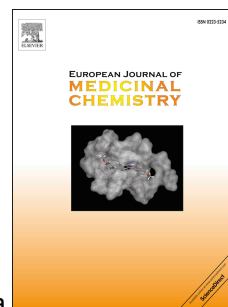


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Thiosemicarbazones as *Aedes aegypti* Larvicidal

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ABSTRACT

A set of aryl- and phoxymethyl-(thio)semicarbazones were synthesized, 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 mosquito 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 world population) are at risk in more than 100 countries [13]. Although many 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 private 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 temephos is producing *A. aegypti* resistant populations [23,24]. 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. aegypti*. 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 (π_{χ}) 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. *Culex 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), piperide (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 synthesize 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 phenoxy 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 analogues 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 synthesized 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

Figure1 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.

Please, insert Figure 1 here.

Compounds (**1-10**) were prepared by reacting commercially 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 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 -OCH₂CH= 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.

Please, insert Table 1 here.

According Chang and co-workers (2003) essential oils or vegetal extracts showing LC_{50} between 50 to 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** ($LC_{50} = 310.8 \mu\text{mol L}^{-1}$) and **13** ($LC_{50} = 287.2 \mu\text{mol L}^{-1}$) should be considered active and compounds **7-10** ($LC_{50} = 134.8, 138.3, 122.8$ and $69.7 \mu\text{mol L}^{-1}$) and **14-17** ($LC_{50} = 92.0, 89.9, 70.1$ and $20.9 \mu\text{mol L}^{-1}$) should be considered highly active.

2.3. Structure-Active Relationship (SAR)

The semicarbazone compounds tested (**1** and **2**) showed low activity ($LC_{50} > 861.8 \mu\text{mol L}^{-1}$). 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 $LC_{50} = 310.8 \mu\text{mol L}^{-1}$ and $LC_{50} = 69.7 \mu\text{mol L}^{-1}$, 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 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 $20.9 \mu\text{mol L}^{-1}$, 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 $69.7 \mu\text{mol L}^{-1}$, 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 $89.9 \mu\text{mol L}^{-1}$, 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**, $LC_{50} = 310.8 \mu\text{mol L}^{-1}$) and 3-Cl (**13**, $LC_{50} = 287.2 \mu\text{mol L}^{-1}$) performed slightly worse. According Hernandez 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 $20.9 \mu\text{mol L}^{-1}$, respectively) in comparison to their corresponding mono-chlorine compounds (**7** and **15** with $LC_{50} =$

134.8 and 89.9 $\mu\text{mol L}^{-1}$, 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**, $\text{LC}_{50} > 1024.4 \mu\text{mol L}^{-1}$) and *para*-OCH₃ (**11**, $\text{LC}_{50} = 503.1 \mu\text{mol L}^{-1}$) 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 cinnamic aldehyde improves larvicide activity [76]. Because of this, a closely related system **8** was synthesized. In fact, this activity was among the most successful ($\text{LC}_{50} = 138.3 \mu\text{mol L}^{-1}$). 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 (-NO₂), however, seems not have contributed to the mortality of *A. aegypti*'s larvae since compound **3** showed a $\text{LC}_{50} > 659.8 \mu\text{mol L}^{-1}$, whereas compound **9** (that does not contain the nitro group) showed a high activity with $\text{LC}_{50} = 122.8 \mu\text{mol L}^{-1}$. One possibility that cannot be neglected is that the nitro group could be bio-reduced to one amino group [78] which, as an electron-donor 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-phenoxyethyl-thiosemicarbazones (top larvicide compounds) are non-toxic.

2.5. QSAR

B3LYP/6-311++G(d,p) calculations to access 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).

Please, insert Table 2 here.

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

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

$$n=10; R = 0.97; R^2 = 0.94; F = 54.61; s = 0.12; p = 0.00005 \quad \text{Equation-1.}$$

Using this equation, Fig. 2.a shows a comparison between predicted and observed larvicide activities. The quality of the adjustment (Equation-1) can be visualized in the residue plot as shown in

Fig. 2.b. 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.

Please, insert Figure 2 here.

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, 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 $\log P$ will improve the larvicide activity. These equation features match the importance, previously observed in the literature, of both hydrophobic and electronic parameters for describing larvicide activity against *A. aegypti*.

In order to check the QSAR model (Equation 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, $\log P$, and the predicted and observed LC_{50} values of these compounds are shown in Table 3.

Please, insert Table 3 here.

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 $16.33 \mu\text{mol L}^{-1}$, leading to a ratio (Cytotoxicity/LC₅₀) equal to 4.1. It is interesting to stress that we stopped determining the experimental LC₅₀ for compound **4** after the concentration of $1024 \mu\text{mol L}^{-1}$. That decision is adequately supported by Equation-1 since the LC₅₀ for the compound **4** is predicted at $6434 \mu\text{mol L}^{-1}$.

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 ($\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$) co-crystallized into AeSCP-2 [81]. These authors found that the polar head of the palmitic acid makes an H-bond interaction with the side chain of the Arg24 residue, whereas the methylenic 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 ($-(\text{CH}_2)_3-\text{NH}(\text{C}=\text{NH})\text{NH}_2$) 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 phenoxyethyl group relative to the thiosemicarbazone moiety resembles the bent conformation of the fatty portion relative to the carboxylic head of the palmitic acid co-crystallized into AeSCP-2.

Because of this, we tested two synthesized thiosemicarbazone derivatives, **4** and **11**, as AeSCP-2 inhibitors (SCPIs) and compared them to a previous tested compound, SCPI-1 [69] (see Fig. 3).

Please, insert Figure 3 here.

Figure 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) μ M 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** ($\log P_{\text{calc}} = 2.09$, Table 2) compared to **4** ($\log P_{\text{calc}} = 0.56$, 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 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.

Please, insert Figure 4 here.

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**. Figure 5a provides a clear illustration of the binding pattern.

Please, insert Figure 5 here.

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. 5.b, particularly for molecules **4**, **11** and **17**. The Docking protocol considered the active flexibility of residue PHE105 and other nine residues during the calculations. Figure 5.b 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 $-\log \text{LC50}$, 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].

Please, insert Figure 6 here.

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).

3. Conclusion

Seventeen compounds belonging to the two classes of aryl-(thio)semicarbazone and phenoxyethyl-thiosemicarbazones derivatives were synthesized 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 phenoxyethyl

group showed a higher larvicide activity. As desirable, the subset formed by eleven aryl- and phenoxyethyl-thiosemicarbazone derivatives showed a large variance of larvicide activity, with the LC_{50} ranging from 21 $\mu\text{mol L}^{-1}$ to 311 $\mu\text{mol L}^{-1}$. A QSAR equation from the LC_{50} against the electric dipole moment (μ) and the logarithm of the partition coefficient ($\log P$) 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 $\mu\text{mol L}^{-1}$, 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-thiosemicarbazone, the corresponding start aldehyde was obtained by reaction between 4-bromobenzaldehyde and nitric acid. Compound (**5**), bis-thiosemicarbazone derivative, was prepared using 2 mols of thiosemicarbazide for the 1,3-dicarbonyl aldehyde. These reactions proceeded well upon refluxing (3-5 h.) with ethanol as a solvent, a rate of 65 to 97% being observed overall.

For the synthesis of phenoxyethyl-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).

Please, insert Scheme 1 here.

Reagents were purchased from Acros Organics, Fluka, Sigma-Aldrich or Vetec and solvents from Vetec or Dinâmica. The deuterated solvents (DMSO-*d*₆, CDCl₃, D₂O) 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 ¹H and ¹³C NMR were performed for all compounds, DEPT analysis as well as the addition of D₂O for locating NH signals in the ¹H NMR were determined. The ¹H and ¹³C NMR spectra were obtained using Unity Plus model Varian instruments (400 MHz for ¹H, ¹³C for 100 MHz) or Bruker AMX (300 MHz to 75.5 MHz for ¹H and ¹³C), using tetramethylsilane as the internal standard. The number of signals in the ¹H 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 (15mL), 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 SiO₂. 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 (NH₂), 3064 (NH), 1708 (C=O), 1591 (C=N) cm⁻¹. ¹H NMR (300 MHz, DMSO-*d*₆): δ 6.51 (s, 2H, NH₂), 7.20 (m, 2H, Ar-H), 7.77 (m, 2H, Ar-H), 7.82 (s, 1H, CH=N), 10.24 (s, 1H, NH). ¹³C 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 C₈H₈FN₃O: 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 (NH₂), 3155 (NH), 1700 (C=O), 1588 (C=N) cm⁻¹. ¹H NMR (300 MHz, DMSO-*d*₆): δ 6.66 (s, 2H, NH₂), 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). ¹³C 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 C₈H₇Cl₂N₃O: 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 (NH₂), 3156 (NH), 1519 (C=N) cm⁻¹. ¹H NMR (300 MHz, DMSO-*d*₆): δ 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, NH₂), 8.34 (s, 1H, NH₂), 8.56 (s, 1H, Ar-H), 11.65 (s, 1H, NH). ¹³C 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 C₈H₇BrN₄O₂S: 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 (NH₂), 3129 (NH), 1509 (C=N) cm⁻¹. ¹H 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, NH₂), 7.94 (s, 1H, CH=N), 8.07 (s, 1H, NH₂), 9.90 (s, 1H, OH), 11.25 (s, 1H, NH). ¹³C 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 C₈H₉N₃OS: 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 (NH₂), 3148 (NH), 1524 (C=N) cm⁻¹. ¹H NMR (300 MHz, DMSO-*d*₆): δ 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, NH₂), 8.22 (s, 1H, Ar-H), 8.26 (s, 2H, NH₂), 11.55 (s, 2H, NH). ¹³C 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 C₁₀H₁₂N₆S₂: 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 (NH₂), 3156 (NH), 1533 (C=N) cm⁻¹. ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.23 (m, 2H, Ar-H), 7.87 (m, 2H, Ar-H), 8.02 (s, 1H, CH=N), 8.03 (s, 1H, NH₂), 8.20 (s d, 1H, NH₂), 11.43 (s, 1H, NH). ¹³C 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 C₈H₈FN₃S: 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 (NH₂), 3164 (NH), 1523 (C=N) cm⁻¹. ¹H NMR (300 MHz, DMSO-*d*₆): δ 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, NH₂), 8.25 (s, 1H, NH₂), 11.49 (s, 1H, NH). ¹³C 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 C₈H₈ClN₃S: 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 (NH₂), 3155 (NH), 1537 (C=N) cm⁻¹. ¹H NMR (300 MHz, DMSO-*d*₆): δ 6.86 (dd, 1H, *J* = 8.9 Hz; *J* = 15.8 Hz, CH=CH), 7.02 (d, 1H, *J* = 15.89 Hz, CH=CH), 7.43-7.24 (m, 3H, Ar-H), 7.55 (d, 2H, *J* = 7.79 Hz, Ar-H), 7.61 (s, 1H, NH₂), 7.89 (d, 1H, *J* = 8.9 Hz, CH=N), 8.17 (s, 1H, NH₂), 11.40 (s, 1H, NH). ¹³C 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 C₁₀H₁₁N₃S: 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 (NH₂), 3165 (NH), 1522 (C=N) cm⁻¹. ¹H 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, NH₂), 8.24 (s broad, 1H, NH₂), 11.49 (s, 1H, NH). ¹³C 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 C₈H₈BrN₃S: 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 (NH₂), 3154 (NH), 1539 (C=N) cm⁻¹. ¹H 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, NH₂), 8.30 (s, 1H, NH₂), 11.57 (s, 1H, NH). ¹³C 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 C₈H₇Cl₂N₃S: 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 K₂CO₃ (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 SiO₂. The hydrolysis of acetal in aldehyde was achieved by adding acetone (5mL), H₂SO₄ (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 SiO₂. 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 (NH₂), 3174 (NH), 1509 (C=N) cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆): δ 3.68 (s, 3H, OCH₃), 4.59 (s, 2H, CH₂), 6.88 (m, 4H, Ar-H), 7.51 (s, 1H, CH=N), 7.68 (s, 1H, NH₂), 8.17 (s, 1H, NH₂), 11.33 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 55.3 (CH₃), 67.3 (CH₂), 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 C₁₀H₁₃N₃O₂S: 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 (NH₂), 3158 (NH), 1536 (C=N) cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆): δ 4.67 (d, 2H, *J* = 5.2 Hz, CH₂), 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, NH₂), 8.18 (s broad, 1H, NH₂), 11.35 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 66.7 (CH₂), 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 C₉H₁₁N₃OS: 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 (NH₂), 3156 (NH), 1513 (C=N) cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆): δ 4.69 (s, 2H, CH₂), 7.08-6.85 (m, 3H, Ar-H), 7.31 (s, 1H, Ar-H), 7.50 (s, 1H, CH=N), 7.69 (s, 1H, NH₂), 8.20 (s, 1H, NH₂), 11.37 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 67.1 (CH₂), 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 C₉H₁₀ClN₃OS: 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 (NH₂), 3154 (NH), 1536 (C=N) cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆): δ 4.66 (s, 2H, CH₂), 6.97 (s, 2H, Ar-H), 7.46 (s, 2H, Ar-H), 7.50 (s, 1H, CH=N), 7.69 (s, 1H, NH₂), 8.21 (s, 1H, NH₂), 11.37 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 67.0 (CH₂), 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 C₉H₁₀BrN₃OS: 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 (NH₂), 3152 (NH), 1532 (C=N) cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆): δ 4.59 (s broad, 2H, CH₂), 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, NH₂), 8.19 (s, 1H, NH₂), 11.36 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 67.1 (CH₂), 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 C₉H₁₀ClN₃OS: 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 (NH₂), 3156 (NH), 1545 (C=N) cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆): δ 4.80 (s, 2H, CH₂), 7.45-7.00 (m, 3H, Ar-H), 7.53 (s, 1H, CH=N), 7.71 (s, 1H, NH₂), 8.23 (s, 1H, NH₂), 11.41 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 68.3 (CH₂), 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 C₉H₉Cl₂N₃OS: 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 (NH₂), 3155 (NH), 1534 (C=N) cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆): δ 4.70 (d, 1H, *J* = 4.7 Hz, CH₂), 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, NH₂), 8.22 (s, 1H, NH₂), 11.38 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 67.4 (CH₂), 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 C₉H₉Cl₂N₃OS: 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 12h. 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 SiO₂ glass dissector under vacuum. The 3-(3,4-dichloro)phenoxy-butan-2-one (6.16 mmol, 1.36g) 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 hours. A yellowish solid was obtained, filtered in Büchner funnel with a sintered disc filter, washed with cold water, and then dried in SiO₂. 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 (NH₂), 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, CH₃), 1.82 (s, 3H, CH₃), 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, NH₂), 8.24 (s largo, 1H, NH₂), 10.19 (s, 1H, NH). ¹³C NMR (75.5 MHz, DMSO-*d*₆): δ 11.08 (CH₃–C=N), 18.48 (CH₃–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–NH₂). *Anal.* Calcd. for C₁₁H₁₃N₃OSCl₂: 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 hours. 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 aldehydes.* In a round bottom flask, acetal intermediate was mixed with acetone (5mL), after an acid solution (H_2SO_4 7 drops and water 10mL) had been slowly added. The mixture was kept under reflux heating ($100^\circ C$) for 4 hours. 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 hours. 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, LogP².

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 solubilising 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 $28 \pm 2^\circ\text{C}$, 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 6×10^5 cells/well in an RPMI-1640 medium supplemented with 10% of FBS and $50 \mu\text{g mL}^{-1}$ of gentamycin. Each test

inhibitor was evaluated in six concentrations (1, 5, 10, 25, 50 and 100 $\mu\text{g mL}^{-1}$) 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% CO₂. 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).

