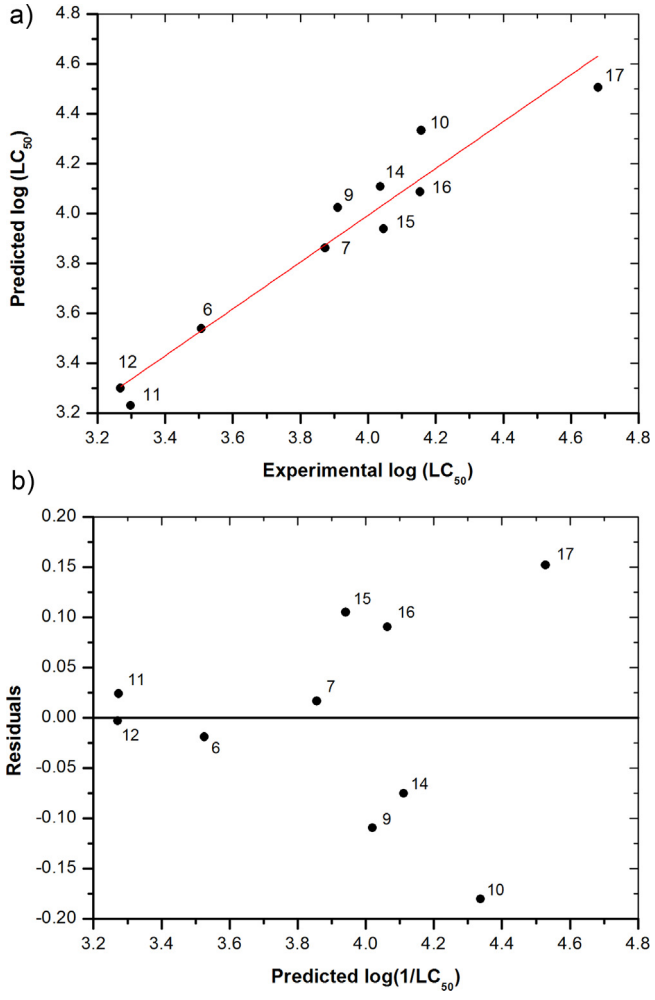



**Fig. 2.** QSAR plots: a) Predicted versus experimental activities and b) residual predicted activity.

The data in this table show that Equation-1 succeeded in predicting correctly the best larvicide activity for **18** and the intermediate larvicide activity for **13**. The cytotoxicity of **18** was evaluated in 16.33 μmol $L^{-1}$, leading to a ratio (Cytotocity/$LC_{50}$) equal to 4.1. It is interesting to stress that we stopped determining the experimental $LC_{50}$ for compound **4** after the concentration of 1024 μmol $L^{-1}$. That decision is adequately supported by Equation-1 since the $LC_{50}$ for the compound **4** is predicted at 6434 μmol $L^{-1}$.

### 2.6. Action mode

In the attempt to improve our comprehension about how the synthesized compounds lead to larvae death in the L4 stage, the *A. aegypti* sterol carrier protein-2 (AeSCP-2) inhibition was tested. Since mosquitoes depend on exogenous sources of cholesterol for biosynthesis of steroid derivatives, it is not surprising to find the high expression of AeSCP-2 in the larvae midgut during the feeding stage [80]. Therefore, compounds that can inhibit this protein have a high potential for becoming useful tool for vector control. In 2003, Lan and co-workers published a high resolution X-ray structure of palmitic acid ($CH_3(CH_2)_{14}COOH$) co-crystallized into AeSCP-2 [81]. These authors found that the polar head of the palmit acid makes an H-bond interaction with the side chain of the Arg24 residue, whereas the metilenic moiety is in a bent conformation inside a hydrophobic pocket. Taking into account the structural features of palmitic acid for binding AeSCP-2, two aspects require attention. First, the side chain of Arg ($-(CH_2)_3-NH(C{=}NH)NH_2$) has an imidourea group at the end portion which is functionally very similar to the (thio)semicarbazone portion of compounds **1**–**17**. Second, the twisted disposition of the phenoxymethyl group relative to the thiosemicarbazone moiety resembles the bent conformation of the fatty portion relative to the carboxylic head of the palmit acid co-crystallized into AeSCP-2. Because of this, we tested two synthetized thiosemicarbazone derivatives, **4** and **11**, as AeSCP-2 inhibitors (SCPIs) and compared them to a previous tested

**Table 3**
Calculated B3LYP/6-311++G(d,p) electric dipole moment and Log P values and predicted (using equation-1) and experimental Log(1/$LC_{50}$) for the thiosemicarbazone derivatives **4**, **13** and **18**.

| Compound (R; SU; X) | Numbering | μ[a] (D) | logP | $LC_{50}$ (pred.) (μ mol $L^{-1}$) | $LC_{50}$ (exp.) (μ mol $L^{-1}$) |
|---|---|---|---|---|---|
| (3,4-diCl; $OCH(CH_3)CCH_3$;S) | **18** | 3.31 | 4.87 | 4.10 | 3.95 |
| (3-Cl; $OCH_2CH$; S) | **13** | 4.66 | 2.650 | 179.38 | 287.2 |
| (4-OH; CH; S) | **4** | 6.02 | 0.56 | 6434.0 | >1024.4 |

[a] [Ref.79].

compound, SCPI-1 [69] (see Fig. 3).

Fig. 3 shows that the competitive binding of compounds **4** and **11** to rAeSCP-2 had a similar dose–response curve as that of the SCPI-1 [69]. The 50% effective concentration ($EC_{50}$) of inhibiting NBD-cholesterol binding to AeSCP-2 was 5.0 (95% Confidence Interval = 2.0–17.0) and 0.6 (95% Confidence Interval = 0.3–1.4) μM for compounds **4** and **11**, respectively. The $EC_{50}$ of compounds **4** and **11** are within the range of identified SCPIs [69]. SCPIs have been shown to suppress dietary cholesterol uptake in both *A. Aegypti* [82] and in the tobacco hornworm [83]. Therefore, the likely mode of action of compounds **4** and **11** was the suppression of dietary cholesterol uptake in treated *A. aegypti* larvae. Although Fig. 3 was fitted to the inhibition of SCP-2 whereas the response function on the QSAR model measured the capacity of whole larvae to die (therefore they are conceptually different things), the higher hydrophobicity of **11** ($logP_{calc}$ = 2.09, Table 2) compared to **4** ($logP_{calc}$ = 0.56, Table 3) matches the higher inhibitory activity of **11** compared to **4**.

### 2.7. Docking studies

In order to improve our comprehension about how the (thio) semicarbazone derivatives interact with SCP-2, we conducted a docking study using as a binding site the palmitic acid contact residues in SCP-2. Fig. 4 shows the superimposition of the best docking solutions obtained for compounds **4**, **11**, **14**, **15**, **16**, **17** on the

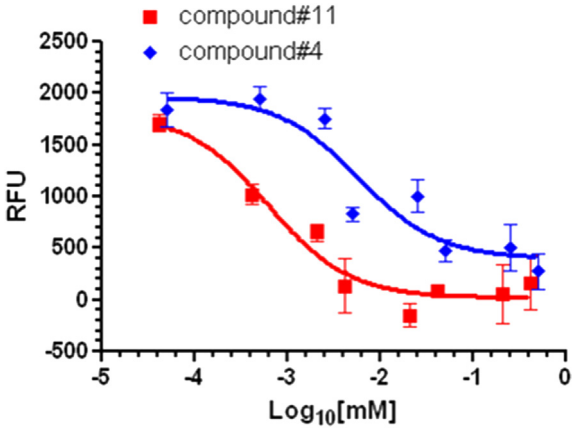



**Fig. 3.** Dose-response curve of inhibition of NBD-cholesterol binding to rAeSCP-2 protein for the thiosemicarbazones tested (4 and 11). RFU = Relative Fluorescent Unit: Fluorescent intensity of (NBD-cholesterol/SCP-2/compound) − fluorescent intensity of (NBD-cholesterol/compound). Vertical Bar stands for one standard deviation.

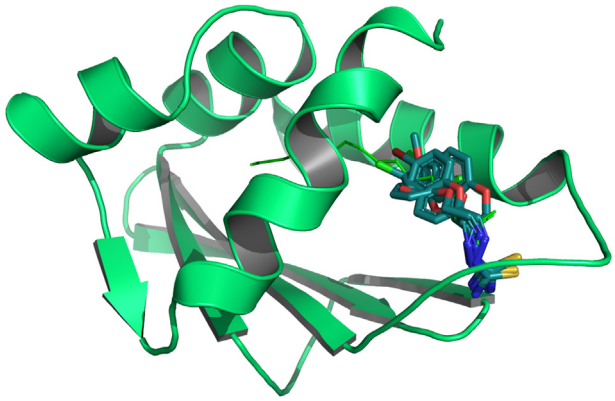


**Fig. 4.** Palmitic acid (green) and thiosemicarbazone superimposed structures docked on the AeSCP-2 target. (PDB: 1PZ4 – http://dx.doi.org/10.2210/pdb1pz4/pdb). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

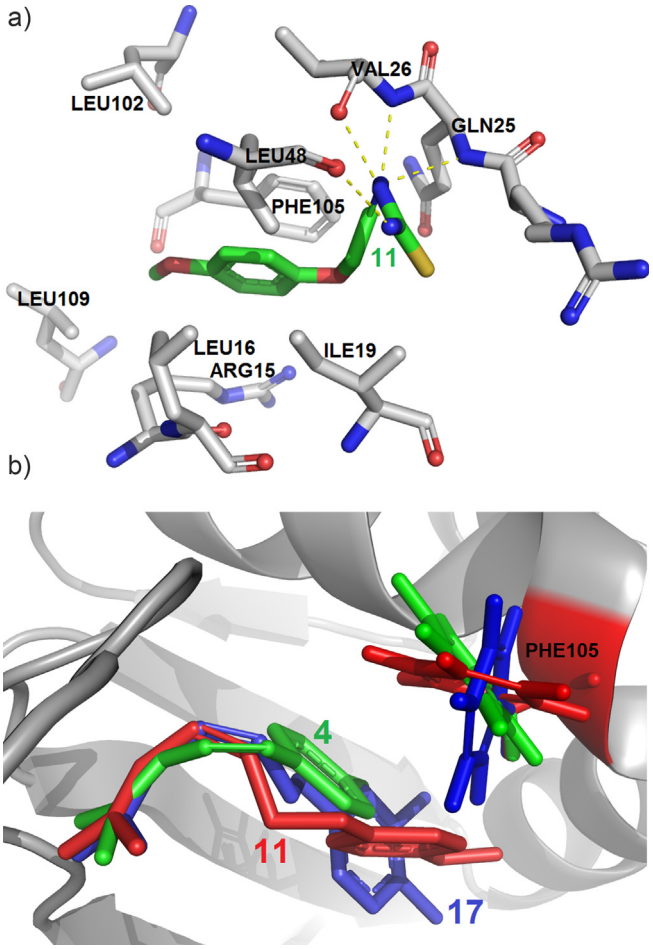



**Fig. 5.** Intermolecular interactions between: a) **11** and hydrophilic and hydrophobic residues of AeSCP-2 and b) aromatic rings of **4**, **11** and **17** and the hydrophobic residue Phe105 of AeSCP-2. Dashed lines represent polar interactions, particularly hydrogen bonds with the ligand. The other residues are involved in hydrophobic interactions with the ligand (11), and the residue PHE105 seems to engage in a π–π stacking interaction with the aromatic ring of the ligand. Other parts of the target are not shown for clarity reasons.

structure of the AeSCP-2 target. The Docking score values for molecules **4**, **11**, **14**, **15**, **16** and **17** are, respectively, 63.5, 64.9, 67.0, 65.3, 69.0 and 70.8.