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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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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. doi: 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, *Malaria J.* (2010) 9:44. <http://www.malariajournal.com/content/9/1/44>. doi: 10.1186/1475-2875-9-44.
- [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. doi: 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. doi: 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. *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. doi: 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. doi: 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. doi: 10.3201/eid1105.041063.
- [9] J. L. Kyle, E. Harris, Global spread and persistence of dengue, *Annu. Rev. Microbiol.* 62 (2008) 71–92. doi: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. doi: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. doi: 10.3201/eid1408.080068.
- [12] R. Allwinn, Significant increase in travel-associated dengue fever in Germany, *Med. Microbiol. Immunol.* 200 (2011) 155–159. doi: 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, *The Lancet* 360 (2002) 1243–1245. doi: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. doi: 10.1126/science.342.6157.415.
- [16] T. M. Ross, Dengue Virus. *Clin. Lab. Med.* 30 (2010) 149–160. doi: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. doi: 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 in Microbiol.* 10 (2002) 100–103. doi: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. doi: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. doi: 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. doi: 10.1111/j.1365-3156.2007.01889.x.
- [22] C. F. S. Andrade, M. Modolo, Susceptibility of *Aedes aegypti* larvae to temephos and *Bacillus thuringiensis var israelensis* in integrated control, *Rev. Saúde Publ.* 25 (1991) 184–187. doi: 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. doi: 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. doi: 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. doi: 10.1016/j.actatropica.2009.10.015.
- [26] M. da Silva, A. Furigo Junior, S. A. Furlan, O. Souza, Production of bio-insecticide *Bacillus thuringiensis var. israelensis* in semicontinuous processes combined with batch processes for sporulation, *Braz. Arch. Biol. Techn.* 54 (2011) 45–52. doi: 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. doi: 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. doi: 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. doi: 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, Bioresource Technol. 100 (2009) 452-456. doi: 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. doi: 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. doi: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. doi: 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. doi: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. doi: 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. doi: 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. doi: 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. doi: 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. doi: 10.1002/aoc.423.
- [41] X. Song, Q. Duong, E. Mitrojjorgji, 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. doi: 10.1002/aoc.660.
- [42] Q. Duong, X. Song, E. Mitrojjorgji, S. Gordon, G. Eng, Larvicidal and structural studies of some triphenyl- and tricyclohexyltin *para*-substituted benzoates, *J. Organomet. Chem.* 691 (2006) 1775-1779. doi: 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. doi: 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. doi: 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. doi: 10.1021/jf8036193.
- [46] N. A. Begum, N. Roy, R. A. Laskar, K. Roy, K, Mosquito larvicidal studies of some chalcone analogues and their derived products: structure–activity relationship analysis, *Med. Chem. Res.* 20 (2011) 184-191. doi: 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. doi: 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. doi: 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. doi: 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. doi: 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. doi: 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. doi: 10.1021/jf011457a.

[53] A. Bluntritt, 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). doi: 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. Akademie Landwirtschaft. – Wissenschaft. DDR, Berlin, 1989, 274, pp. 157-165.

[56] H. I Tomokazu, A. O Nobuharu, H. K. Hiroshi, K. K. Atsushi, 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. doi: 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. doi: 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. doi: 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. doi: 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. doi: 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. doi: 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. Heterocyclic Chem.* 29 (1992) 1097-1100. doi: 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. doi: 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. doi: 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-arylpiperazines, *Bioorgan. Med. Chem.* 16 (2008) 4509-4515. doi: 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-Nitrophenoxycetylhydrazone Derivatives

Obtained from Natural Safrole: New Lead-Agents with Analgesic and Antipyretic Properties, Bioorgan. Med. Chem. 14 (2006) 7924–7935. doi: 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. doi: 10.1046/j.1365-2583.2003.00386.x.

[69] M. Kim, V. Wessely, Q. Lan, Q. Identification of mosquito sterol carrier protein-2 inhibitors, J. Lipid Res. 46 (2005) 650-657. doi: 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. doi: 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, Bioresource Technol. 89 (2003) 99-102. doi: 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. doi: 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. doi: 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. doi: 10.1021/jm9900467.

[75] M. Z. Hernandez, 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. doi: 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. doi: 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. doi: 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. doi: 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. doi: 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 thiolase and its regulation by homeotic genes in the midgut of *Drosophila* embryos, *Dev. Growth Differ.* 38 (1996) 373–381. doi: 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. doi: 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. doi: 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.* 10:9 (2010). doi: 10.1186/1472-6793-10-9.
- [84] D. R. M. Moreira, S. P. M. Costa, M. Z. Hernandez, 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. doi: 10.1021/jm301518v.

[85] M. V. O. Cardoso, L. R. P. de Siqueira, E. B. da Silva, L. B. Costa, M. Z. Hernandez, 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. doi: 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. Hernandez, 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 PPAR γ ligands, *Bioorg. Med. Chem.* 18 (2010) 3805–3811. doi: 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. doi: 10.1016/S0223-5234(02)01447-2.

[88] L. Somogyi, Reactions of flavonoid thiosemicarbazones under acetylating conditions, *Tetrahedron* 47 (1991) 9305-9316. doi: 10.1016/S0040-4020(01)96219-2.

[89] R. E. Bruns, I. S. Scarminio, B. de Barros Neto, Statistical Design, Chemometrics, Elsevier: Amsterdam, 2006.

[90] L. Eriksson, E. Johansson, Multivariate design and modeling in QSAR, *Chemometr. Intell. Lab.* 34 (1996) 1-19. doi: 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. doi: 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. doi: 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. doi: 10.1016/j.jmgm.2011.01.004.
- [98] DeLano, W. L. 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.

Figure and Scheme Caption

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

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

Figure 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.

Figure 4. Palmitic acid (green) and thiosemicarbazone superimposed structures docked on the AeSCP-2 target. (PDB: 1PZ4 - <http://dx.doi.org/10.2210/pdb1pz4/pdb>).

Figure 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.

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

Scheme 1. Synthesis of compounds (**1-18**). Reagents and conditions: (a) HCl or H₂SO₄, ethanol, room temperature, 2-3 h.

Graphical Abstract

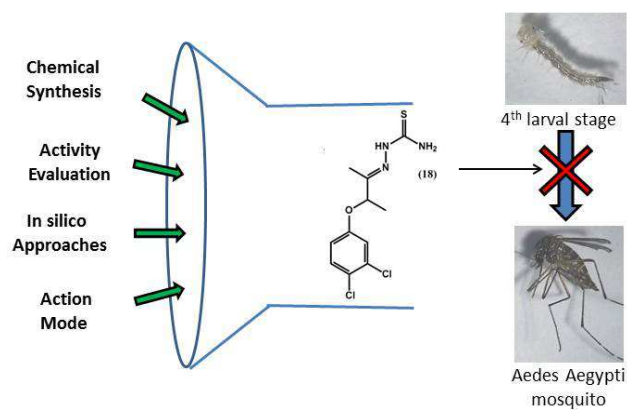


Table 1 – Larvicide activity (LC₅₀) and cytotoxicity for the synthetic (thio)semicarbazones derivatives (1-17).

Compound (R;SU;X) ^a	Numbering	Conc. range ^b (ppm)	LC ₅₀ (ppm)	LC ₅₀ (μmolL ⁻¹)	Log(1/LC ₅₀)	Cytotoxicity ^c (μmolL ⁻¹)
<i>Aryl-(thio)semicarbazones</i>						
(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
<i>Phenoxymethyl-thiosemicarbazones</i>						
(4-OCH ₃ ;OCH ₂ CH;S)	11	80-160	120.4	503.1	3.298	20.9
(H;OCH ₂ CH;S)	12	80-160	112.7	538.5	3.268	119
(3-Cl;OCH ₂ CH;S)	13	50-80	70.0	287.2	3.542	103
(4-Br;OCH ₂ CH;S)	14	20-40	26.5	92.0	4.036	> 347
(4-Cl;OCH ₂ CH;S)	15	20-30	21.9	89.9	4.046	103
(2,3-diCl;OCH ₂ CH;S)	16	10-30	19.5	70.1	4.154	17.9
(3,4-diCl;OCH ₂ CH;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.

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

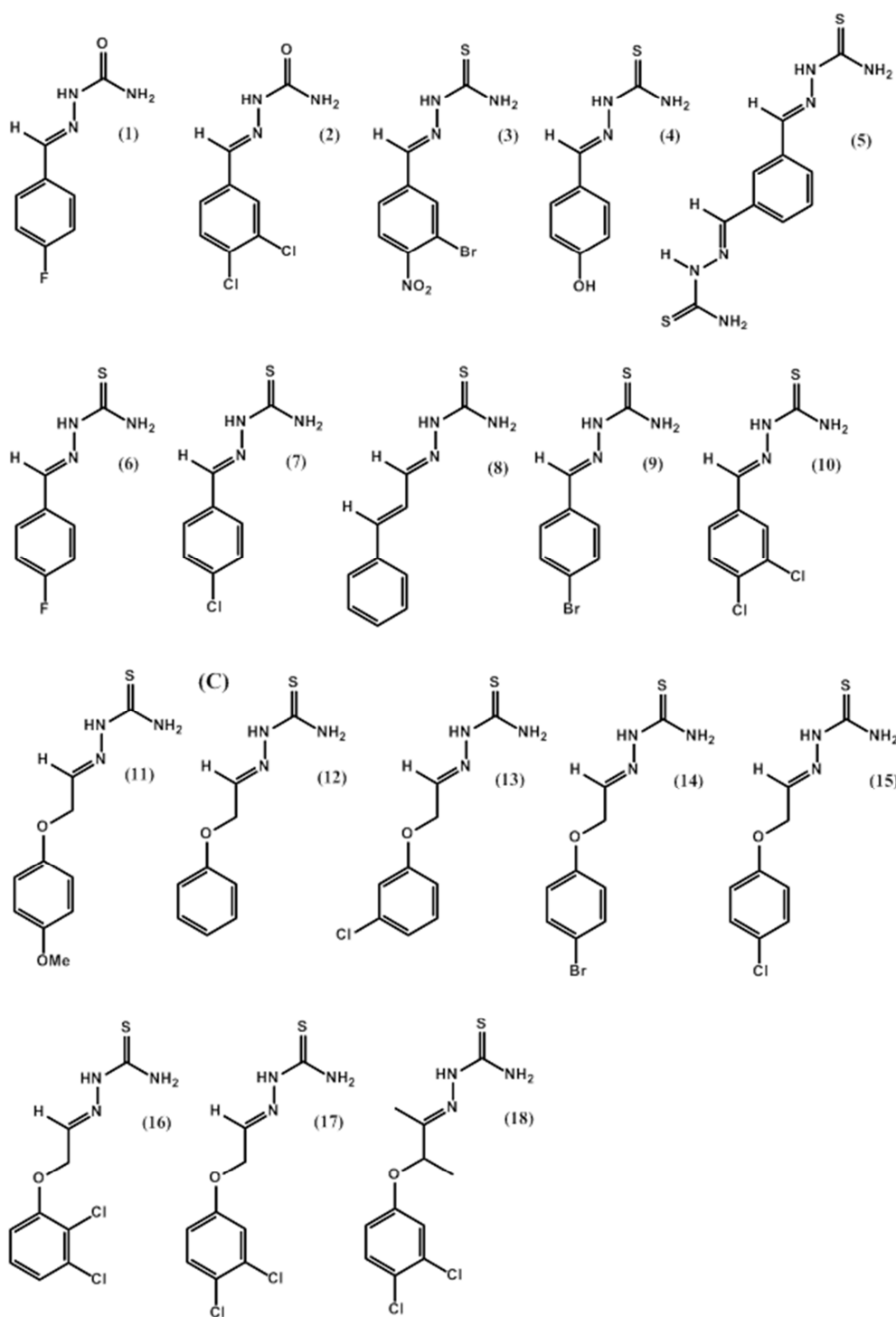
Numbering	$\log(1/LC_{50})$	μ^a (D)	ϵ_{HOMO}^a (eV)	ϵ_{LUMO}^a (eV)	$\Delta\epsilon^a$ (eV)	$\Sigma q_{benzene}^a$ (e)	qC_1^a (e)	qN_1^a (e)	qN_2^a (e)	$\log P^b$	$(\log P)^2^b$
6	3.507	3.702	-6.045	-2.224	3.821	-0.370	0.778	0.192	-0.033	2.180	4.752
7	3.873	3.503	-6.078	-2.336	3.742	-1.050	0.522	0.179	-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

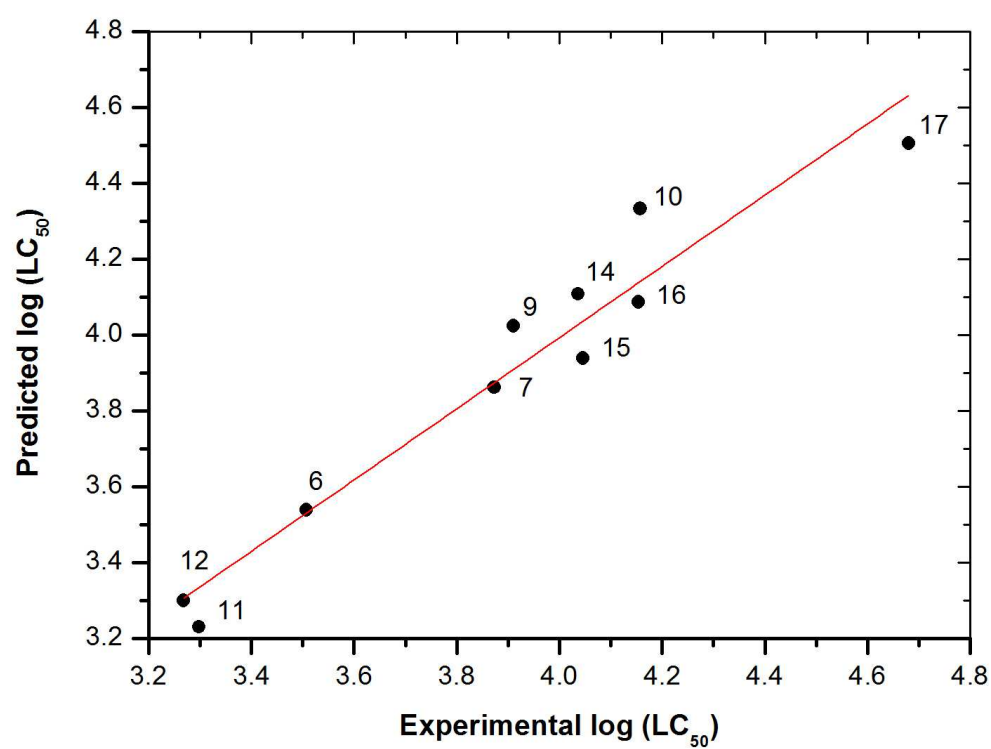
^a Ref. 79, ^b this work.

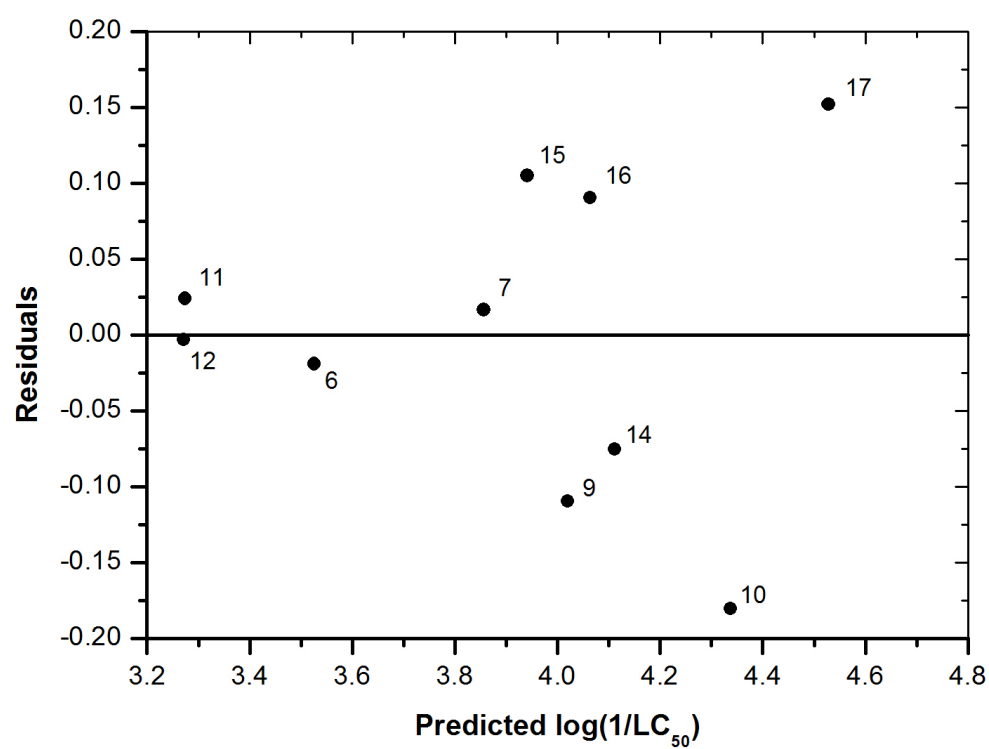
Table 3 – Calculated B3LYP/6-311++G(d,p) electric dipole moment and Log P values and predicted (using equation-1) and experimental Log(1/LC₅₀) for the thiosemicarbazone derivatives **4**, **13** and **18**.

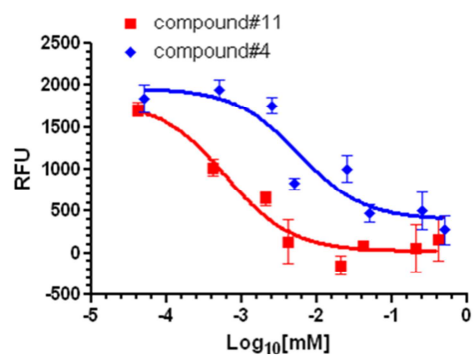
Compound (R;SU;X)	Numbering	μ^a (D)	logP	LC ₅₀ (pred.) (μ mol L ⁻¹)	LC ₅₀ (exp.) (μ mol L ⁻¹)
(3,4-diCl;OCH(CH ₃)CCH ₃ ;S)	18	3.31	4.87	4.10	3.95
(3-Cl;OCH ₂ CH;S)	13	4.66	2.650	179.38	287.2
(4-OH;CH;S)	4	6.02	0.56	6434.0	>1024.4

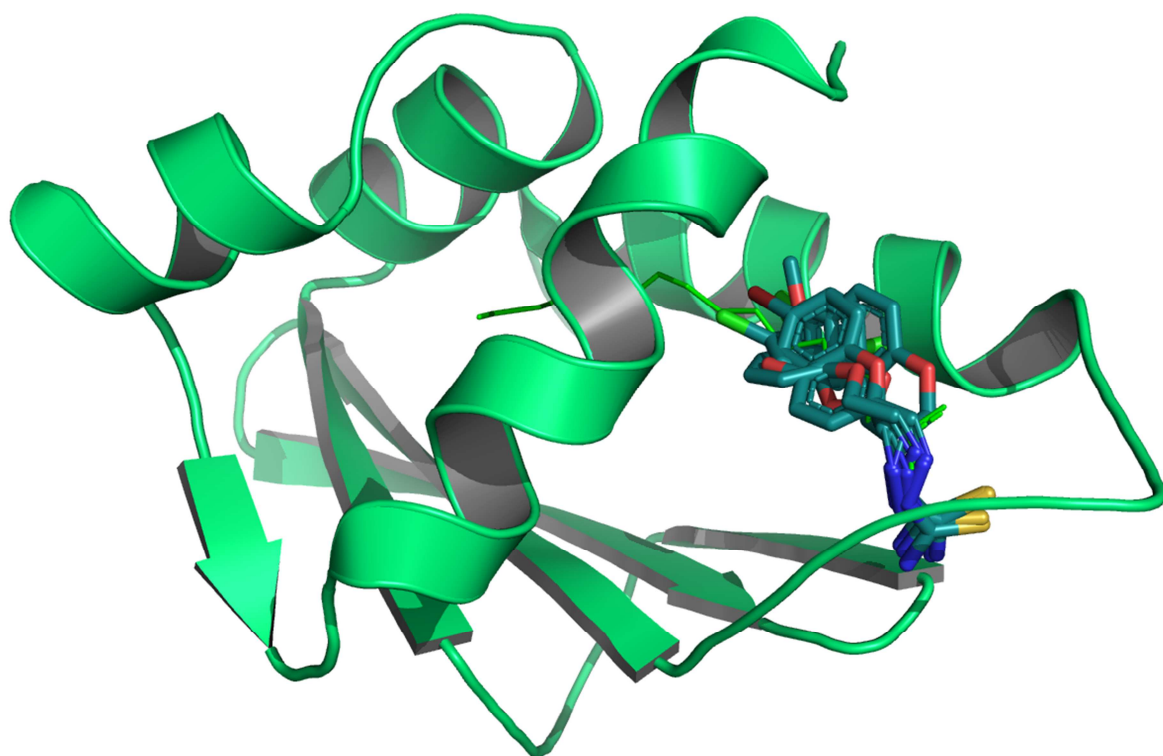
^a Ref. 79.

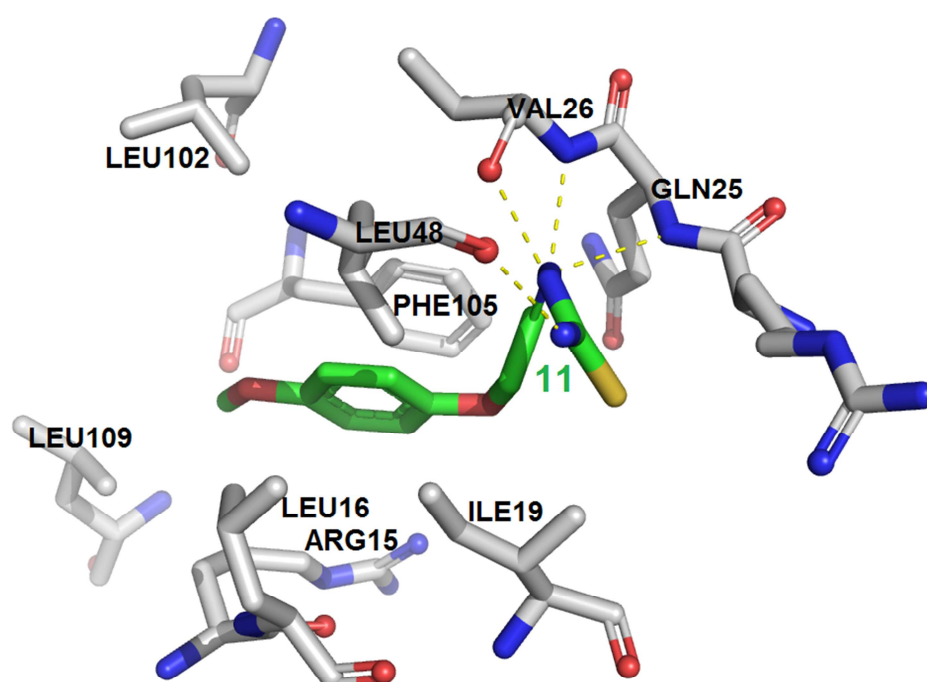


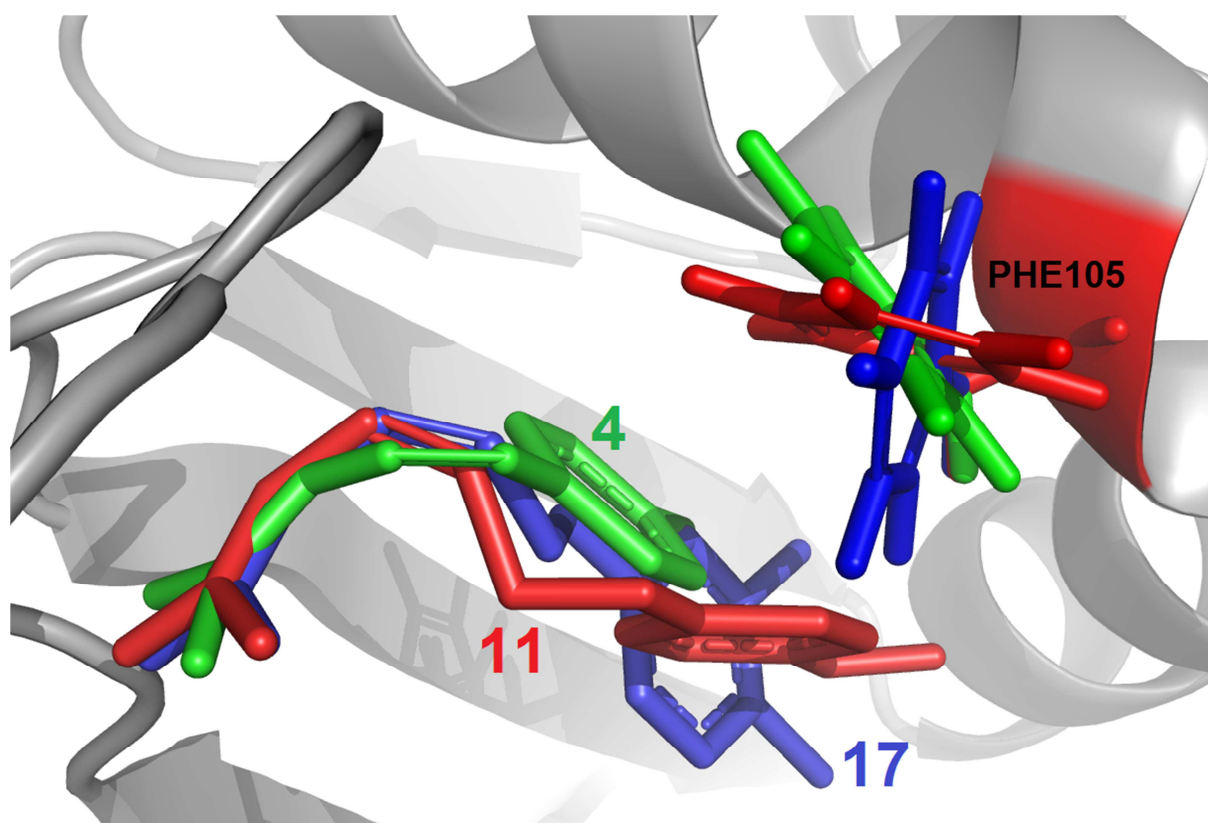


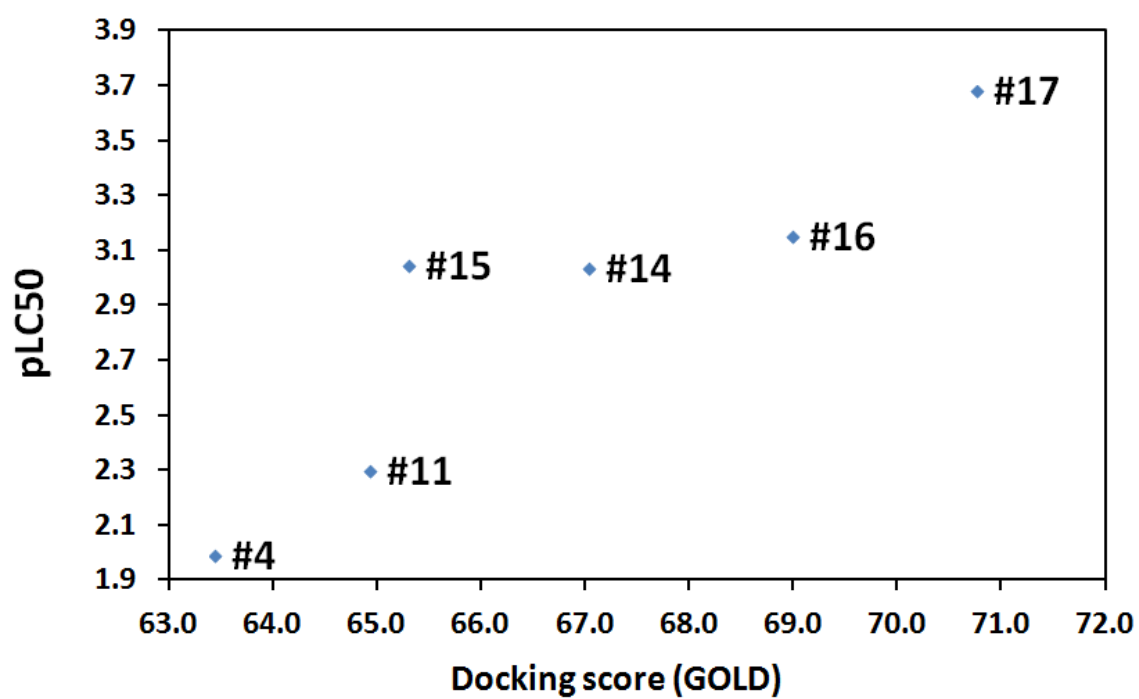












- Thiosemicarbazones (TSCZ) derivatives works as *A. aegypti* larvicidal
- QSAR model was successfull predicting new activities
- The dose-response profile was obtained for AeSCP-2
- Docking studies corroborate AeSCP-2 as a potential target for TSCZ
- The most active TSCZ derivatives are non-toxic for mammal cells

Supplementary Material

Thiosemicarbazones as *Aedes aegypti* Larvicidal

João Bosco P. da Silva^{a,*}, Daniela Maria do A. F. Navarro^{a,*}, Aluizio G. da Silva^a, Geanne K. N. Santos^a, Kamilla A. Dutra^a, Diogo Rodrigo Moreira^{a,1}, Mozart N. Ramos^a, José Wanderlan P. Espíndola^b, Ana Daura T. de Oliveira^b, Dalci José Brondani^b, Ana Cristina L. Leite^b, Marcelo Zaldini Hernandez^b, Valéria R. A. Pereira^c, Lucas F. da Rocha^c, Maria Carolina A. B. de Castro^c, Beatriz C. de Oliveira^c, Que Lan^d, Kenneth M. Merz Jr.^{e,2}

^a Departamento de Química Fundamental, Centro de Ciências Exatas e da Natureza, Universidade Federal de Pernambuco, 50670-901, Recife, PE, Brazil.