In order to elucidate the binding mode of these molecules into AeSCP-2, an investigation of the intermolecular interactions was conducted. One can see in Fig. 5a the important residues mainly involved in the interactions between AeSCP-2 and molecule **11**. Fig. 5a provides a clear illustration of the binding pattern.

It is important to emphasize an important π–π stacking interaction that was found between the side-chain ring of the PHE105 residue and practically all the ligands investigated. Three examples can be seen in Fig. 5b, particularly for molecules **4**, **11** and **17**. The Docking protocol considered the active flexibility of residue PHE105 and other nine residues during the calculations. Fig. 5b shows the alternative conformations adopted by PHE105 in order to establish and stabilize the π–π stacking interaction with the respective ligand, searching for a maximum of planarity between the two aromatic rings (PHE105 and ligand). Every other molecule studied binds to AeSCP-2 in a very similar way, in comparison to molecule **11**, as one can see in the panoramic view of all the Docking solutions presented in Fig. 4. Furthermore, the important residues of AeSCP-2 involved in interactions (polar and hydrophobic) with the docked ligands are practically the same residues involved in the interaction with palmitic acid (ARG24, GLN25, LEU102 and PHE105) in the X-Ray structure, demonstrating that the choice of the flexible side chains for the Docking calculations was pertinent.

Finally, in order to compare the *in silico* results against larvicidal activities, the LC50 values were first converted into pLC50 (equals −log LC50, at molar concentration). The plot on Fig. 6 shows the trend observed between the *in silico* Docking scores and the pLC50 data. This indicates that most of the larvicidal compounds (those with the highest values for pLC50) are usually those with the higher docking scores, demonstrating that the molecules with more stable or positive Docking scores (i.e., greater *in silico* affinity for the AeSCP-2 target) are also the most active larvicidal compounds (i.e., greater pLC50 values). This kind of trend between *in vitro* and *in silico* data, showing the corroboration among experimental and theoretical results, was also found in other studies undertaken by our group [84–86].

Additionally, the *in vitro* values (EC50) for the inhibition of NBD-cholesterol binding to rAeSCP-2 protein (see Fig. 3), available for molecules **4** and **11**, are 5.0 μmolL$^{-1}$ and 0.6 μmolL$^{-1}$, respectively. These results also corroborate with the Docking score values for the same two molecules in the AeSCP-2 target, which are 63.5 e 64.9, respectively. Between these two compounds, molecule **11** presents the highest *in vitro* inhibition potency, and has also the greater *in silico* affinity (high Docking score) for the AeSCP-2 target, by showing a trend among *in silico* and *in vitro* results, indicating that the molecules with more stable or positive Docking scores (i.e., greater *in silico* affinity for the AeSCP-2 target) are also the most active larvicidal compounds (i.e., greater *in vitro* pLC50 values).

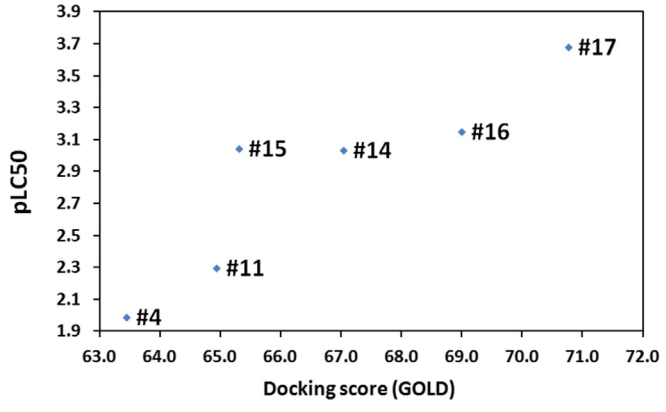



**Fig. 6.** Experimental (*in vitro*) larvicidal activity of thiosemicarbazone derivatives versus the Docking score (*in silico*) for these compounds in AeSCP-2.

## 3. Conclusion

Seventeen compounds belonging to the two classes of aryl-(thio)semicarbazone and phenoxymethyl-thiosemicarbazones derivatives were synthetized and tested against the L4 stage for *A. aegypti*. In general thiosemicarbazone exhibited an improved larvicide activity compared to the corresponding semicarbazone compounds. For the thiosemicarbazones, those with the phenoxymethyl group showed a higher larvicide activity. As desirable, the subset formed by eleven aryl- and phenoxymethyl-thiosemicarbazone derivatives showed a large variance of larvicide activity, with the $LC_{50}$ ranging from 21 μmol L$^{-1}$ to 311 μmol L$^{-1}$. A QSAR equation from the $LC_{50}$ against the electric dipole moment (μ) and the logarithm of the partition coefficient (logP) was obtained using the multiple linear regression technique. Using this equation we were doubly successful. First, in predicting the larvicide activity of two compounds not used to create the model, with the more active one, **18**, at 4.1 μM. Second, it was possible to explain why deciding to stop the analysis on the experimental $LC_{50}$ for the compound **4** was correct - the predicted $LC_{50}$ 6434 μ mol L$^{-1}$, is too high. The dose–response profile observed for the compounds **4** and **11** on the inhibition of AeSCP-2 are supported by the QSAR model. The docking calculations corroborate the hypothesis of the (thio)semicarbazone derivatives acting through the inhibition of the AeSCP-2 target.

## 4. Experimental

### 4.1. General chemistry

The arylhydrazone (**1**–**10**) were prepared essentially as reported previously [87,88] from commercially available aldehydes. Only compound (**3**) a nitro aryl-thiosemiccarbazone, the corresponding start aldehyde was obtained by reaction between 4-bromobenzaldeyde and nitric acid. Compound (**5**), bis-thiosemicarbazone derivative, was prepared using 2 mols of thiosemicarbazide for the 1.3-dicarbaldeyde. These reactions proceeded well upon refluxing (3–5 h) with ethanol as a solvent, a rate of 65–97% being observed overall.

For the synthesis of phenoxymethyl-thiosemicarbazones derivatives (**11**–**17**), the aldehydes were obtained from different phenols. The step process involved ether formation and acid hydrolysis of the acetal intermediary with moderate yields (36–56%). To accomplish the synthesis of 3-phenoxybutan-2-ones derivatives (18–20), start 3-phenoxybutan-2-ones intermediates were obtained by a reaction between 4-*tert*-butylphenol and 3-chloro-2-butanone using potassium carbonate and potassium iodide (Scheme 1).

Reagents were purchased from Acros Organics, Fluka, Sigma–Aldrich or Vetec and solvents from Vetec or Dinâmica. The deuterated solvents (DMSO-$d_6$, $CDCl_3$, $D_2O$) were supplied from CIL (Tédia Brazil). The reactions were monitored in thin layer chromatography (TLC) using silica gel 60 containing a fluorescent indicator F254. The chromatographic plates were visualized under UV light (at dual wavelength 365 or 254 nm). Melting points were measured using a Thomas Hoover capillary instrument and the values were not subsequently corrected. The $^1$H and $^{13}$C NMR were performed for all compounds, DEPT analysis as well as the addition of $D_2O$ for locating NH signals in the $^1$H NMR were determined. The

$^1$H and $^{13}$C NMR spectra were obtained using Unity Plus model Varian instruments (400 MHz for $^1$H, $^{13}$C for 100 MHz) or Bruker AMX (300 MHz–75.5 MHz for $^1$H and $^{13}$C), using tetramethylsilane as the internal standard. The number of signals in the $^1$H NMR spectra was designated as follows: s/singlet; /d doublet, t/triplet, dd/double doublet, q/quartet, m/multiplet. Infrared spectroscopy was performed with a Bruker instrument (model IFS 66) using KBr pellets. The Elemental Analysis was performed with a Carlo Erba instrument model E-1110 or Perkin Elmer 2400 seriesii. High-resolution electrospray ionization mass spectra (HRESIMS) were acquired on a nanoUPLC-Xevo G2 Tof (Waters) in the positive ionization mode.

### 4.2. Synthesis of compounds (**1**–**10**). Example for compound (**1**)

In a round bottom flask for 100 mL, 4-fluorobenzaldehyde (2.5 mmol) was dissolved in ethanol (15 mL), then HCl (03 drops) were added to the reaction at room temperature. Semicarbazide hydrochloride (2.5 mmol) was added and the mixture was maintained under magnetic stirring for 3 h at room temperature. After this time, the mixture was cooled at 0 °C and the precipitate was filtered in a Büchner funnel with a sintered disc filter, washed with cold water, *n*-hexane and then dried over $SiO_2$. Compounds were recrystallized in hot ethanol, to provide compounds with acceptable purity.