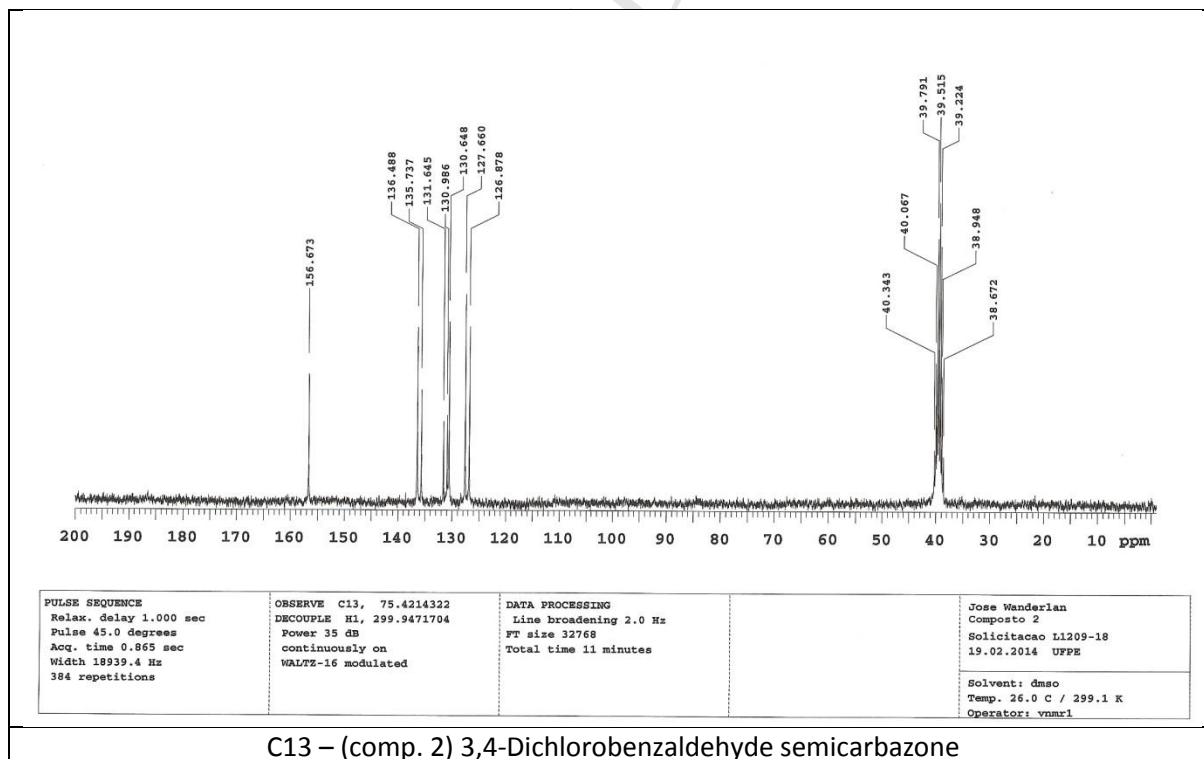
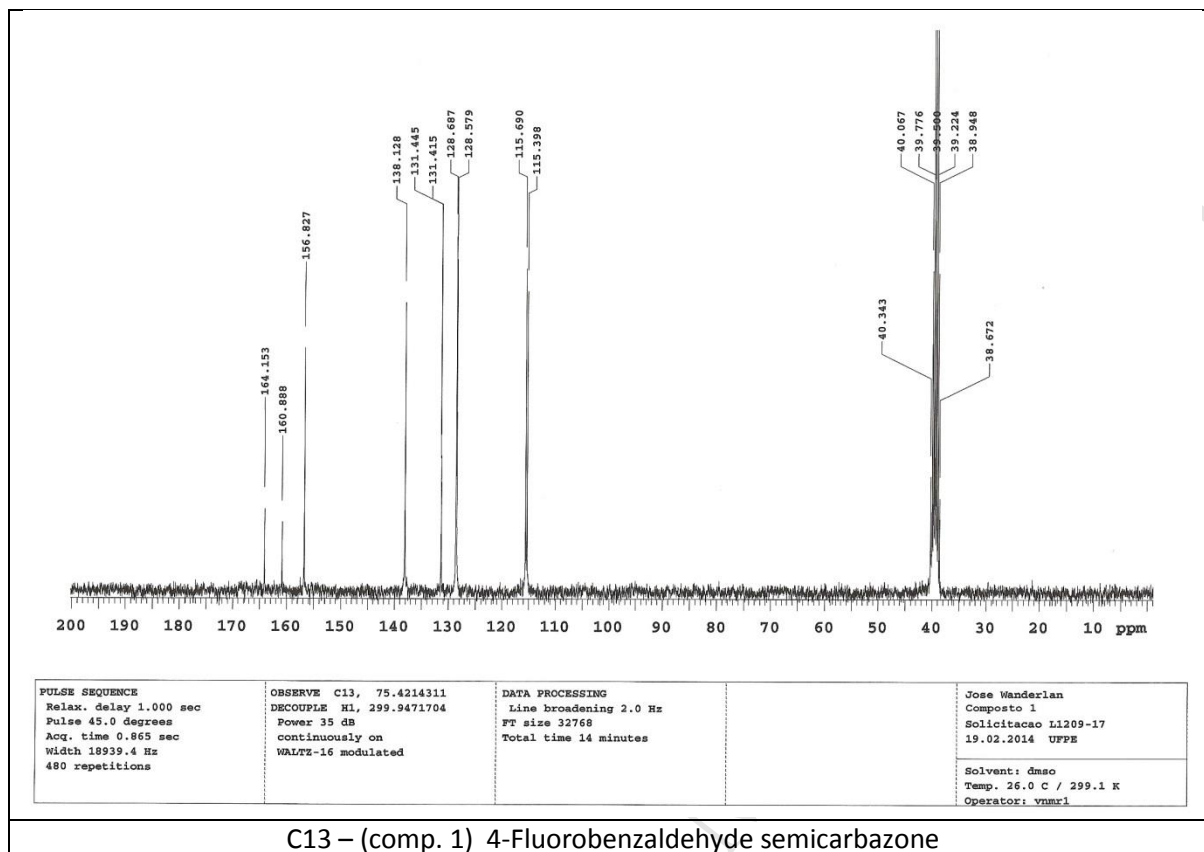
^b Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Pernambuco, 50740-521, Recife, PE, Brazil.

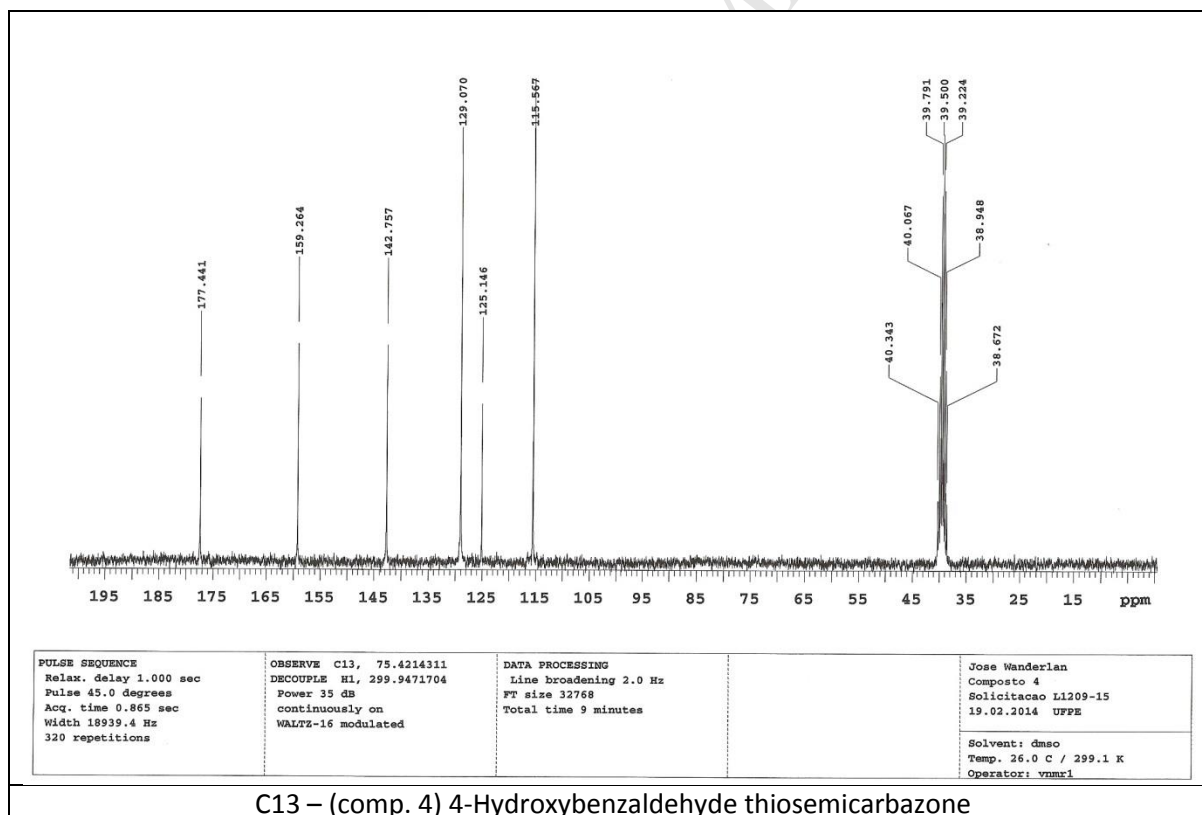
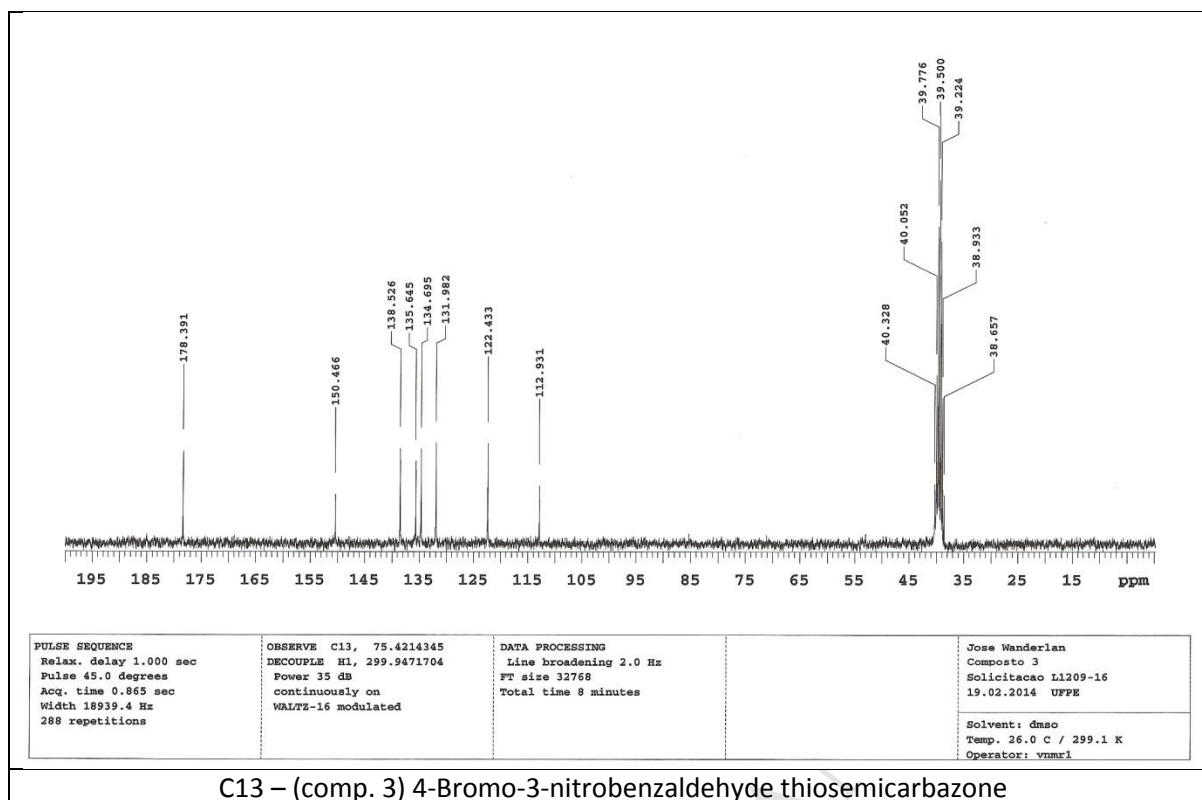
^c Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz, 50670-420, Recife, PE, Brazil.

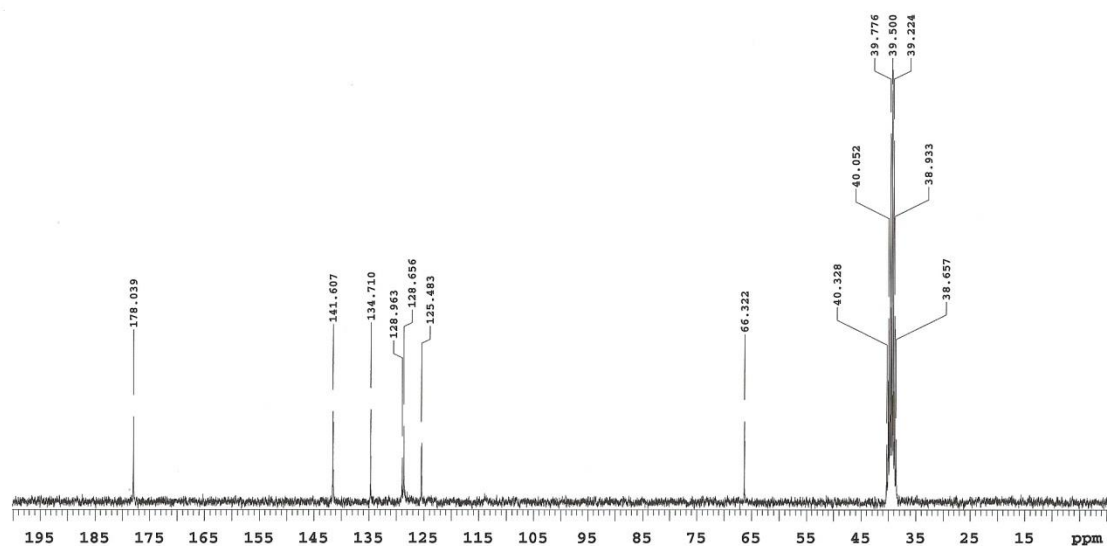
^d Department of Entomology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706, USA.