#### 4.2.1. 4-Fluorobenzaldehyde semicarbazone (**1**)

Colorless crystals, yield = 84%; mp (°C): 230–232; IR (KBr): 3463 and 3275 ($NH_2$), 3064 (NH), 1708 (C=O), 1591 (C=N) cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 6.51 (s, 2H, $NH_2$), 7.20 (m, 2H, Ar–H), 7.77 (m, 2H, Ar–H), 7.82 (s, 1H, CH=N), 10.24 (s, 1H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 164.1 (C Ar), 156.8 (C=O), 138.1 (CH=N), 131.4 (C Ar), 128.6 (CH Ar), 115.5 (CH Ar). *Anal.* Calcd for $C_8H_8FN_3O$: C, 53.04; H, 4.45; N, 23.19; Found: C, 53.06; H 4.56; N 22.39. HRESIMS *m/z*: 180.0590 [M−H]$^+$.

#### 4.2.2. 3,4-Dichlorobenzaldehyde semicarbazone (**2**)

Colorless crystals, yield = 88%; mp (°C): 246–249; IR (KBr): 3465 and 3279 ($NH_2$), 3155 (NH), 1700 (C=O), 1588 (C=N) cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 6.66 (s, 2H, $NH_2$), 7.61 (d, 1H, $J$ = 8.3 Hz, Ar–H), 7.66 (d, 1H, $J$ = 8.3 Hz, Ar–H), 7.78 (s, 1H, Ar–H), 8.12 (s, 1H, CH=N), 10.42 (s, 1H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 156.6 (C=O), 136.4 (CH=N), 135.7 (C Ar), 131.6 (C Ar), 130.9 (C Ar), 130.6 (CH Ar), 127.6 (CH Ar), 126.8 (CH Ar). *Anal.* Calcd for $C_8H_7Cl_2N_3O$: C, 41.41; H, 3.04; N, 18.11; Found: C, 41.61; H, 3.23; N, 17.48. HRESIMS *m/z*: 231.9981 [M]$^+$.

#### 4.2.3. 4-Bromo-3-nitrobenzaldehyde thiosemicarbazone (**3**)

Yellowish crystals, yield = 83%; mp (°C): 238–240; IR (KBr): 3417 and 3258 ($NH_2$), 3156 (NH), 1519 (C=N) cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$ 7.91 (d, 1H, $J$ = 8.0 Hz, Ar–H), 7.93 (d, 1H, $J$ = 8.0 Hz, Ar–H), 8.04 (s, 1H, CH=N), 8.28 (s, 1H, $NH_2$), 8.34 (s, 1H, $NH_2$), 8.56 (s,1H, Ar–H), 11.65 (s, 1H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 178.3 (C=S), 150.4 (C Ar), 138.5 (CH=N), 135.6 (C Ar), 134.6 (CH Ar), 131.9 (CH Ar), 122.4 (CH Ar), 112.9 (C Ar). *Anal.* Calcd for $C_8H_7BrN_4O_2S$: C, 31.70; H, 2.33; N, 18.48; Found: C, 30.29; H, 2.30; N, 16.76. HRESIMS *m/z*: 303.9576 [M+H]$^+$.

#### 4.2.4. 4-Hydroxybenzaldehyde thiosemicarbazone (**4**)

Brownish crystal, yield = 84%; mp (°C): 221–223; IR (KBr): 3467 and 3359 ($NH_2$), 3129 (NH), 1509 (C=N) cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): δ 6.77 (d, 2H, $J$ = 8.3 Hz, Ar–H), 7.60 (d, 2H, $J$ = 8.3 Hz, Ar–H), 7.84 (s, 1H, $NH_2$), 7.94 (s, 1H, CH=N), 8.07 (s, 1H, $NH_2$), 9.90 (s, 1H, OH), 11.25 (s, 1H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 177.4 (C=S), 159.2 (C Ar), 142.7 (CH=N), 129.0 (CH Ar), 125.1 (C Ar), 115.5 (CH Ar). *Anal.* Calcd for $C_8H_9N_3OS$: C, 49.22; H, 4.65; N, 21.52; Found: C, 49.20; H, 4.68; N, 20.61. HRESIMS *m/z*: 194.0405 [M−H]$^+$.

#### 4.2.5. 2,2'-(1,3-Phenylenebis(methanylylidene)) bis(thiosemicarbazide) (**5**)

Colorless crystals, yield = 65%; mp (°C): 255–258; IR (KBr): 3423 and 3235 ($NH_2$), 3148 (NH), 1524 (C=N) cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.42 (t, 1H, $J$ = 7.7 Hz, Ar–H), 7.79 (d, 2H, $J$ = 7.7 Hz, Ar–H), 8.05 (s, 2H, CH=N), 8.10 (s broad, 2H, $NH_2$), 8.22 (s, 1H, Ar–H), 8.26 (s, 2H, $NH_2$), 11.55 (s, 2H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 178.0 (C=S), 141.6 (CH=N), 134.7 (C Ar), 128.9 (CH Ar), 128.6 (CH Ar), 125.4 (CH Ar). *Anal.* Calcd for $C_{10}H_{12}N_6S_2$: C, 42.84; H, 4.31; N, 29.98; Found: C, 36.38; H, 5.11; N, 23.68. HRESIMS *m/z*: 281.0589 [M+H]$^+$.

#### 4.2.6. 4-Fluorobenzaldehyde thiosemicarbazone (**6**)

Beige crystals, yield = 97%; mp (°C): 189–191; IR (KBr): 3391 and 3235 ($NH_2$), 3156 (NH), 1533 (C=N) cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.23 (m, 2H, Ar–H), 7.87 (m, 2H, Ar–H), 8.02 (s, 1H, CH=N), 8.03 (s, 1H, $NH_2$), 8.20 (s d, 1H, $NH_2$), 11.43 (s, 1H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 177.9 (C=S), 164.6 (C Ar), 141.0 (CH=N), 130.8 (C Ar), 129.4 (CH Ar), 115.7 (CH Ar). *Anal.* Calcd for $C_8H_8FN_3S$: C, 48.72; H, 4.09; N, 21.31; Found: C, 47.90; H, 4.21; N, 20.08. HRESIMS *m/z*: 198.0450 [M+H]$^+$.

#### 4.2.7. 4-Chlorobenzaldehyde thiosemicarbazone (**7**)

Colorless crystals, yield: 77%; mp (°C): 217–220; IR (KBr): 3435 and 3279 ($NH_2$), 3164 (NH), 1523 (C=N) cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.45 (d, 2H, $J$ = 8.3 Hz, Ar–H), 7.83 (d, 2H, $J$ = 8.3 Hz, Ar–H), 8.01 (s, 1H, CH=N), 8.08 (s, 1H, $NH_2$), 8.25 (s, 1H, $NH_2$), 11.49 (s, 1H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 178.0 (C=S), 140.8 (CH=N), 134.2 (C Ar), 133.1 (C Ar), 128.9 (CH Ar), 128.6 (CH Ar). *Anal.* Calcd for $C_8H_8ClN_3S$: C, 44.97; H, 3.77; N, 19.67; Found: C, 45.30; H, 3.96; N, 19.12. HRESIMS *m/z*: 214.0140 [M+H]$^+$.

#### 4.2.8. 2-(3-Phenylallylidene)thiosemicarbazide (**8**)

Yellowish crystals, yield = 90%; mp (°C):110–113. IR (KBr): 3418 and 3260 ($NH_2$), 3155 (NH), 1537 (C=N)cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 6.86 (dd, 1H, $J$ = 8.9 Hz; $J$ = 15.8 Hz, CH=C<u>H</u>), 7.02 (d, 1H, $J$ = 15.89 Hz, C<u>H</u>=CH), 7.43–7.24 (m, 3H, Ar–H), 7.55 (d, 2H, $J$ = 7.79 Hz, Ar–H), 7.61 (s, 1H, $NH_2$), 7.89 (d, 1H, $J$ = 8.9 Hz, CH=N), 8.17 (s, 1H, $NH_2$), 11.40 (s, 1H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 177.6 (C=S), 144.7 (CH=N), 138.8 (CH=N), 135.8 (C Ar), 128.8 (CH Ar), 126.9 (CH Ar), 125.0 (CH Ar). *Anal.* Calcd for $C_{10}H_{11}N_3S$: C, 58.51; H, 5.40; N, 20.47; Found: C, 53.93; H, 6.20; N, 17.65. HRESIMS *m/z*: 206.0749 [M+H]$^+$.

#### 4.2.9. 4-Bromobenzaldehyde thiosemicarbazone (**9**)

Beige crystals, yield = 82%; mp (°C): 209–211, IR (KBr): 3436 and 3287 ($NH_2$), 3165 (NH), 1522 (C=N) cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): δ 7.58 (d, 2H, $J$ = 8.3 Hz, Ar–H), 7.76 (d, 2H, $J$ = 8.3 Hz, Ar–H), 8.00 (s, 1H, CH=N), 8.08 (s, 1H, $NH_2$), 8.24 (s broad, 1H, $NH_2$), 11.49 (s, 1H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 178.1 (C=S), 140.9 (CH=N), 134.5 (C Ar), 131.5 (CH Ar), 129.1 (CH Ar), 122.9 (C Ar). *Anal.* Calcd for $C_8H_8BrN_3S$: C, 37.22; H, 3.12; N, 16.28; Found: C, 57.32; H, 3.24; N, 15.78. HRESIMS *m/z*: 259.9630 [M+H]$^+$.

#### 4.2.10. 3,4-Dichlorobenzaldehyde thiosemicarbazone (**10**)

White crystals, yield = 62%; mp (°C): 212–215. IR (KBr): 3396 and 3255 ($NH_2$), 3154 (NH), 1539 (C=N)cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): δ 7.63 (d, 1H, $J$ = 8.3 Hz, Ar–H), 7.71 (dd, 1H, $J$ = 1.7 Hz, $J$ = 8.3 Hz, Ar–H), 7.98 (s, 1H, CH=N), 8.24 (d, 1H, $J$ = 1.7 Hz, Ar–H), 8.27 (s, 1H, $NH_2$), 8.30 (s, 1H, $NH_2$), 11.57 (s, 1H, NH). $^{13}$C NMR (75.5 MHz, ppm, DMSO-$d_6$): δ 178.2 (C=S), 139.3 (CH=N), 135.0 (C

Ar), 131.8 (C Ar), 131.7 (C Ar), 130.7 (CH Ar), 128.1 (CH Ar), 127.7 (CH Ar). *Anal.* Calcd for $C_8H_7Cl_2N_3S$: C, 38.73; H, 2.84; N, 16.94; Found: C, 39.09; H, 3.03; N, 16.41. HRESIMS m/z: 247.9636 [M−H]+.

### 4.3. Synthesis of compounds (**11**–**17**). Example for compound (**11**)

In a round bottom flask, phenol (3.1 mmol), 5 mL DMF and $K_2CO_3$ (7.8 mmol) were added together. The reaction mixture was maintained under magnetic stirring at room temperature for 30 min. Then, bromoacetaldehyde diethylacetal (9.3 mmol) was added in portions and the reaction mixture was heated under reflux for 72 h. After that, the product was extracted with dichloromethane and the solvent was removed under reduced pressure and dried in $SiO_2$. The hydrolysis of acetal in aldehyde was achieved by adding acetone (5 mL), $H_2SO_4$ (7 drops) and 10 mL water. The reaction mixture was stirred under reflux heating for 4 h. The product was extracted with ethyl acetate and the solvent was removed under reduced pressure and then dried in $SiO_2$. The aldehyde obtained was reacted with thiosemicarbazide as described above. Products were purified by recrystallization using ethanol/water (1:1).

#### 4.3.1. 2-(4-Methoxyphenoxy)acetaldehyde thiosemicarbazone (**11**)

Brownish crystals, yield = 56%; mp (°C): 145–147; IR (KBr): 3372 and 3279 ($NH_2$), 3174 (NH), 1509 (C=N) $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 3.68 (s, 3H, $OCH_3$), 4.59 (s, 2H, $CH_2$), 6.88 (m, 4H, Ar–H), 7.51 (s, 1H, CH=N), 7.68 (s, 1H, $NH_2$), 8.17 (s, 1H, $NH_2$), 11.33 (s, 1H, NH). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 55.3 ($CH_3$), 67.3 ($CH_2$), 114.6 (CH Ar), 115.7 (CH Ar), 141.4 (CH=N), 151.8 (C Ar), 153.73 (C Ar), 178.3 (C=S). *Anal.* Calcd. For $C_{10}H_{13}N_3O_2S$: C, 50.19; H, 5.48; N, 17.56; Found: C, 50.03; H, 5.28; N, 17.34. HRESIMS *m/z*: 240.3020 $[M+H]^+$.

#### 4.3.2. 2-Phenoxyacetaldehyde thiosemicarbazone (**12**)

White crystals, yield = 40%; mp (°C): 142–143; IR (KBr): 3449 and 3323 ($NH_2$), 3158 (NH), 1536 (C=N) $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 4.67 (d, 2H, $J$ = 5.2 Hz, $CH_2$), 6.95 (t, 1H, $J$ = 7.5 Hz, Ar–H), 6.98 (d, 2H, $J$ = 8.4 Hz, Ar–H), 7.29 (dd, 2H, $J$ = 7.5 Hz, $J$ = 8.4 Hz, Ar–H), 7.53 (t, 1H, $J$ = 5.2 Hz CH=N), 7.68 (s broad, 1H, $NH_2$), 8.18 (s broad, 1H, $NH_2$), 11.35 (s, 1H, NH). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 66.7 ($CH_2$),114.6 (CH Ar), 121.0 (CH Ar), 129.5 (CH Ar), 141.0 (CH=N), 157.8 (C Ar), 178.3 (C=S). *Anal.* Calcd. For $C_9H_{11}N_3OS$: C, 51.66; H, 5.30; N, 20.08; Found: C, 51.58; H, 5.08; N, 19.76. HRESIMS *m/z*: 210.0621 $[M+H]^+$.

#### 4.3.3. 2-(3-Chlorophenoxy)acetaldehyde thiosemicarbazone (**13**)

Beige crystals, yield = 40%; mp (°C): 147–149; IR (KBr): 3406 and 3239 ($NH_2$), 3156 (NH), 1513 (C=N) $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 4.69 (s, 2H, $CH_2$), 7.08–6.85 (m, 3H, Ar–H), 7.31 (s, 1H, Ar–H), 7.50 (s, 1H, CH=N), 7.69 (s, 1H, $NH_2$), 8.20 (s, 1H, $NH_2$), 11.37 (s, 1H, NH). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 67.1 ($CH_2$), 113.7 (CH Ar), 114.8 (CH Ar), 121.0 (CH Ar), 130.9 (CH Ar), 133.7 (C Ar), 140.4 (CH=N), 158.8 (C Ar), 178.4 (C=S). *Anal.* Calcd. For $C_9H_{10}ClN_3OS$: C, 44.36; H, 4.14; N, 17.24; Found: C, 43.99; H, 4.04; N, 16.82. HRESIMS *m/z*: 244.0229 $[M+H]^+$.

#### 4.3.4. 2-(4-Bromophenoxy)acetaldehyde thiosemicarbazone (**14**)

White crystals, yield = 45%; mp (°C): 166–168; IR (KBr): 3388 and 3261 ($NH_2$), 3154 (NH), 1536 (C=N) $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): δ4.66 (s, 2H, $CH_2$), 6.97 (s, 2H, Ar–H), 7.46 (s, 2H, Ar–H), 7.50 (s, 1H, CH=N), 7.69 (s, 1H, $NH_2$), 8.21 (s, 1H, $NH_2$), 11.37 (s, 1H, NH). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 67.0 ($CH_2$), 112.4 (C Ar), 117.0 (CH Ar), 132.1 (CH Ar), 140.4 (CH=N), 157.1 (C Ar), 178.3 (C=S). *Anal.* Calcd. For $C_9H_{10}BrN_3OS$: C, 37.51; H, 3.50; N, 14.58; Found: C, 37.22; H, 3.76; N, 14.23. HRESIMS *m/z:* 288.2792 $[M]^+$.

#### 4.3.5. 2-(4-Chlorophenoxy)acetaldehyde thiosemicarbazone (**15**)

Beige crystals, yield = 52%; mp (°C): 178–181; IR (KBr): 3402 and 3273 ($NH_2$), 3152 (NH), 1532 (C=N) $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$):δ4.59 (s broad, 2H, $CH_2$), 7.01 (d, 2H, $J$ = 8.7 Hz, Ar–H), 7.33 (d, 2H, $J$ = 8.7 Hz, Ar–H), 7.50 (s, 1H, CH=N), 7.69 (s, 1H, $NH_2$), 8.19 (s, 1H, $NH_2$), 11.36 (s, 1H, NH). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): δ67.1 ($CH_2$), 116.5 (CH Ar), 124.8 (C Ar), 129.3 (CH Ar), 140.5 (CH=N), 156.7 (C Ar), 178.4 (C=S). *Anal.* Calcd. For $C_9H_{10}ClN_3OS$: C, 44.36; H, 4.14; N, 17.24; Found: C, 44.26; H, 3.98; N, 16.71. HRESIMS *m/z*: 244.0230$[M+H]^+$.

#### 4.3.6. 2-(2,3-Dichlorophenoxy)acetaldehyde thiosemicarbazone (**16**)

Beige crystals, yield = 40%; mp (°C): 189–192; IR (KBr): 3430 and 3251 ($NH_2$), 3156 (NH), 1545 (C=N) $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 4.80 (s, 2H, $CH_2$), 7.45–7.00 (m, 3H, Ar–H), 7.53 (s, 1H, CH=N), 7.71 (s, 1H, $NH_2$), 8.23 (s, 1H, $NH_2$), 11.41 (s, 1H, NH). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 68.3 ($CH_2$), 112.8 (CH Ar), 120.1 (C Ar), 122.6 (CH Ar), 128.5 (CH Ar), 132.4 (C Ar), 139.7 (CH=N), 154.7 (C Ar), 178.5 (C=S). *Anal.* Calcd. For $C_9H_9Cl_2N_3OS$: C, 38.86; H, 3.26; N, 15.11; Found: C, 38.49; H, 3.44; N, 14.83. HRESIMS *m/z*: 277.9840 $[M]^+$.

#### 4.3.7. 2-(3,4-Dichlorophenoxy)acetaldehyde thiosemicarbazone (**17**)

White crystals, yield = 56%; mp (°C): 169–172; IR (KBr): 3408 and 3264 ($NH_2$), 3155 (NH), 1534 (C=N) $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 4.70 (d, 1H, $J$ = 4.7 Hz, $CH_2$), 7.02 (dd, 1H, $J$ = 2.3 Hz, Ar–H, $J$ = 9.1 Hz, Ar–H), 7.31 (d, 1H, $J$ = 2.3 Hz, Ar–H), 7.49 (t, 1H, $J$ = 4.7 Hz, CH=N), 7.52 (d, 1H, $J$ = 9.1 Hz, Ar–H), 7.68 (s, 1H, $NH_2$), 8.22 (s, 1H, $NH_2$), 11.38 (s, 1H, NH). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 67.4 ($CH_2$), 115.6 (CH Ar), 116.7 (CH Ar), 122.9 (C Ar), 131.0 (CH Ar), 131.6 (C Ar), 139.9 (CH=N), 157.3 (C Ar), 178.4 (C=S). *Anal.* Calcd. For $C_9H_9Cl_2N_3OS$: C, 38.86; H, 3.26; N, 15.11; Found: C, 38.55; H, 3.52; N, 14.91. HRESIMS *m/z*: 277.9822 $[M]^+$.

### 4.4. Synthesis of compounds (**18**)

3-((3,4-dichloro)phenoxy)butan-2-one was obtained by reacting 3,4-dichlorophenol (6.4 mmol, 1.0 g) with 3-chloro-2-butanone (6.66 mmol, 0.71 g) in potassium carbonate (9.98 mmol, 1.38 g), potassium iodide (0.66 mmol, 0.11 g) and 15 mL of acetone. This mixture was maintained under magnetic stirring at room temperature for 12 h. The precipitate was filtered in a Büchner funnel with a sintered disc filter and discarded. The solvent was completely evaporated and then was extracted first into diethyl ether and water and subsequently in diethyl ether and sodium hydroxide to 0.1 M. The compound was then dried in a $SiO_2$ glass dissector under vacuum. The 3-(3,4-dichloro)phenoxy-butan-2-one (6.16 mmol, 1.36 g) obtained was reacted with thiosemicarbazide (6.16 mmol, 0.56 g), 4 drops of hydrochloric acid and 10 mL of ethanol in a 150 mL round bottom flask under magnetic stirring for 2 h. A yellowish solid was obtained, filtered in Büchner funnel with a sintered disc filter, washed with cold water, and then dried in $SiO_2$. The products were purified by crystallization using ethanol as solvent.