^e Quantum Theory Project, University of Florida, 2234 New Physics Building, Gainesville, PO Box 118435, Florida and Institute for Cyber Enabled Research, Department of Chemistry and the Department of Biochemistry and Molecular Biology, Michigan State University, 578 S. Shaw Lane, East Lansing, MI 48824, USA

NMR Spectra

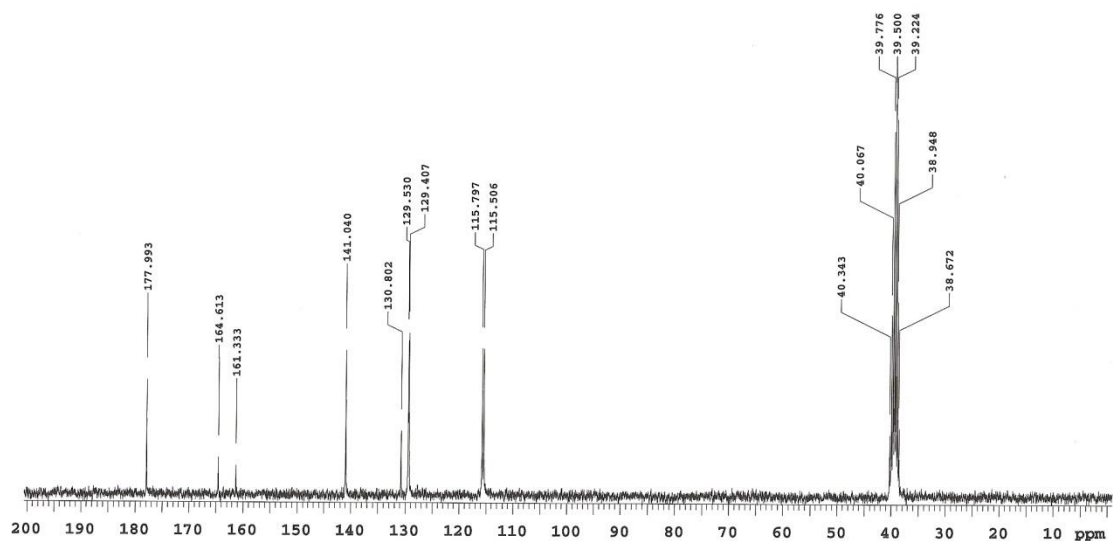






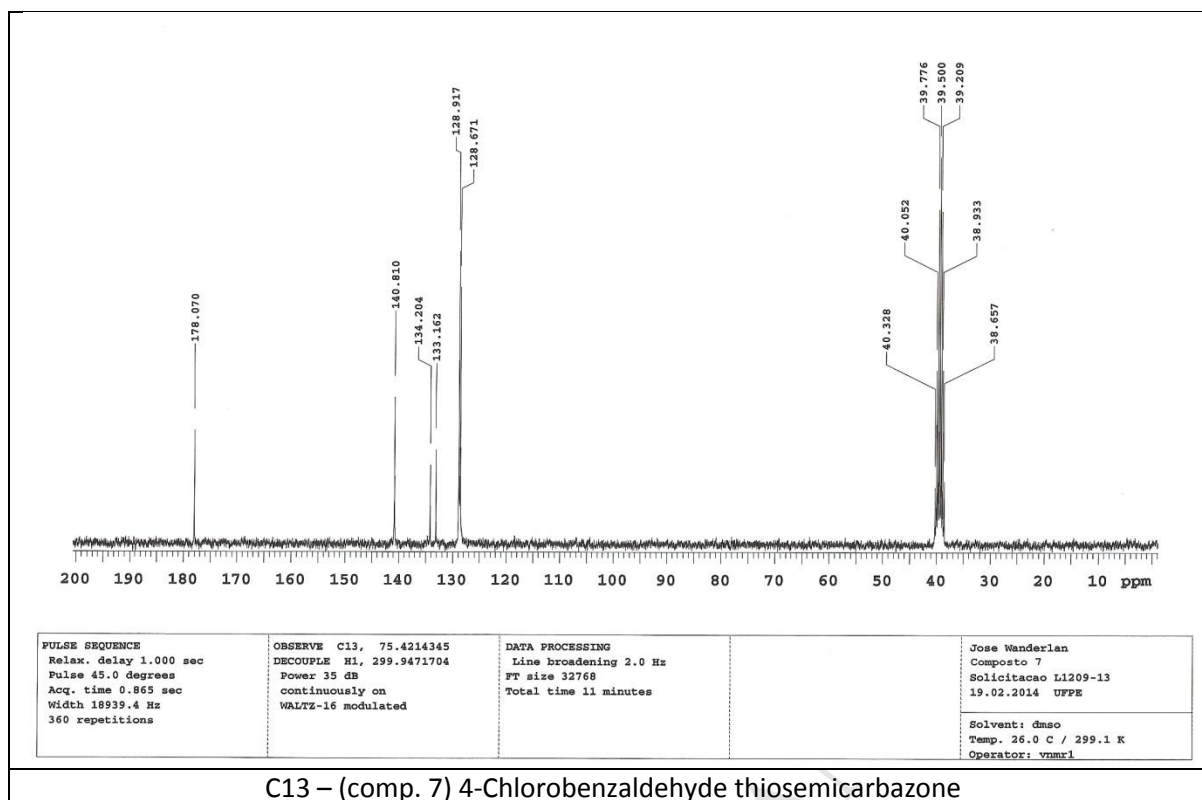
PULSE SEQUENCE Relax. delay 1.000 sec Pulse 45.0 degrees Acq. time 0.865 sec Width 18939.4 Hz 400 repetitions	OBSERVE C13, 75.4214345 DECOUPLE H1, 299.9471704 Power 35 dB continuously on WALTZ-16 modulated	DATA PROCESSING Line broadening 2.0 Hz FT size 32768 Total time 12 minutes	Jose Wanderlan Composto 5 Solicitacao LI209-9 19.02.2014 UFPE Solvent: dmsc Temp. 26.0 C / 299.1 K Operator: vnmr1
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C13 – (comp. 5) 2,2'-(1,3-Phenylenebis(methanylylidene))bis(thiosemicarbazide)

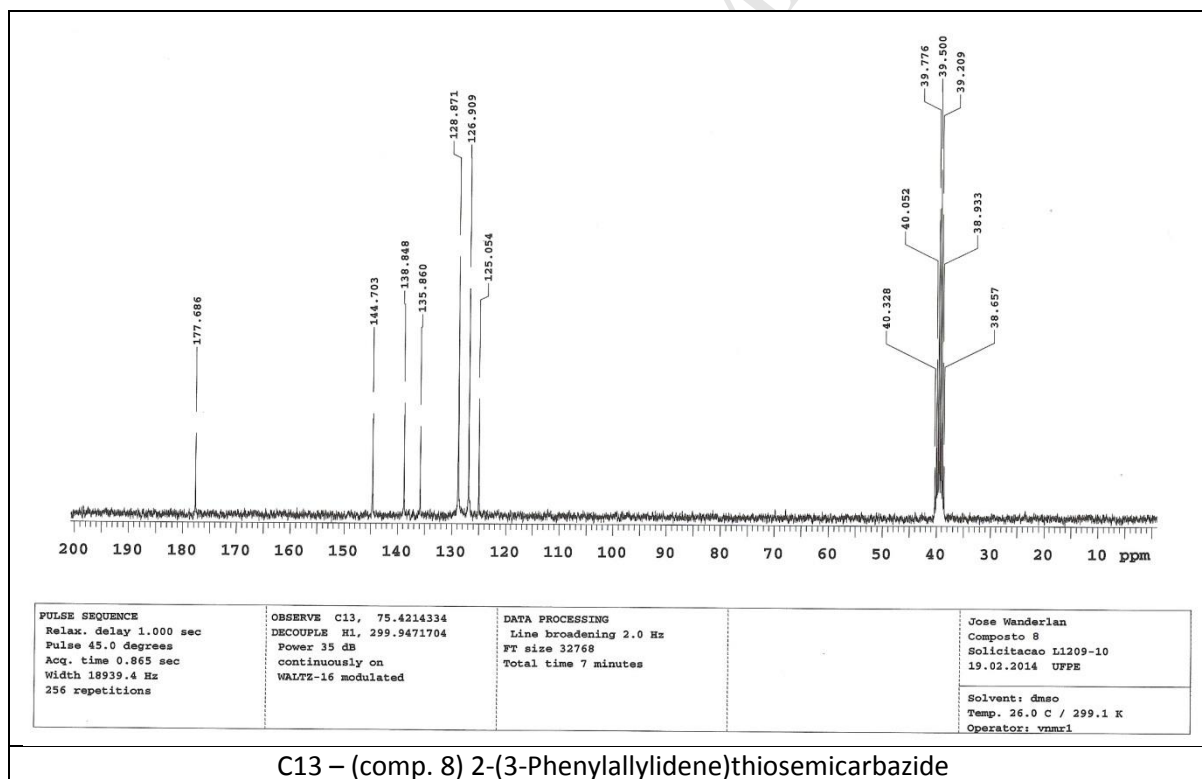


PULSE SEQUENCE Relax. delay 1.000 sec Pulse 45.0 degrees Acq. time 0.865 sec Width 18939.4 Hz 480 repetitions	OBSERVE C13, 75.4214334 DECOUPLE H1, 299.9471704 Power 35 dB continuously on WALTZ-16 modulated	DATA PROCESSING Line broadening 2.0 Hz FT size 32768 Total time 14 minutes	Jose Wanderlan Composto 6 Solicitacao LI209-12 19.02.2014 UFPE Solvent: dmsc Temp. 26.0 C / 299.1 K Operator: vnmr1
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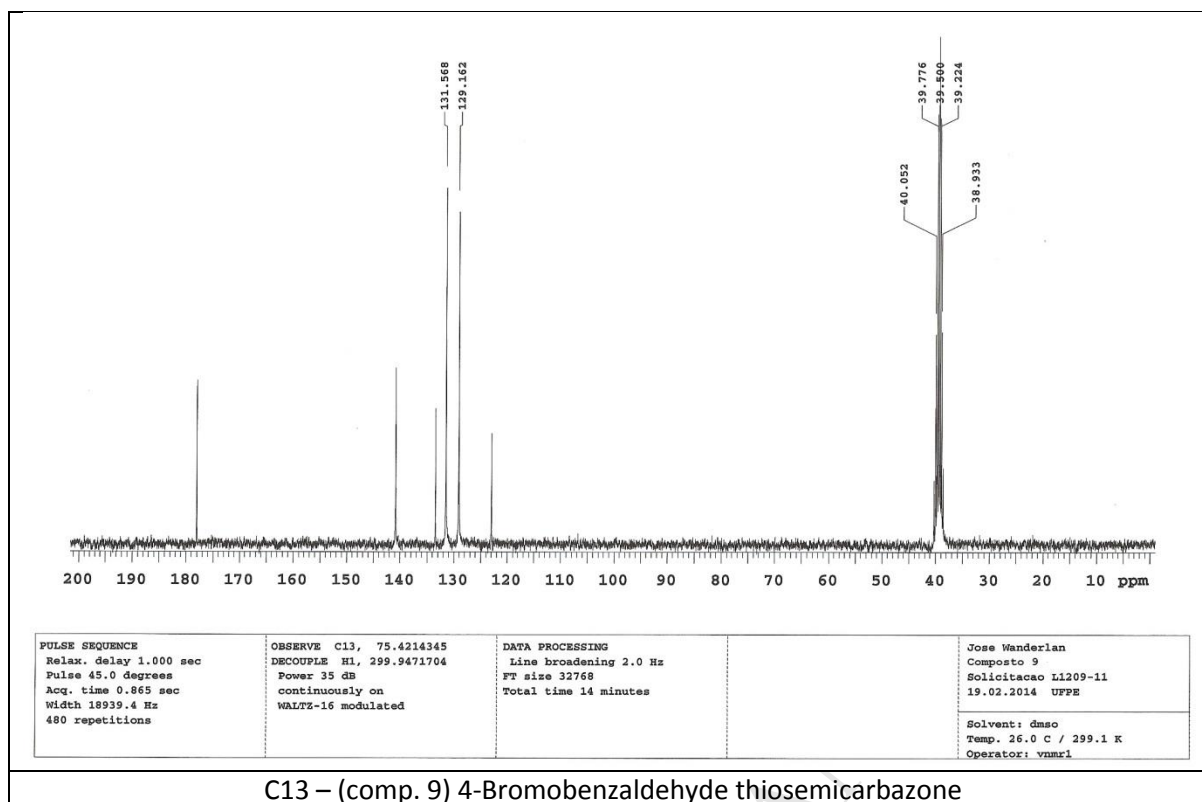
C13 – (comp. 6) 4-Fluorobenzaldehyde thiosemicarbazone



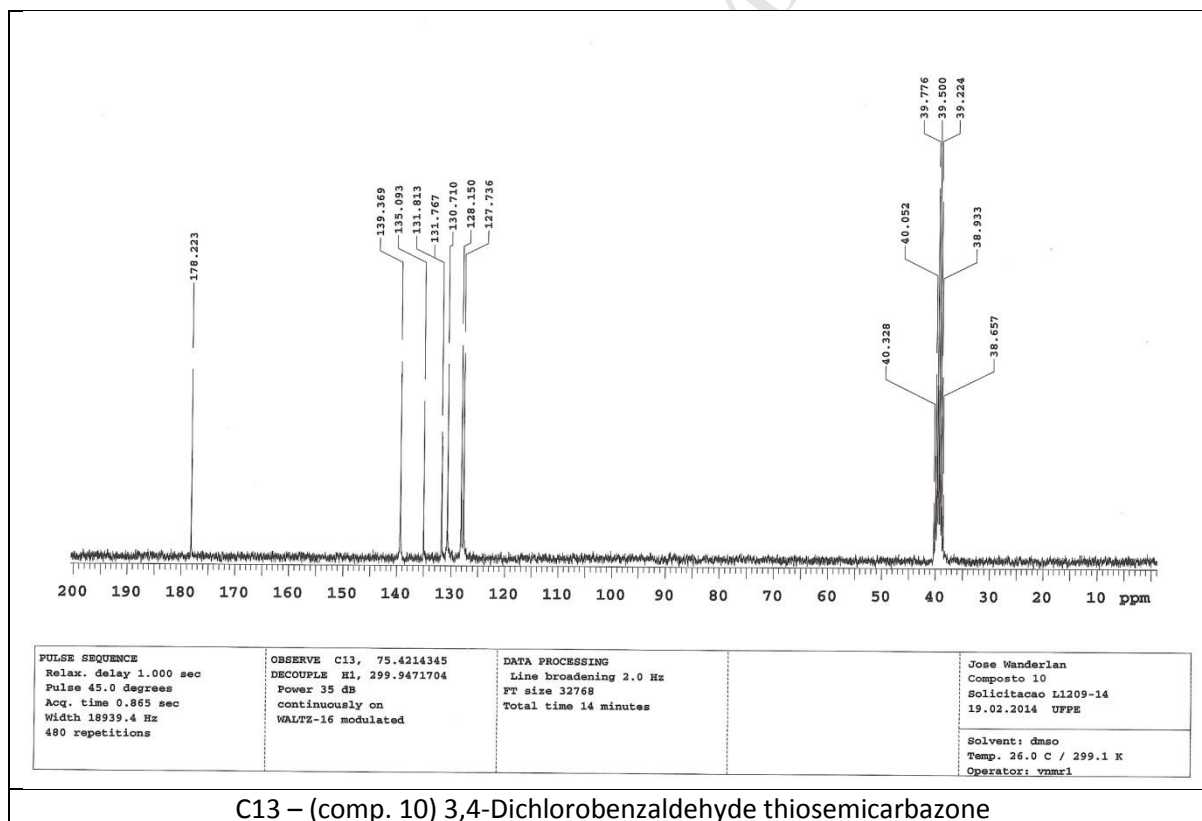
C13 – (comp. 7) 4-Chlorobenzaldehyde thiosemicarbazone