#### 4.4.1. 3-(3,4-Dichlorophenoxy)butan-2-one thiosemicarbazone (**18**)

Yellowish crystals yield = 1.37 g, 74.41%. mp (°C): 154–156. IR (KBr): 3420 (N–H), 3259 and 3155 ($NH_2$), 1593 (C=N), 1282 (C–O), 1084 (C=S) $cm^{-1}$.$^1H$ NMR (300 MHz, DMSO-$d_6$): δ 1.42 (d, $J$ = 6.6 Hz, 3H, $CH_3$), 1.82 (s, 3H, $CH_3$), 5.00 (q, 1H, $J$ = 6.6 Hz, H–C), 7.00 (dd, 1H, $J$ = 3.0 Hz, $J$ = 9.0 Hz, Ar–H), 7.27 (d, 1H, $J_4$ = 3.0 Hz, Ar–H), 7.47 (d, 1H, $J$ = 9.0 Hz, Ar–H), 7.85 (s largo, 1H, $NH_2$), 8.24 (s

largo, 1H, $NH_2$), 10.19 (s, 1H, NH). $^{13}C$ NMR (75.5 MHz, DMSO-$d_6$): δ 11.08 ($CH_3$–C═N), 18.48 ($CH_3$–C–O), 77.12 (CH–O), 116.52 (CH, Ar), 117.75 (CH, Ar), 122.94 (C–Cl, Ar), 130.99 (CH, Ar), 131.55 (C–Cl, Ar), 150.58 (C═N), 156.71 (C–O, Ar), 179.30 (S═C–$NH_2$). *Anal.* Calcd. for $C_{11}H_{13}N_3OSCl_2$: C, 43.15; H, 4.28; N, 13.72; Found: C, 43.39; H, 4.09; N, 13.69. HRESIMS *m/z*: 306.0320 $[M+H]^+$.

### 4.5. Synthesis of diethyl acetal intermediate

In a round bottom flask, phenol (3.12 mmol) was added to dry DMF and $K_2CO_3$ (7.8 mmol). The mixture was kept under magnetic stirring at room temperature for 30 min. Then bromoacetaldehyde diethyl acetal (9.36 mmol) was added and the brownish mixture was kept under reflux heating for 72 h. The reactions were monitored by thin-layer chromatographic plate (TLC). After that, the product was extracted with dichloromethane and solvent was removed under reduced pressure and then dried in $SiO_2$.

### 4.6. Synthesis of aldheydes

In a round bottom flask, acetal intermediate was mixed with acetone (5 mL), after an acid solution ($H_2SO_4$ 7 drops and water 10 mL) had been slowly added. The mixture was kept under reflux heating (100 °C) for 4 h. The reaction was monitored using a thin-layer chromatographic plate (TLC). At the end, the product was extracted with ethyl acetate and the solvent was removed under reduced pressure and then dried in $SiO_2$.

### 4.7. Synthesis of intermediated compounds 3-phenoxybutan-2-ones intermediates to afford compound ***18***

**3-((4-*tert*butyl)phenoxy)butan-2-one:**4-*tert*-butylphenol (6.66 mmol, 1.0 g), 3-chloro-2-butanone (6.66 mmol, 0.71 g), potassium carbonate (9.98 mmol, 1.38 g), potassium iodide (0.66 mmol, 0.11 g) and 15 mL of acetone were mixed in a 150 mL round bottom flask and placed under magnetic stirring for 12 h. The precipitate was filtered in a Büchner funnel with a sintered disc filter and discarded. The solvent was completely evaporated and then was extracted first into diethyl ether and water and subsequently in diethyl ether and sodium hydroxide to 0.1 M. Compound was then dried in $SiO_2$ glass dissector under vacuum.

### 4.8. QSAR

In this work we opted to employ a set of QSAR descriptors, named electronic and hydrophobic descriptors, that our review of the literature (see introduction section) had pointed out as being important for representing the larvicide activity of *A. aegypti*. In particular, we selected descriptors of easy chemical interpretation and at the same time belonging to the ligand, such as: i) atomic charge or sum of atomic charge, ii) the electric dipole moment, iii) HOMO, LUMO and HOMO-LUMO energy difference, iv) Log P and its square value, $LogP^2$.

In order to obtain the QSAR model, Multiple Linear Regressions (MLR) [89] between the ligand descriptors and the larvicide activity for *A. aegypti* were used due to the simplicity of their interpretation To avoid collinearity problems between descriptors in MLR [90], before obtaining the QSAR equation, the correlation coefficient matrix (related to the information contained in Table 2) was determined. Only those descriptors that correlated to the larvicide activity above 0.7 and at the same time did not correlate with each other over 0.5 were used in the MLR. In order to check the quality of the MLR the cross-validation method was employed [91]. In all cases the Statistica program [92] was employed.

### 4.9. Docking studies for A. aegypti sterol carrier protein-2 (AeSCP-2)

The *in vitro* inhibition of AeSCP-2 was measured for molecules **4** and **11**, and the other molecules (**14**–**17**), shown in Table 1 and discussed above as highly active in larvicide bioassays, provided a base for the selection of compounds (ligands) **4**, **11**, **14**, **15**, **16** and **17** for docking calculations. The optimized structures of all the ligands were obtained by application of the RM1 method [93], available as part of the SPARTAN 08′ program [94], using internal default settings for convergence criteria. The target structure for docking calculations and analysis was taken from Protein Data Bank (http://www.pdb.org) under the PDB code 1PZ4 for *A. aegypti* sterol carrier protein-2 (AeSCP-2) [81]. The active site was defined as all atoms within a radius of 6.0 Å from the co-crystallized ligand (palmitic acid, labeled as PLM in PDB). The concern to take into account the Induced Fit effects led us to treat the side chains of ten residues as flexible during the docking calculations, following current trends in this area. Residues ARG15, LEU16, ILE19, ASP20, ASN23, ARG24, GLN25, LEU48, LEU102 and PHE105 were selected for the AeSCP-2 target. The CHEMPLP score function [95] of the GOLD 5.1 program [96] was used for docking calculations, followed by the Binana program [97], which was used to analyze the molecular interactions present in the best docking solutions, using a default setting, except for H-bond distance, which was changed to a maximum of 3.5 Å. The figures were generated with Pymol [98].

### 4.10. Larvicidal bioassay

The larvicidal activity of the thiosemicarbazones and semicarbazones was evaluated using an adaptation [54,32] of the method recommended by the World Health Organization [99]. Stock solutions were prepared by solubilizing 5 mg of the compounds with the appropriated co-solvents (Tween80); the resulting solution was then dissolved in 50 mL of distilled water. Dilution of the stock solutions allowed the preparation of suitable concentrations to be tested. Fourth larvae stage *A. aegypti* were added to beakers (20 larvae per beaker) containing these solutions (20 mL). Four replicate assays were carried out for every sample concentration, and for each assay a negative control was included and prepared as described without the active compounds. Mortality of the larvae was determined after 48 h incubation at 28 ± 2 °C, 70 ± 10 relative humidity. Larvae were considered dead when they did not respond to stimulus or did not rise to the surface of the solution. The lethal concentration value $LC_{50}$ was calculated by probit analysis using StatusPlus2006 software [32,33].

### 4.11. Cytotoxicity to mouse splenocytes

BALB/c mouse splenocytes were placed into 96-well plates at a cell density of $6 \times 10^5$ cells/well in an RPMI-1640 medium supplemented with 10% of FBS and 50 μg $mL^{-1}$ of gentamycin. Each test inhibitor was evaluated in six concentrations (1, 5, 10, 25, 50 and 100 μg $mL^{-1}$) in triplicate. To each well, an aliquot of test inhibitor suspended in DMSO was added. Controls included wells only containing either solvent (untreated cells) or saponin (positive control). The plate was incubated for 24 h at 37 °C and 5% $CO_2$. After incubation, 1.0 μCi of $^3H$-thymidine (Perkin Elmer, Waltham, USA) was added to each well, and the plate was returned to the incubator. The plate was then transferred to a beta-radiation counter (Multilabel Reader, Finland), and the percent of $^3H$-thymidine was determined. Cell viability was measured as the percent of $^3H$-thymidine incorporation for treated-cells in comparison to untreated cells. Highest non-toxic concentration for each compound was estimated.

### 4.12. Inhibitory cholesterol binding test

Recombinant AeSCP-2 protein was purified and an NBD-cholesterol (Molecular probes, Eugene, OR, USA)/rAeSCp-2/compound competition assay was performed as described [69]. A separate set of tests were performed using NBD cholesterol with increasing concentration of a SCPI to assess whether the SCPI interfered with NBD cholesterol fluorescence. If a compound interfered with NBD cholesterol fluorescence, the background control was NBD cholesterol along with the SCPI. The net change in NBD cholesterol fluorescence intensity was calculated by subtracting the fluorescence of background controls from the NDB cholesterol/AeSCP-2 complex in the presence of a compound. The data were plotted with the relative NBD cholesterol intensity (bound NBD cholesterol) as the Y-axis and molarity of inhibitor as the X-axis using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA).

## Authors' contributions

DMAFN coordinated the larvicidal bioassay, wrote the first draft and revised the manuscript. MNR coordinated the first electronic structure calculations and advised AGS on the analysis of the results and development of the QSAR model. GKNS and KAD developed the larvicidal bioassays. DRM made the compound synthesis and revised the synthetic experimental methodology. JWPE worked on the compound synthesis and wrote part of the manuscript. ADTO synthesized compound **18**. ACLL coordinated the synthesis and spectroscopic characterization of all compounds. DJB orientated the synthesis. MZH developed the docking studies. VRAP coordinated and analyzed the cytotoxicity assays in BALB/c mice splenocytes developed by LFR, MCABC and BCO. QL developed the experiments of mode-of-action on AeSCP-2. KMMJr discussed the electronic structure results and JBPS was involved in the electronic structure calculations, QSAR model, comparison between calculated and experimental results and wrote the manuscript. All authors read and agreed with the final version of this manuscript.

## Notes

The authors declare no competing financial interest.

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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.2015.04.061.

## References

[1] L. Rêgis, A.F. Furtado, C.M.F. de Oliveira, C.B. Bezerra, L.R.F. da Silva, J. Araújo, A. Maciel, M.H. Silva-Filha, S.B. Silva, Integrated control of the filariasis vector with community participation in an urban area of Recife, Pernambuco, Brazil, Cad. Saúde Públ. 12 (1996) 473–482, http://dx.doi.org/10.1590/S0102-311X1996000400005.

[2] R. N'Guessan, P. Boko, A. Odjo, J. Chabi, M. Akogbeto, M. Rowland, Control of pyrethroid and DDT-resistant Anopheles gambiae by application of indoor residual spraying or mosquito nets treated with a long-lasting organophosphate insecticide, chlorpyrifos-methyl, Malar. J. 9 (2010) 44, http://dx.doi.org/10.1186/1475-2875-9-44. http://www.malariajournal.com/content/9/1/44.

[3] T. Mizutani, M. Kobayashi, Y. Eshita, K. Shirato, T. Kimura, Y. Ako, H. Miyoshi, T. Takasaki, I. Kurane, H. Kariwa, T. Umemura, I. Takashima, Involvement of the JNK-like protein of the *Aedes albopictus* mosquito cell line, C6/36, in phagocytosis, endocytosis and infection of West Nile virus, Insect Mol. Biol. 12 (2003) 491–499, http://dx.doi.org/10.1046/j.1365-2583.2003.00435.x.

[4] G. Pialoux, B.-A. Gaüzère, S. Jauréguiberry, M. Strobel, Chikungunya, an epidemic arbovirosis, Lancet Infect. Dis. 7 (2007) 319–327, http://dx.doi.org/10.1016/S1473-3099(07)70107-X.

[5] M.B. Nathan, R. Dayal-Drager, M. Guzman, Chapter 1: epidemiology, burden of disease and transmission, in: World Health Organization (Ed.), Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control, New Edition, WHO Library Cataloguing-in-Publication Data, Geneva, 2009, pp. 3–17.

[6] D.M. Morens, A.S. Fauci, Dengue and hemorrhagic fever: a potential threat to public health in United States, J. Am. Med. Assoc. 299 (2008) 214–216, http://dx.doi.org/10.1001/jama.2007.31-a.

[7] J.G. Rigau-Perez, M.K. Laufer, Dengue-related deaths in Puerto Rico, 1992–1996: diagnosis and clinical alarm signals, Clin. Infect. Dis. 42 (2006) 1241–1246, http://dx.doi.org/10.1086/501355.

[8] P.V. Effler, L. Pang, P. Kitsutani, V. Vorndam, M. Nakata, T. Ayers, J. Elm, T. Tom, P. Reiter, J.G. Rigau-Perez, J.M. Hayes, K. Mills, M. Napier, G.G. Clark, D.J. Gubler, Dengue fever, Hawaii, 2001–2002, Emerg. Infect. Dis. 11 (2005) 742–749, http://dx.doi.org/10.3201/eid1105.041063.

[9] J.L. Kyle, E. Harris, Global spread and persistence of dengue, Annu. Rev. Microbiol. 62 (2008) 71–92, http://dx.doi.org/10.1146/annurev.micro.62.081307.163005.

[10] N.W. Beebe, R.D. Cooper, P. Mottram, A.W. Sweeney, Australia's dengue risk driven by human adaptation to climate change, PLoS Negl. Trop. Dis. 3 (2009) 1–9, http://dx.doi.org/10.1371/journal.pntd.0000429.

[11] M.J.P. Delgado, J.M. Gutierrez, L.B. Radic, T. Maretic, S. Zekan, T. Avšič-Županc, E.S. Aymar, A. Trilla, J.G. Brustenga, Imported dengue hemorrhagic fever, Europe, Emerg. Infect. Dis. 14 (2008) 1329–1330, http://dx.doi.org/10.3201/eid1408.080068.

[12] R. Allwinn, Significant increase in travel-associated dengue fever in Germany, Med. Microbiol. Immunol. 200 (2011) 155–159, http://dx.doi.org/10.1007/s00430-011-0185-2.

[13] WHO, Fact Sheet N 117, January, 2012. http://www.who.int/mediacentre/factsheets/fs117/en/. August 23, 2014.

[14] S.B. Halstead, J. Deen, The future of dengue vaccines, Lancet 360 (2002) 1243–1245, http://dx.doi.org/10.1016/S0140-6736(02)11276-1.

[15] D. Normile, Surprising new dengue virus throws a spanner in disease control efforts, Science 342 (2013) 415, http://dx.doi.org/10.1126/science.342.6157.415.

[16] T.M. Ross, Dengue virus, Clin. Lab. Med. 30 (2010) 149–160, http://dx.doi.org/10.1016/j.cll.2009.10.007.

[17] A.J. Stevens, M.E. Gahan, S. Mahalingam, P.A. Keller, The medicinal chemistry of dengue fever, J. Med. Chem. 52 (2009) 7911–7926, http://dx.doi.org/10.1021/jm900652e.

[18] D.J. Gubler, Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century, Trends Microbiol. 10 (2002) 100–103, http://dx.doi.org/10.1016/S0966-842X(01)02288-0.

[19] D.J. Gubler, The economic burden of dengue, Am. J. Trop. Med. Hyg. 86 (2012) 743–744, http://dx.doi.org/10.4269/ajtmh.2012.12-0157.

[20] R.I. Rose, Pesticides and public health: integrated methods of mosquito management, Emerg. Infect. Dis. 7 (2001) 17–23, http://dx.doi.org/10.3201/eid0701.700017.

[21] J.A. Suya, D.S. Shepard, M.-S. Chang, M. Caram, S. Hoyer, D. Socheat, N. Chantha, M.B. Nathan, Cost-effectiveness of annual targeted larviciding campaigns in Cambodia against the dengue vector *Aedes aegypti*, Trop. Med. Int. Health 12 (2007) 1026–1036, http://dx.doi.org/10.1111/j.1365-3156.2007.01889.x.

[22] C.F.S. Andrande, M. Modolo, Susceptibility of *Aedes aegypti* larvae to temephos and *Bacillus thuringiensis var israelensis* in integrated control, Rev. Saúde Publ. 25 (1991) 184–187, http://dx.doi.org/10.1590/S0034-89101991000300004.

[23] I.A. Braga, J.B.P. Lima, S.S. Soares, D. Valle, *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe, and Alagoas, Brazil, Mem.do Inst. Oswaldo Cruz 99 (2004) 199–203, http://dx.doi.org/10.1590/S0074-02762004000200015.

[24] A.J. Martins, C.D.M. Ribeiro, D.F. Bellinato, A.A. Peixoto, D. Valle, J.B.P. Lima, Effect of insecticide resistance on development, longevity and reproduction of field or laboratory selected *Aedes aegypti* populations, PLoS One 7 (2012) 1–9, http://dx.doi.org/10.1371/journal.pone.0031889.

[25] M.A.V. Melo-Santos, J.J.M. Varjal-Melo, A.P. Araújo, T.C.S. Gomes, M.H.S. Paiva, L.N. Regis, A.F. Furtado, T. Magalhaes, M.L.G. Macoris, M.T.M. Andrighetti, C.F.J. Ayres, Resistance to the organophosphate temephos: mechanisms, evolution and reversion in an *Aedes aegypti* laboratory strain from Brazil, Acta Trop. 113 (2010) 180–189, http://dx.doi.org/10.1016/j.actatropica.2009.10.015.

[26] M. da Silva, A. Furigo Jr., S.A. Furlan, O. Souza, Production of bio-inseticide *Bacillus thuringiensis var. israelensis* in semicontinuous processes combined with batch processes for sporulation, Braz. Arch. Biol. Tech. 54 (2011) 45–52,