C13 – (comp. 8) 2-(3-Phenylallylidene)thiosemicarbazide

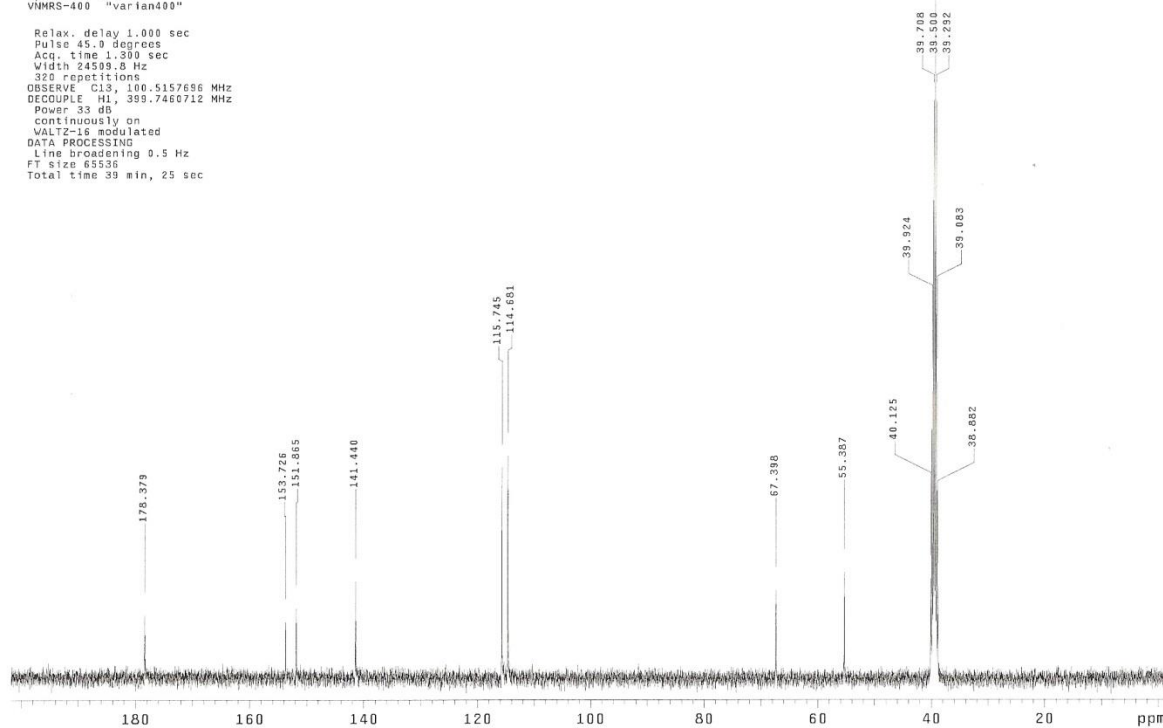


C13 – (comp. 9) 4-Bromobenzaldehyde thiosemicarbazone



C13 – (comp. 10) 3,4-Dichlorobenzaldehyde thiosemicarbazone

Vanderlan
Composto 11
Solicitação N. 16617_9
05.07.10 UFPE
File: exp
Pulse Sequence: s2pu1
Solvent: dmsd
Temp. 25.0 C / 298.1 K
Operator: ricardo
VNMRS-400 "varian400"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.200 sec
Width 24599.8 Hz
320 repetitions
OBSERVE C13, 100.5157696 MHz
DECOUPLE H1, 399.7460712 MHz
Power 33 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 39 min, 25 sec



C13 – (comp. 11) 2-(4-Methoxyphenoxy)acetaldehyde thiosemicarbazone

Wanderlan
Composto 12
Solicitacao N. I0720_11
29.07.19 UFPE

File: exp

Pulse Sequence: s2pul

Solvent: dmsd

Temp: 27.0 C / 300.1 K

Operator: ricardo

VNMRS-400 "Varian400"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.300 sec

Width 24509.8 Hz

272 repetitions

OBSERVE C13, 100.5157756 MHz

DECOUPLE H1, 399.7460712 MHz

Power 33.00

Continuously on

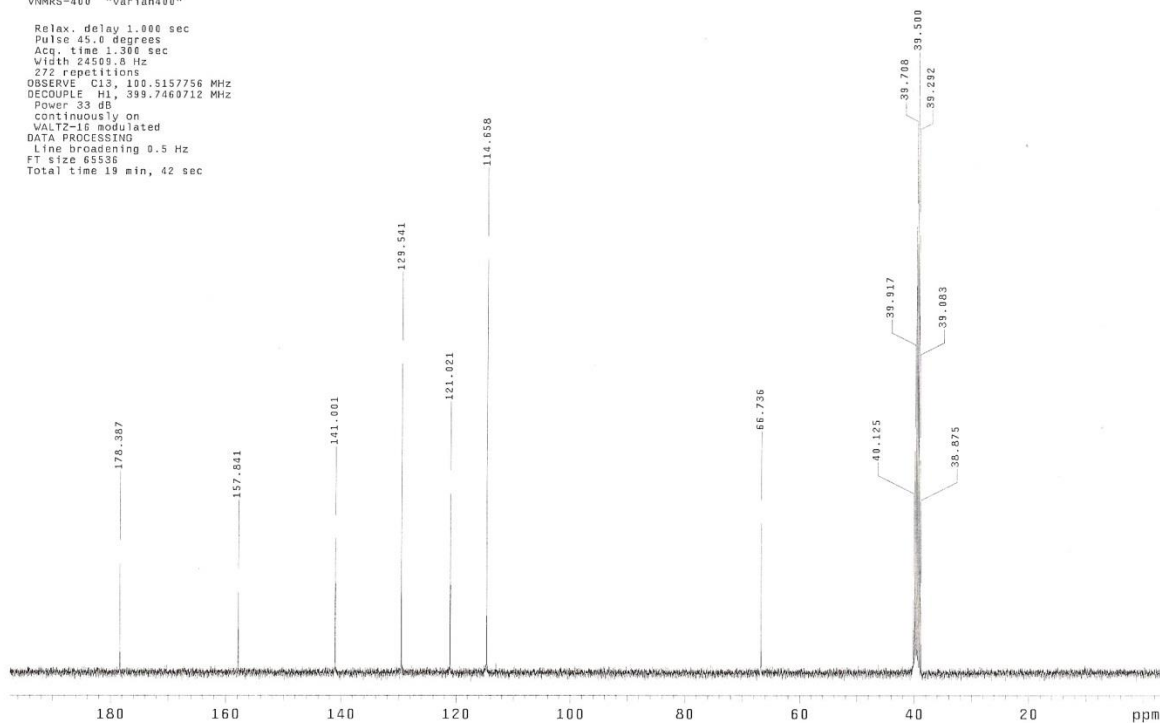
WALTZ-16 modulated

DATA PROCESSING

Line broadening 0.5 Hz

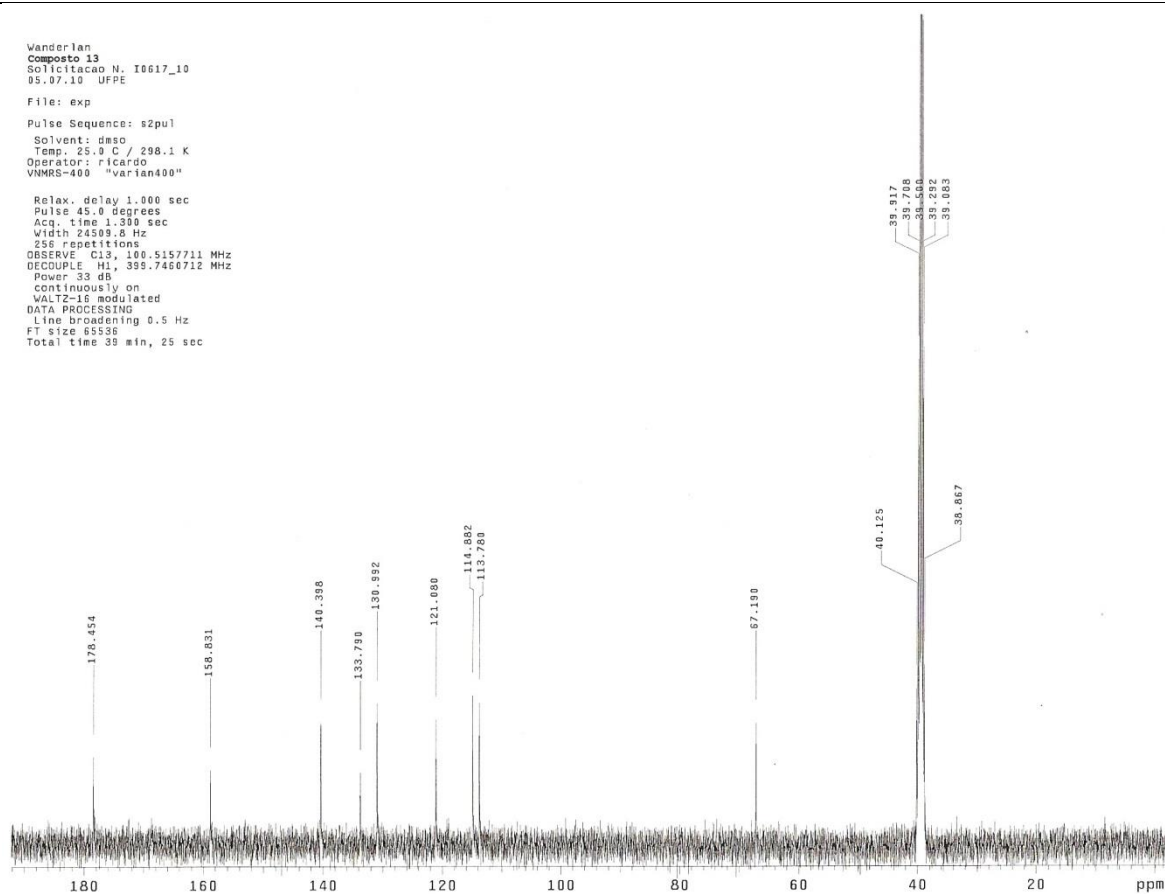
FT size 65536

Total time 19 min, 42 sec

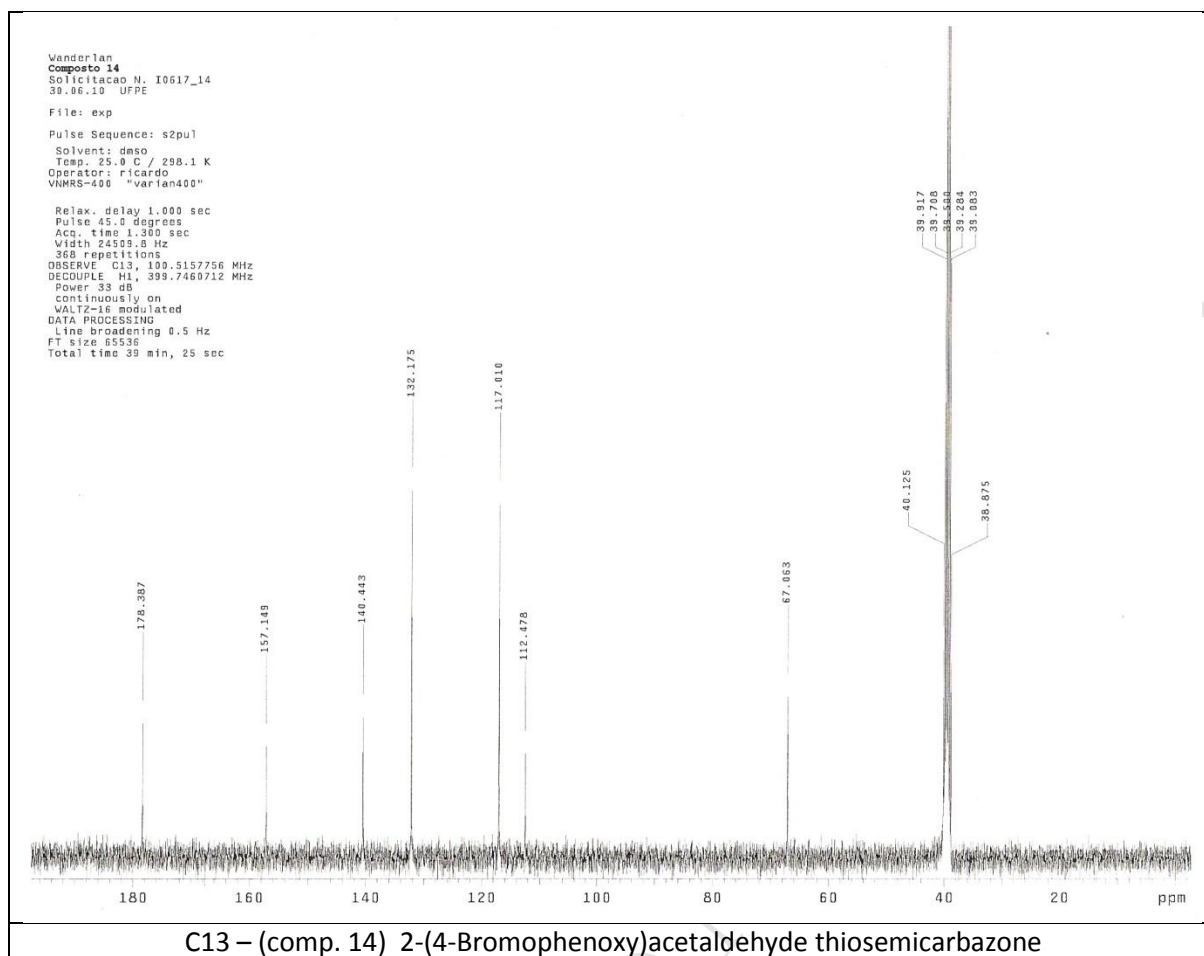


C13 – (comp. 12) 2-Phenoxyacetaldehyde thiosemicarbazone

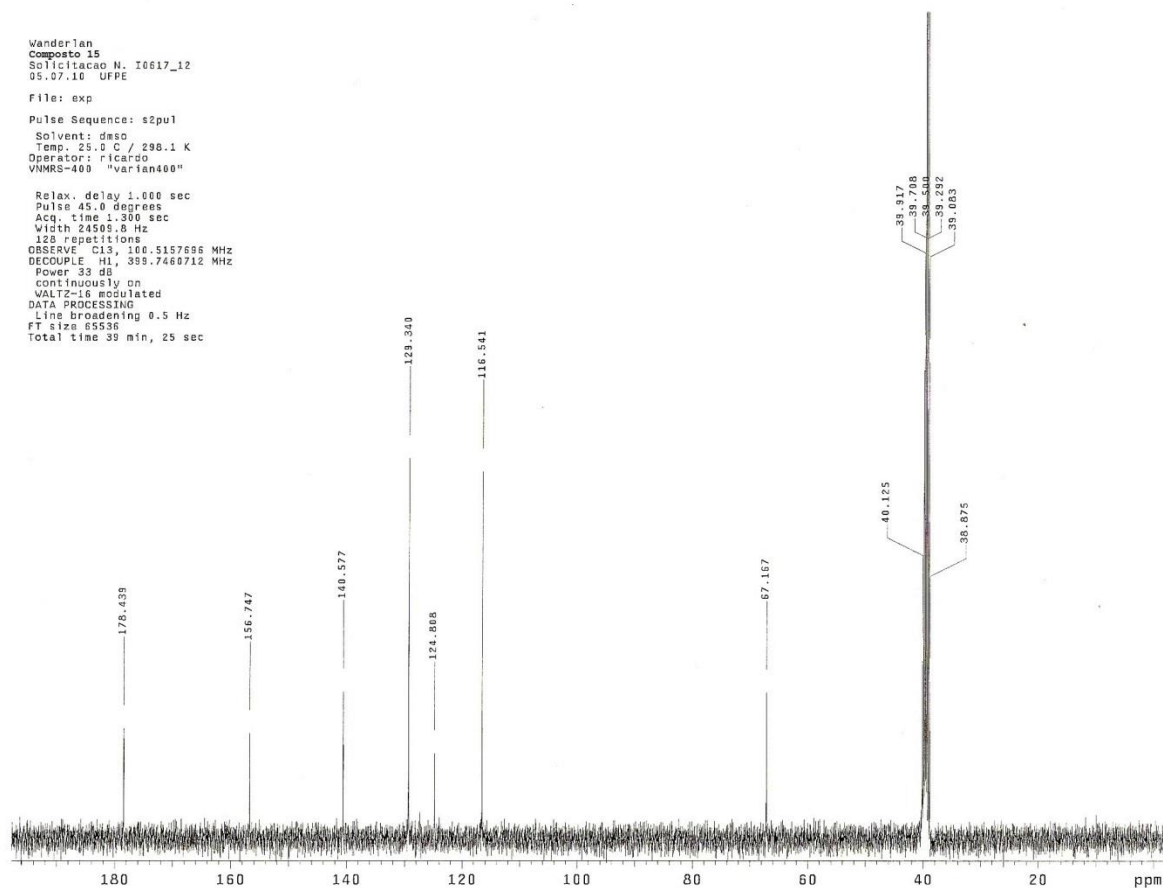
Vanderlan
Composto 13
Solicitacao N. I0617_10
05.07.10 UFPE
File: exp
Pulse Sequence: s2pul
Solvent: dms
Temp. 25.0 C / 298.1 K
Operator: ricardo
VNMRS-400 "Varian400"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 24509.8 Hz
256 repetitions
OBSERVE C13, 100.5157711 MHz
DECOUPLE H1, 399.7456712 MHz
Power 33 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 39 min, 25 sec



C13 - (comp. 13) 2-(3-Chlorophenoxy)acetaldehyde thiosemicarbazone

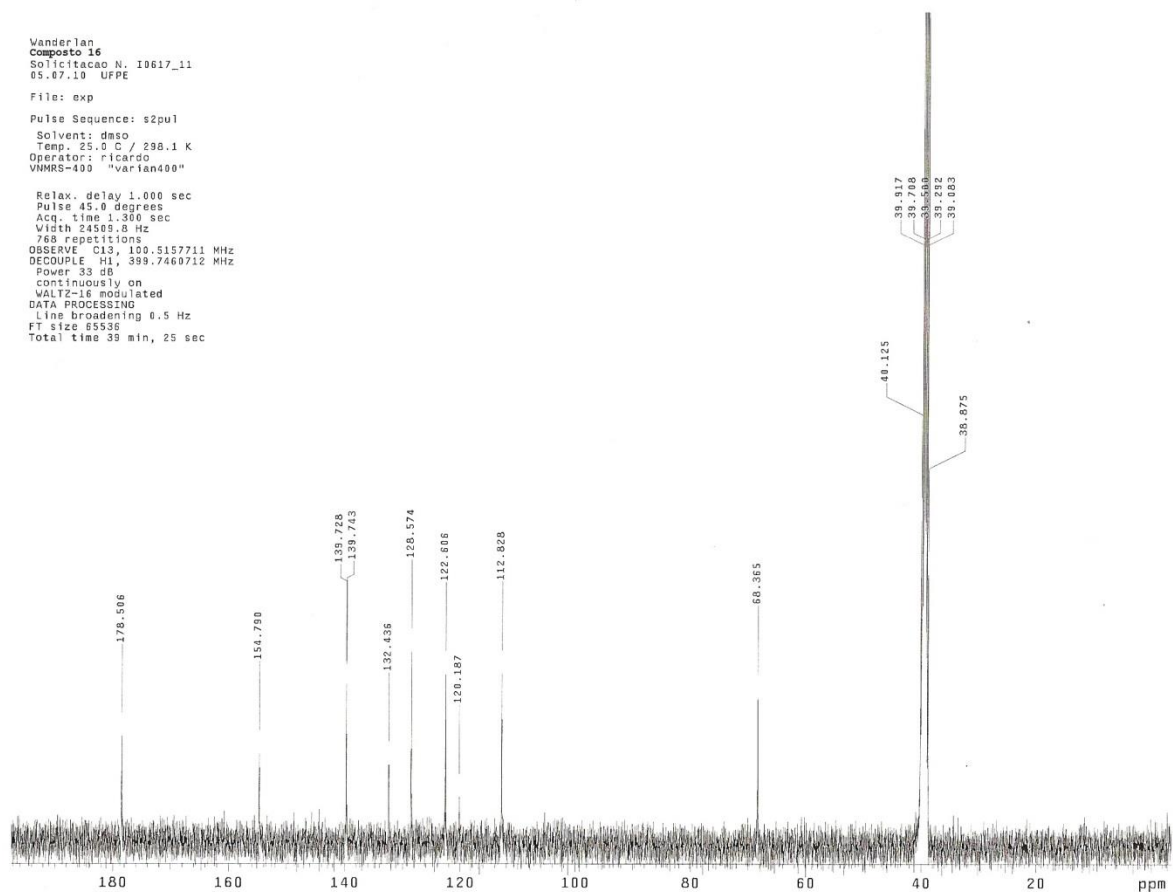


Vanderlan
Composto 15
Solicitação N. 16617_12
05.07.10 UFPE
File: exp
Pulse Sequence: s2pu1
Solvent: dms
Temp. 25.0 C / 298.1 K
Operator: ricardo
VNMRS-400 "varian400"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 24500.0 Hz
128 repetitions
OBSERVE C13, 100.5157696 MHz
DECOUPLE H1, 399.7469712 MHz
Power 33 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 39 min, 25 sec



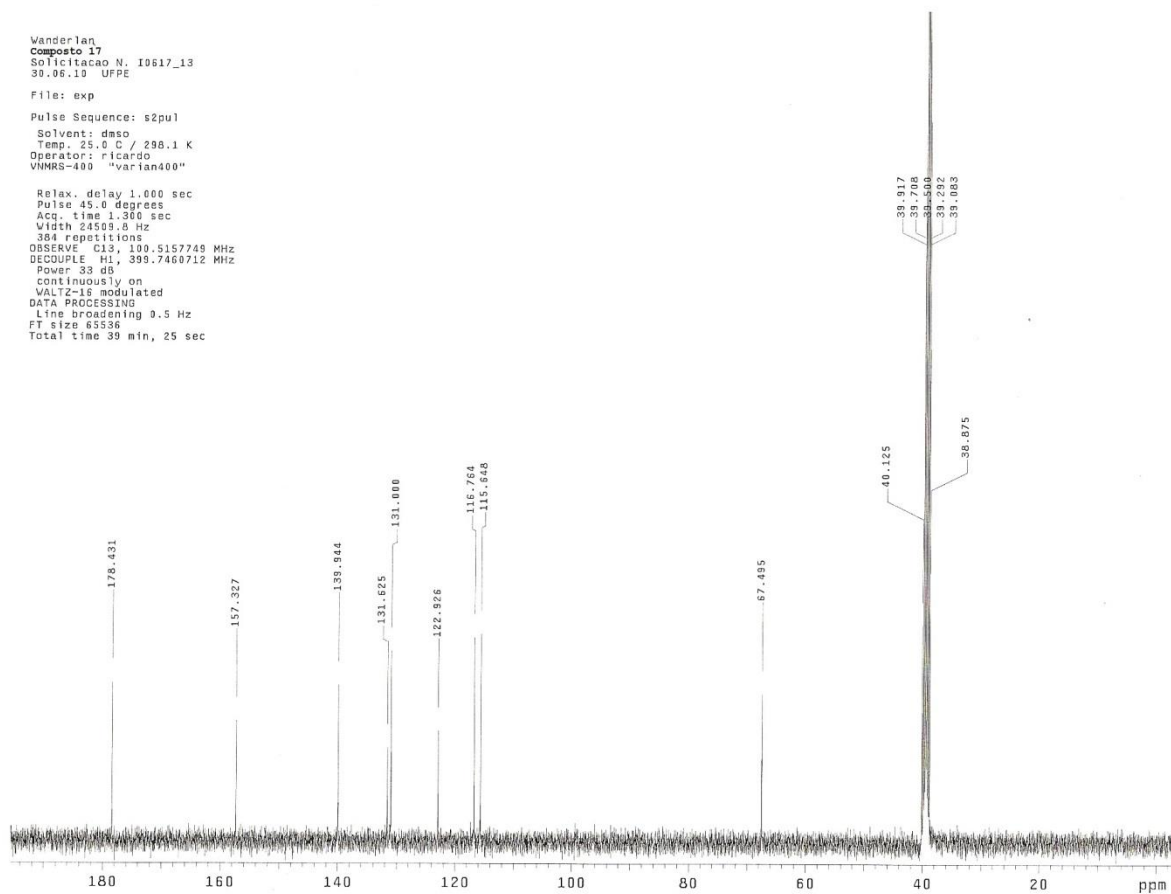
C13 – (comp. 15) 2-(4-Chlorophenoxy)acetaldehyde thiosemicarbazone

Vanderlan
Composto 16
Solicitação N. 10617_11
05.07.10 UFPE
File: exp
Pulse Sequence: s2pul
Solvent: dms0
Temp. 25.0 C / 298.1 K
Operator: ricardo
VNMRS-400 "vian400"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Vinch 24508.8 Hz
768 repetitions
OBSERVE C13, 100.5157711 MHz
DECOUPLE H1, 399.7460712 MHz
Power 33 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 6.5 Hz
FT size 65536
Total time 39 min, 25 sec



C13 – (comp. 16) 2-(2,3-Dichlorophenoxy)acetaldehyde thiosemicarbazone

Vanderlan
Composto 17
Solicitação N. 10617_13
30.06.10 UFPE
File: exp
Pulse Sequence: s2pul
Solvent: dmsd
Temp. 25.0 C / 298.1 K
Operator: ricardo
VNMRS-400 "Varian400"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.360 sec
Width 24509.6 Hz
384 repetitions
OBSERVE C13, 100.5157749 MHz
DECOUPLE H1, 399.7460712 MHz
Power 33 dB
Continuously on
VOLTAGE modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 39 min, 25 sec



C13 – (comp. 17) 2-(3,4-Dichlorophenoxy)acetaldehyde thiosemicarbazone

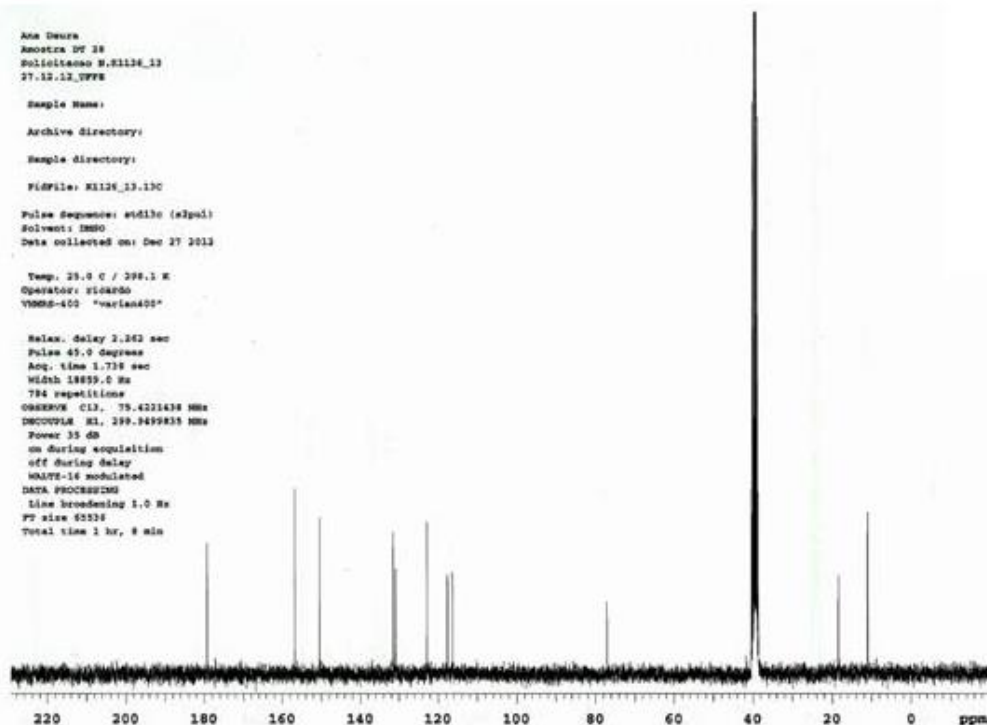
Ana Deura
 Amostra DT 18
 Solicitacao N.81138_13
 27.12.12 UFPE

Sample Name:
 Archive directory:
 Sample directory:
 FidFile: 81138_13.130

Pulse Sequence: sdd1b (s2pul)
 Solvent: DMSO
 Data collected on: Dec 27 2013

Temp. 29.0 C / 398.1 K
 Operator: ricardo
 VNMRS-400 "varian400"

Relax. delay 2.262 sec
 Pulse 45.0 degrees
 Acq. time 1.738 sec
 Width 18859.0 Hz
 784 repetitions
 OBSERVE C13. 75.4231438 MHz
 DECOUPLE H1. 299.8499835 MHz
 Power 35 dB
 on during acquisition
 off during delay
 WALTZ-16 modulated
 DATA PROCESSING
 Line broadening 1.0 Hz
 FT size 65536
 Total time 1 hr, 8 min



C13 – (comp. 18) 3-(3,4-Dichlorophenoxy)butan-2-one thiosemicarbazone

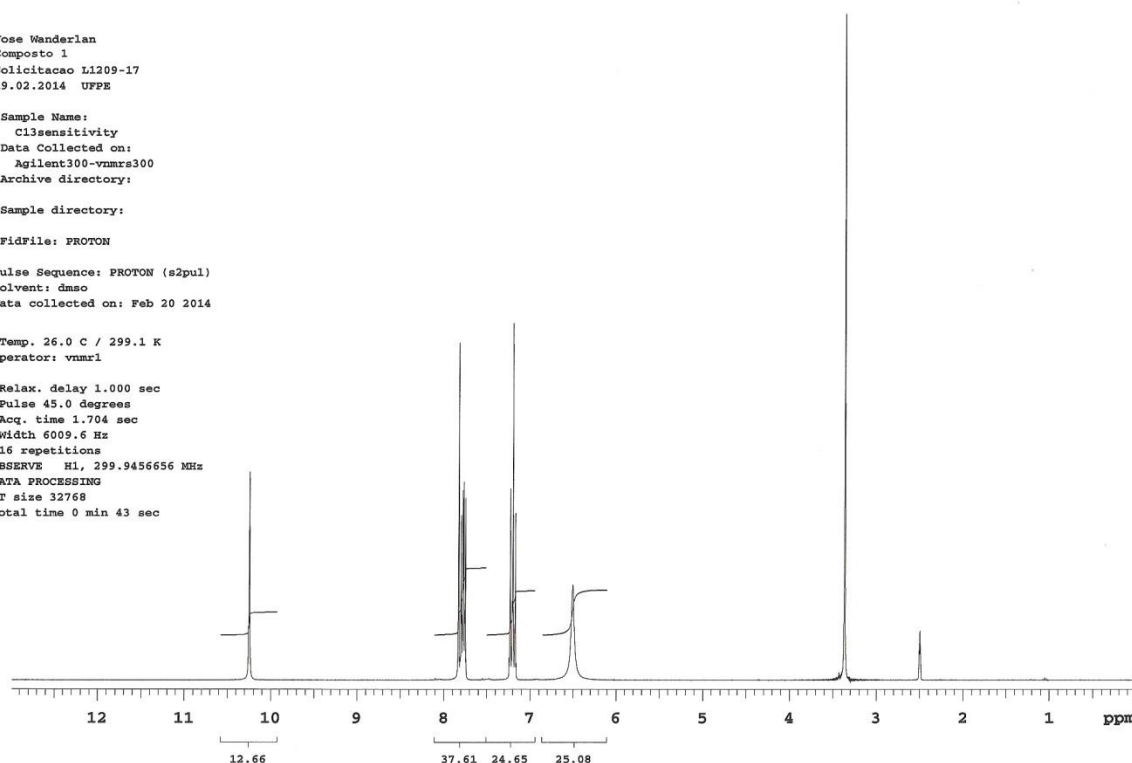
Jose Wanderlan
 Composto 1
 Solicitacao L1209-17
 19.02.2014 UFPE

Sample Name:
 C13sensitivity
 Data Collected on:
 Agilent300-vnmrs300
 Archive directory:
 Sample directory:
 FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
 Solvent: dmsd
 Data collected on: Feb 20 2014