http://dx.doi.org/10.1590/S1516-89132011000100006.
[27] P. Subbiah, B. Archana, Optimization of medium composition for the production of mosquitocidal toxins from *Bacillus thuringiensis* subsp. *Israelensis*, Indian J. Exp. Biol. 50 (2012) 65–71.
[28] M. Paris, C. Melodelima, E. Coissac, G. Tetreau, S. Reynaud, J.-P. David, L. Despres, Transcription profiling of resistance to Bti toxins in the mosquito *Aedes aegypti* using next-generation sequencing, J. Invertebr. Pathol. 109 (2012) 201–208, http://dx.doi.org/10.1016/j.jip.2011.11.004.
[29] A.M. Pohlit, A.R. Rezende, E.L.L. Baldin, N.P. Lopes, V.F.A. Neto, Plant extracts, isolated phytochemicals, and plant-derived agents which are lethal to arthropod vectors of human tropical diseases – a review, Planta Med. 77 (2011) 618–630, http://dx.doi.org/10.1055/s-0030-1270949.
[30] S. Rajkumar, A. Jebanesan, Chemical composition and larvicidal activity of leaf essential oil from Clausena dentata (Willd) M. Roam. (Rutaceae) against the chikungunya vector, Aedes aegypti Linn. (Diptera: Culicidae), J. Asia-Pacific Ento. 13 (2010) 107–109, http://dx.doi.org/10.1016/j.aspen.2010.02.001.
[31] S.S. Cheng, C.G. Huang, Y.J. Chen, J.J. Yu, W.J. Chen, S.T. Chang, Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species, Bioresour. Technol. 100 (2009) 452–456, http://dx.doi.org/10.1016/j.biortech.2008.02.038.
[32] G.K.N. Santos, K.A. Dutra, R.A. Barros, C.A.G. Câmara, D.D. Lira, N.B. Gusmão, D.M.A.F. Navarro, Essential oils from *Alpinia purpurata* (Zingiberaceae): chemical composition, oviposition deterrence, larvicidal and antibacterial activity, Ind. Crop. Prod. 40 (2012) 254–260, http://dx.doi.org/10.1016/j.indcrop.2012.03.020.
[33] E.M. Bianco, L. Pires, G.K.N. Santos, K.A. Dutra, T.N.V. Reis, E.R.T.P.P. Vasconcelos, A.L.M. Cocentino, D.M.A.F. Navarro, Larvicidal activity of seaweeds from northeastern Brazil and of a halogenated sesquiterpene against the dengue mosquito (*Aedes aegypti*), Ind. Crop. Prod. 43 (2013) 270–275, http://dx.doi.org/10.1016/j.indcrop.2012.07.032.
[34] A. Leo, C. Hansch, C. Church, Comparison of parameters currently used in the study of structure-activity relationships, J. Med. Chem. 12 (1969) 766–771, http://dx.doi.org/10.1021/jm00305a010.
[35] C. Hansch, P.P. Maloney, T. Fujita, R.M. Muir, Correlation of biological activity of phenoxyacetic acids with hammett substituent constants and partition coefficients, Nature 194 (1962) 178–180, http://dx.doi.org/10.1038/194178b0.
[36] S.R.L. Santos, V.B. Silva, M.A. Melo, J.D.F. Barbosa, R.L.C. Santos, D.P. Sousa, S.C.H. Cavalcanti, Toxic effects on and structure-toxicity relationships of phenylpropanoids, terpenes, and related compounds in *Aedes aegypti* larvae, Vector Borne Zoonotic Dis. 10 (2010) 1049–1054, http://dx.doi.org/10.1089/vbz.2009.0158.
[37] D.P. de Sousa, Y.W. Vieira, M.;P. Uliana, M.A. Melo, T.J. Brocksom, S.C.H. Cavalcanti, Larvicidal activity of *para*-Benzoquinones, Parasitol. Res. 107 (2010) 741–745, http://dx.doi.org/10.1007/s00436-010-1942-7.
[38] S.R.L. Santos, M.A. Melo, A.V. Cardoso, R.L.C. Santos, D.P. de Sousa, S.C.H. Cavalcanti, Structure–activity relationships of larvicidal monoterpenes and derivatives against *Aedes aegypti Linn*, Chemosphere 84 (2011) 150–153, http://dx.doi.org/10.1016/j.chemosphere.2011.02.0.
[39] C.L. Cantrell, J.W. Pridgeon, F.R. Fronczek, J.J. Becnel, Structure–activity relationship studies on derivatives of eudesmanolides from inula helenium as toxicants against *Aedes aegypti* larvae and adults, Chem. Biodivers. 7 (2010) 1681–1697, http://dx.doi.org/10.1002/cbdv.201000031.
[40] G. Eng, X. Song, Q. Duong, D. Strickman, J. Glass, L. May, Synthesis, structure characterization and insecticidal activity of some triorganotin dithiocarbamates, Appl. Organomet. Chem. 17 (2003) 218–225, http://dx.doi.org/10.1002/aoc.423.
[41] X. Song, Q. Duong, E. Mitrojorgji, A. Zapata, N. Nguyen, D. Strickman, J. Glass, G. Eng, Synthesis, structure characterization and larvicidal activity of some tris-(*para*-substitutedphenyl)tins, Appl. Organomet. Chem. 18 (2004) 363–368, http://dx.doi.org/10.1002/aoc.660.
[42] Q. Duong, X. Song, E. Mitrojorgji, S. Gordon, G. Eng, Larvicidal and structural studies of some triphenyl- and tricyclohexyltin *para*-substituted benzoates, J. Organomet. Chem. 691 (2006) 1775–1779, http://dx.doi.org/10.1016/j.jorganchem.2005.12.005.
[43] X. Song, A. Zapata, J. Hoener, A.C. Dios, L. Casabianca, G. Eng, Synthesis larvicidal QSAR and structural studies of some triorganotin 2,2,3,3-tetramethylcyclopropanecarboxylates, Appl. Organomet. Chem. 21 (2007) 545–550, http://dx.doi.org/10.1002/aoc.1241.
[44] C. Hansch, R.P. Verma, Larvicidal activities of some organotin compounds on mosquito larvae: a QSAR study, Eur. J. Med. Chem. 44 (2009) 260–273, http://dx.doi.org/10.1016/j.ejmech.2008.02.040.
[45] Z. Huang, Q. Cui, L. Xiong, Z. Wang, K. Wang, Q. Zhao, F. Bi, Q. Wang, Synthesis and insecticidal activities and SAR studies of novel benzoheterocyclic diacylhydrazine derivatives, J. Agric. Food Chem. 57 (2009) 2447–2456, http://dx.doi.org/10.1021/jf8036193.
[46] N.A. Begum, N. Roy, R.A. Laskar, K. Roy, Mosquito larvicidal studies of some chalcone analogues and their derived products: structure–activity relationship analysis, Med. Chem. Res. 20 (2011) 184–191, http://dx.doi.org/10.1007/s00044-010-9305-6.
[47] G. Pasquale, G.P. Romanelli, J.C. Autino, J. García, E.V. Ortiz, P.R. Duchowicz, Quantitative structure-activity relationships of mosquito larvicidal chalcone derivatives, J. Agric. Food Chem. 60 (2012) 692–697, http://dx.doi.org/10.1021/jf203374r.
[48] M. Debboun, J. Wagman, In vitro repellency of *N,N*-diethyl-3-methylbenzamide and *N,N*-diethylphenylacetamide analogs against *Aedes aegypti* and *Anopheles stephensi (Diptera: Culicidae)*, J. Med. Entomol. 41 (2004) 430–434, http://dx.doi.org/10.1603/0022-2585-41.3.430.
[49] A.R. Katritzky, D.A. Dobchev, I. Tulp, M. Karelson, D.A. Carlson, QSAR study of mosquito repellents using Codessa Pro, Bioorg. Med. Chem. Lett. 16 (2006) 2306–2311, http://dx.doi.org/10.1016/j.bmcl.2005.11.113.
[50] G. Paluch, J. Grodnitzky, L. Bartholomay, J. Coast, Quantitative structure–activity relationship of botanical sesquiterpenes: spatial and contact repellency to the yellow fever mosquito, *Aedes aegypti*, J. Agric. Food Chem. 57 (2009) 7618–7625, http://dx.doi.org/10.1021/jf900964e.
[51] S.C. Basak, R. Natarajan, W. Novak, P. Miszta, J.A. Klun, Three dimensional structure-activity relationships (3D-QSAR) for insect repellency of diastereoisomeric compounds: a hierarchical molecular overlay approach, Sar. QSAR Environ. Res. 18 (2007) 237–250, http://dx.doi.org/10.1080/10629360701303784.
[52] I.K. Park, S.G. Lee, S.G. Shin, J.D. Park, Y.J. Ahn, Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species, J. Agric. Food Chem. 50 (2002) 1866–1870, http://dx.doi.org/10.1021/jf011457a.
[53] Bluntritt, A., Master Dissertation, Universidade Federal de Pernambuco, 2006.
[54] R.A.W. Neves Filho, C.A. Silva, C.S.B. Silva, V.P. Brunstein, D.M.A.F. Navarro, F.A.B. Santos, L.C. Alves, M.G.S. Cavalcanti, R.M. Srivastava, M.G. Carneiro-da-Cunha, Improved microwave-mediated synthesis of 3-(3-Aryl-1,2,4-oxadiazol-5-yl)propionic acids and their larvicidal and fungal growth inhibitory properties, Chem. Pharm. Bull. 57 (2009), http://dx.doi.org/10.1248/cpb.57.819.
[55] B. Bordas, A.B. Demilo, A. Lopata, S.B. Haught, Insecticides-mechanisms of action and resistance tagungbericht, Tag.Ber. Akad. Landwirtsch. – Wiss. DDR, Berlim 274 (1989) 157–165.
[56] Tomokazu, H. I., Nobuharu, A. O., Hiroshi, H. K., Atsushi, K. K., United States Patent 5304573, 1994.
[57] J. Wu, B.A. Song, D.Y. Hu, M. Yue, S. Yang, Design, synthesis and insecticidal activities of novel pyrazole amides containing hydrazone substructures, Pest Manag. Sci. 68 (2012) 801–810, http://dx.doi.org/10.1002/ps.2329.
[58] Z. Afrasiabi, E. Sinn, J. Chen, Y. Ma, A.L. Rheingold, L.N. Zakharov, N. Rath, S. Padhye, Appended 1,2-naphthoquinones as anticancer agents 1: synthesis, structural, spectral and antitumor activities of ortho-naphthaquinone thiosemicarbazone and its transition metal complexes, Inorg. Chim. Acta 357 (2004) 271–278, http://dx.doi.org/10.1016/S0020-1693(03)00484-5.
[59] D. Kovala-Demertzi, M.A. Demertzis, E. Filiou, A.A. Pantazaki, P.N. Yadav, J.R. Miller, Y. Zheng, D.A. Kyriakdis, Platinum(II) and palladium(II) complexes with 2-Acetyl pyridine 4N-ethyl thiosemicarbazone able to overcome the *cis*-Platin resistance. Structure, antibacterial activity and DNA strand breakage, BioMetals 16 (2003) 411–418, http://dx.doi.org/10.1023/A:1022543718598.
[60] T.R. Bal, B. Anand, P. Yogeeswari, D. Sriram, Synthesis and evaluation of anti-HIV activity of isatin β-thiosemicarbazone derivatives, Bioorg. Med. Chem. Lett. 15 (2005) 4451–4455, http://dx.doi.org/10.1016/j.bmcl.2005.07.046.
[61] N. Bharti, K. Husain, M.T.G. Garza, D.E. Cruz-Vega, J. Castro-Garza, B.D. Mata-Cardenas, F. Naqvi, A. Azam, Synthesis and in vitro antiprotozoal activity of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazone derivatives, Bioorg. Med. Chem. Lett. 12 (2002) 3475–3478, http://dx.doi.org/10.1016/S0960-894X(02)00703-5.
[62] N. Karali, Synthesis and primary cytotoxicity evaluation of new 5-nitroindole-2,3-dione derivatives, Eur. J. Med. Chem. 37 (2002) 909–918, http://dx.doi.org/10.1016/S0223-5234(02)01416-2.
[63] P.C. Unangst, D.T. Connor, Synthesis and transformations of 2,6-bis(1,1-dimethylethyl)-4-[2-(thiazolyl)ethenyl]phenols, J. Heterocycl. Chem. 29 (1992) 1097–1100, http://dx.doi.org/10.1002/jhet.5570290511.
[64] N. Ergenç, G. Çapan, N.S. Gunay, S. Özkirimli, M. Gungor, S. Özbey, E. Kendi, Synthesis and hypnotic activity of new 4-thiazolidinone and 2-thioxo-4,5-imidazolidinedione derivatives, Arch. Pharm. 332 (1999) 343–347, http://dx.doi.org/10.1002/(SICI)1521-4184(199910)332:10<343::AID-ARDP343>3.0.CO;2–0.
[65] A. Verma, S.K. Saraf, 4-thiazolidinone – a biologically active scaffold, Eur. J. Med. Chem. 43 (2008) 897–905, http://dx.doi.org/10.1016/j.ejmech.2007.07.017.
[66] M. Behrouzi-Fardmoghadam, F. Poorrajab, S.K. Ardestani, S. Emami, A. Shafieea, A. Foroumadi, Synthesis and in vitro anti-leishmanial activity of 1-[5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl]- and 1-[5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl]-4-aroylpiperazines, Bioorg. Med. Chem. 16 (2008) 4509–4515, http://dx.doi.org/10.1016/j.bmc.2008.02.052.
[67] H.J.C. Bezerra-Netto, D.I. Lacerda, A.L.P. Miranda, H.M. Alves, E.J. Barreiro, C.A.M. Fraga, Design and synthesis of 3,4-methylenedioxy-6-nitrophenoxyacetylhydrazone derivatives obtained from natural safrole: new lead-agents with analgesic and antipyretic properties, Bioorg. Med. Chem. 14 (2006) 7924–7935, http://dx.doi.org/10.1016/j.bmc.2006.07.046.
[68] K.C. Krebs, Q. Lan, Isolation and expression of a sterol carrier protein-2 gene from the yellow fever mosquito, *Aedes aegypti*, Insect. Mol. Biol. 12 (2003) 51–60, http://dx.doi.org/10.1046/j.1365-2583.2003.00386.x.
[69] M. Kim, V. Wessely, Q. Lan, Identification of mosquito sterol carrier protein-2 inhibitors, J. Lipid Res. 46 (2005) 650–657, http://dx.doi.org/10.1194/jlr.M400389-JLR200.
[70] V.C. Kramer, D.J. Schnell, K.W. Nickerson, Relative toxicity of organic solvents to *Aedes aegypti* larvae, J. Invertebr. Pathol. 42 (1983) 285–287, http://dx.doi.org/10.1016/0022-2011(83)90076-9.

[71] S.S. Cheng, H.T. Chang, S.T. Chang, K.H. Tsai, W.J. Chen, Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae, Bioresour. Technol. 89 (2003) 99–102, http://dx.doi.org/10.1016/S0960-8524(03)00008-7.
[72] H. Beraldo, Semicarbazones and thiosemicarbazones: their wide pharmacological profile and clinical applications, Quím. Nova 27 (2004) 461–471, http://dx.doi.org/10.1590/S0100-40422004000300017.
[73] H. Beraldo, D. Gambino, The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes, Mini-Rev. Med. Chem. 4 (2004) 31–39, http://dx.doi.org/10.2174/1389557043487484.
[74] B. Zacharie, M. Lagraoui, M. Dimarco, C.L. Penney, L. Gagnon, Thioamides: synthesis, stability, and immunological activities of thioanalogues of imreg. preparation of new thioacylating agents using fluorobenzimidazolone derivatives, J. Med. Chem. 42 (1999) 2046–2052, http://dx.doi.org/10.1021/jm9900467.
[75] M.Z. Hernandes, S.M.T. Cavalcanti, D.R.M. Moreira, W.F. Azevedo Junior, A.C.L. Leite, Halogen atoms in the modern medicinal chemistry: hints for the drug design, Curr. Drug Targets 11 (2010) 303–314, http://dx.doi.org/10.2174/138945010790711996.
[76] N.K. Simas, E.C. Lima, S.R. Conceição, R.M. Kuster, A.M. Oliveira Filho, Natural products for dengue transmission control– larvicidal activity of *myroxylon balsamum* (red oil) and of terpenoids and phenylpropanoids, Quím. Nova 27 (2004) 46–49, http://dx.doi.org/10.1590/S0100-40422004000100009.
[77] F.R. Paula, S.H.P. Serrano, L.C. Tavares, Aspects of bioactivity and toxicity of nitrocompounds, Quím. Nova 32 (2009) 1013–1020, http://dx.doi.org/10.1590/S0100-40422009000400032.
[78] B.H. Min, W.A. Garland, Determination of clonazepam and its 7-amino metabolite in plasma and blood by gas chromatography-chemical ionization mass spectrometry, J. Chromatogr. A 139 (1977) 121–133, http://dx.doi.org/10.1016/S0021-9673(01)84132-7.
[79] J.B.P. da Silva, F. Hallwass, A.G. da Silva, D.R. Moreira, M.N. Ramos, J.W.P. Espíndola, A.D.T. de Oliveira, D.J. Brondani, A.C.L. Leite, K.M. Merz Jr., J. Mol. Struct. 1093 (2015) 219–227, http://dx.doi.org/10.1016/j.molstruc.2015.03.011.
[80] T. Kitamura, S. Kobayashi, M. Okada, Regional expression of the transcript encoding sterol carrier protein x related thiolaseand its regulation by homeotic genes in the midgut of *Drosophila* embryos, Dev. Growth Differ. 38 (1996) 373–381, http://dx.doi.org/10.1046/j.1440-169X.1996.t01-3-00005.x.
[81] D.H. Dyer, S. Lovell, J.B. Thoden, H.M. Holden, I. Rayment, Q. Lan, The structural determination of an insect sterol carrier protein-2 with a ligand-bound C16 fatty acid at 1.35-Å resolution, J. Biol. Chem. 278 (2003) 39085–39091, http://dx.doi.org/10.1074/jbc.M306214200.
[82] R.T. Larson, V. Wessely, Z. Jiang, Q. Lan, Larvicidal activity of sterol carrier protein-2 inhibitor in four species of mosquitoes, J. Med. Entomol. 45 (2008) 439–444, http://dx.doi.org/10.1093/jmedent/45.3.439.
[83] M.–S. Kim, Q. Lan, Sterol carrier protein-x gene and effects of sterol carrier protein-2 inhibitors on lipid uptake in manduca sexta, BMC-Physiol. 10 (2010) 9, http://dx.doi.org/10.1186/1472-6793-10-9.
[84] D.R.M. Moreira, S.P.M. Costa, M.Z. Hernandes, M.M. Rabello, G.B. de Oliveira Filho, C.M.L. de Melo, L.F. da Rocha, C.A. de Simone, R.S. Ferreira, J.R.B. Fradico, C.S. Meira, E.T. Guimarães, R.M. Srivastava, V.R.A. Pereira, M.B.P. Soares, A.C.L. Leite, Structural investigation of anti-trypanosoma cruzi 2-iminothiazolidin-4-ones allows the identification of agents with efficacy in infected mice, J. Med. Chem. 55 (2012) 10918–10936, http://dx.doi.org/10.1021/jm301518v.
[85] M.V.O. Cardoso, L.R.P. de Siqueira, E.B. da Silva, L.B. Costa, M.Z. Hernandes, M.M. Rabello, R.S. Ferreira, L.F. da Cruz, D.R.M. Moreira, V.R.A. Pereira, M.C.A.B. de Castro, P.V. Bernhardt, A.C.L. Leite, 2-Pyridyl thiazoles as novel anti-Trypanosoma cruzi agents: structural design, synthesis and pharmacological evaluation, Eur. J. Med. Chem. 86 (2014) 48–59, http://dx.doi.org/10.1016/j.ejmech.2014.08.012.
[86] C.D. Barros, A.A. Amato, T.B. de Oliveira, K.B.R. Iannini, A.L. da Silva, T.G. da Silva, E.S. Leite, M.Z. Hernandes, M.C.A. de Lima, S.L. Galdino, F.A.R. Neves, I.R. Pitta, Synthesis and anti-inflammatory activity of new arylidene-thiazolidine-2,4-diones as PPARγ ligands, Bioorg. Med. Chem. 18 (2010) 3805–3811, http://dx.doi.org/10.1016/j.bmc.2010.04.045.
[87] B.S. Holla, K.V. Malini, B.S. Rao, B.K. Sarojini, N.S. Kumari, Synthesis of some new 2,4-disubstituted thiazoles as possible antibacterial and anti-inflammatory agents, Eur. J. Med. Chem. 38 (2003) 313–318, http://dx.doi.org/10.1016/S0223-5234(02)01447-2.
[88] L. Somogyi, Reactions of flavonoid thiosemicarbazones under acetylating conditions, Tetrahedron 47 (1991) 9305–9316, http://dx.doi.org/10.1016/S0040-4020(01)96219-2.
[89] R.E. Bruns, I.S. Scarminio, B. de Barros Neto, Statistical Design, Chemometrics, Elsevier, Amsterdan, 2006.
[90] L. Eriksson, E. Johansson, Multivariate design and modeling in QSAR, Chemom. Intell. Lab. 34 (1996) 1–19, http://dx.doi.org/10.1016/0196-7439(96)00023-8.
[91] K.R. Beebe, R.J. Pell, M.B. Seasholtz, Chemometrics: a Practical Guide, Wiley&Sons Interscience, New York, 1998.
[92] STATISTICA (Data Analysis Software System), Version 6.1, StatSoft, Inc, 2004. www.statsoft.com.
[93] G.B. Rocha, R.O. Freire, A.M. Simas, J.J.P. Stewart, RM1: a reparameterization of AM1 for H, C, N, O, P, S, F, Cl, Br, and I, J. Comput. Chem. 27 (2006) 1101–1111, http://dx.doi.org/10.1002/jcc.20425.
[94] Spartan '08 Tutorial and User's Guide, Wavefunction, Irvine, CA, 2008. http://www.wavefun.com/products/spartan.html.
[95] O. Korb, T. Stutzle, T.E. Exner, Empirical scoring functions for advanced protein–ligand docking with PLANTS, J. Chem. Inf. Model 49 (2009) 84–96, http://dx.doi.org/10.1021/ci800298z.
[96] Gold Software, Version 5.1, Cambridge Crystallographic Data Centre. http://www.ccdc.cam.ac.uk.
[97] J.D. Durrant, J.A. Mccammon, BINANA: a novel algorithm for ligand-binding characterization, J. Mol. Graph. Model 29 (2011) 888–893, http://dx.doi.org/10.1016/j.jmgm.2011.01.004.
[98] W.L. DeLano, The PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA, 2002. http://www.pymol.org.
[99] World Health Organization, Guidelines for Laboratory and Field Testing of Mosquito Larvicides, World Health Organization, Geneva, 2005.