Temp. 26.0 C / 299.1 K
 Operator: vnmr1

Relax. delay 1.000 sec
 Pulse 45.0 degrees
 Acq. time 1.704 sec
 Width 6009.6 Hz
 16 repetitions
 OBSERVE H1. 299.9456656 MHz
 DATA PROCESSING
 FT size 32768
 Total time 0 min 43 sec



H1 - (comp. 1) 4-Fluorobenzaldehyde semicarbazone

Jose Wanderlan
Composto 2
Solicitacao LI209-18
19.02.2014 UFPE

Sample Name:
C13sensitivity
Data Collected on:
Agilent300-vnmrs300
Archive directory:

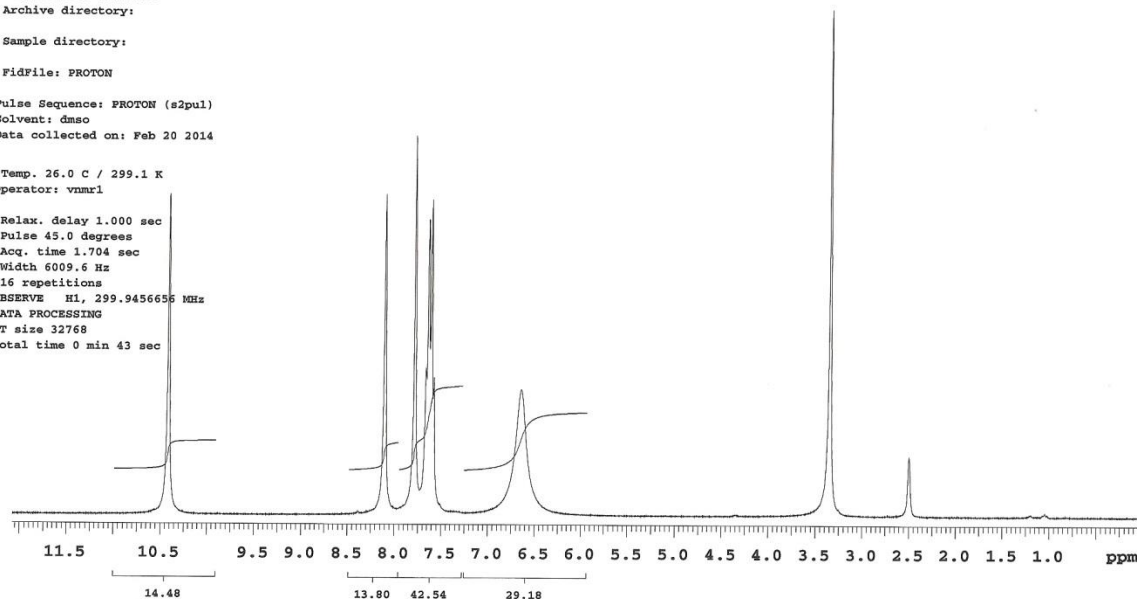
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmsc
Data collected on: Feb 20 2014

Temp. 26.0 C / 299.1 K
Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 6009.6 Hz
16 repetitions
OBSERVE H1, 299.9456653 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 43 sec



H1 - (comp. 2) 3,4-Dichlorobenzaldehyde semicarbazone

Jose Wanderlan
Composto 3
Solicitacao LI209-16
19.02.2014 UFPE

Sample Name:
C13sensitivity
Data Collected on:
Agilent300-vnmrs300
Archive directory:

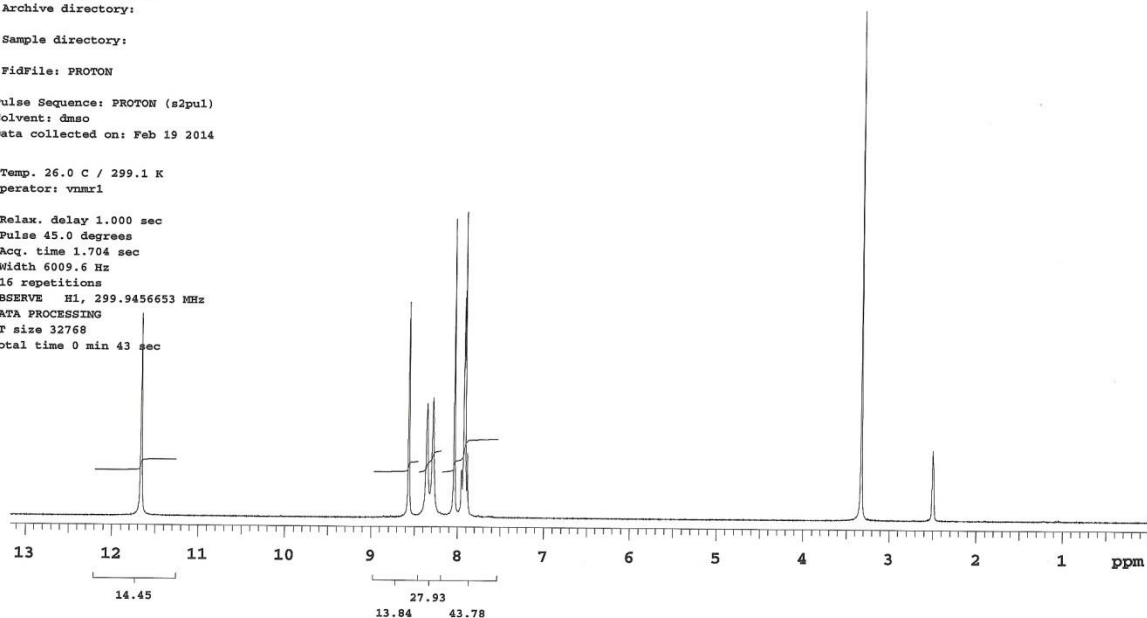
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmsc
Data collected on: Feb 19 2014

Temp. 26.0 C / 299.1 K
Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 6009.6 Hz
16 repetitions
OBSERVE H1, 299.9456653 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 43 sec



H1 - (comp. 3) 4-Bromo-3-nitrobenzaldehyde thiosemicarbazone

Jose Wanderlan
Composto 4
Solicitacao LI209-15
19.02.2014 UFPE

Sample Name:
Cl3sensitivity
Data Collected on:
Agilent300-vnmr300
Archive directory:

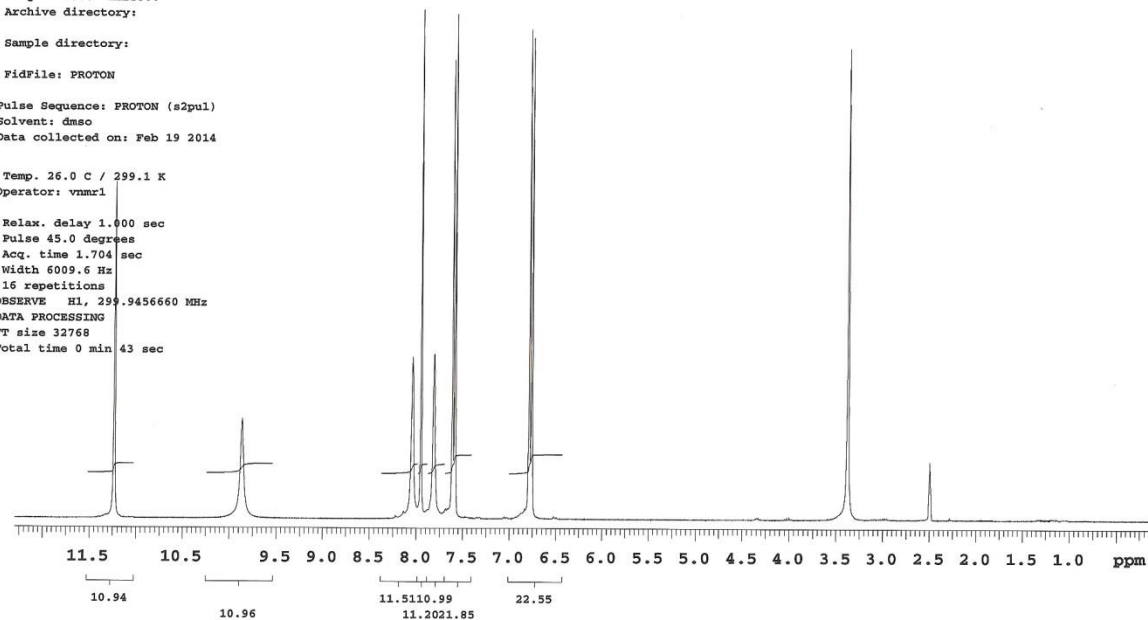
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmsd
Data collected on: Feb 19 2014

Temp. 26.0 C / 299.1 K
Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 6009.6 Hz
16 repetitions
OBSERVE H1, 299.9456660 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 43 sec



H1 - (comp. 4) 4-Hydroxybenzaldehyde thiosemicarbazone

Jose Wanderlan
Composto 5
Solicitacao LI209-9
19.02.2014 UFPE

Sample Name:
Cl3sensitivity
Data Collected on:
Agilent300-vnmr300
Archive directory:

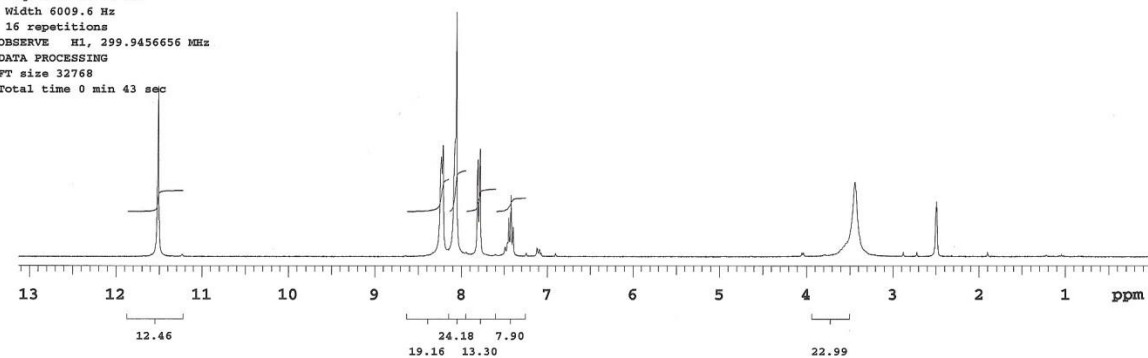
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmsd
Data collected on: Feb 19 2014

Temp. 26.0 C / 299.1 K
Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 6009.6 Hz
16 repetitions
OBSERVE H1, 299.9456656 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 43 sec



H1 - (comp. 5) 2,2'-(1,3-Phenylenebis(methanylylidene))bis(thiosemicarbazide)

Jose Wanderlan
Composto 6
Solicitacao LI209-12
19.02.2014 UFPE

Sample Name:
Cl3sensitivity
Data Collected on:
Agilent300-vnmr300
Archive directory:

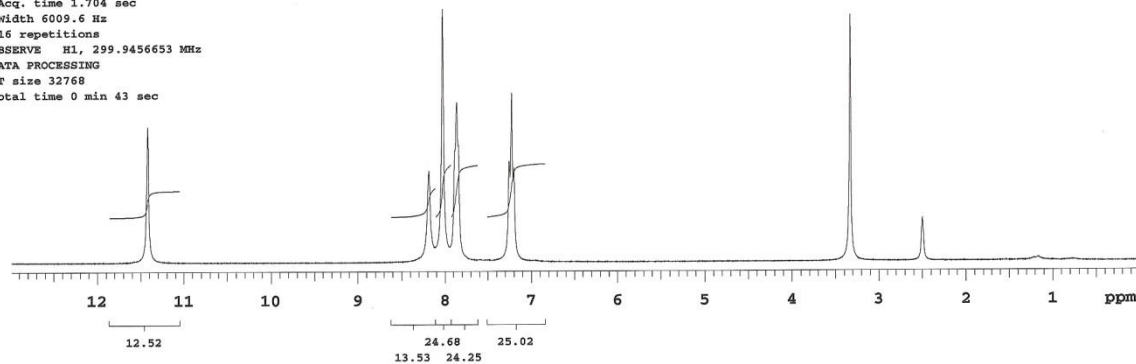
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmsc
Data collected on: Feb 19 2014

Temp. 26.0 C / 299.1 K
Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 6009.6 Hz
16 repetitions
OBSERVE H1, 299.9456653 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 43 sec



H1 - (comp. 6) 4-Fluorobenzaldehyde thiosemicarbazone

Jose Wanderlan
Composto 7
Solicitacao LI209-13
19.02.2014 UFPE

Sample Name:
Cl3sensitivity
Data Collected on:
Agilent300-vnmr300
Archive directory:

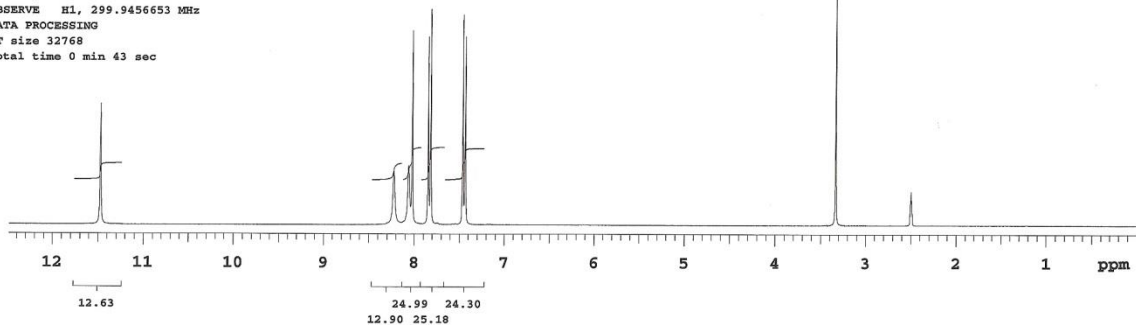
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmsc
Data collected on: Feb 19 2014

Temp. 26.0 C / 299.1 K
Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 6009.6 Hz
16 repetitions
OBSERVE H1, 299.9456653 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 43 sec



H1 - (comp. 7) 4-Chlorobenzaldehyde thiosemicarbazone

Jose Wanderlan
Composto 8
Solicitacao LI209-10
19.02.2014 UFPE

Sample Name:
Cl3sensitivity
Data Collected on:
Agilent300-vmmr300
Archive directory:

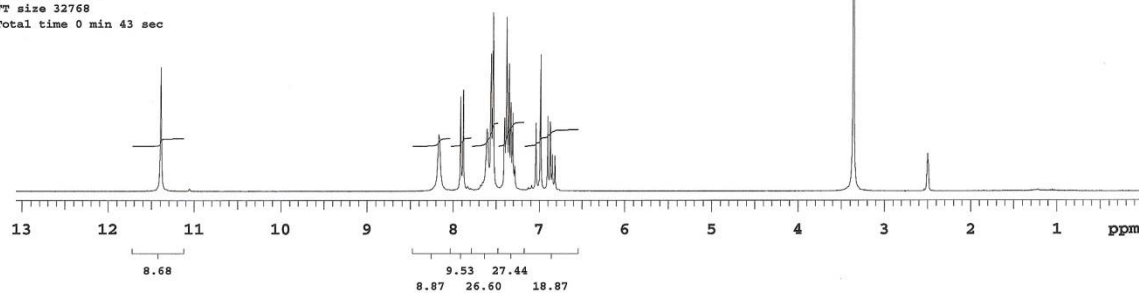
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmsc
Data collected on: Feb 19 2014

Temp. 26.0 C / 299.1 K
Operator: vmmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 6009.6 Hz
16 repetitions
OBSERVE H1, 299.9456653 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 43 sec



H1 - (comp. 8) 2-(3-Phenylallylidene)thiosemicarbazide

Jose Wanderlan
Composto 9
Solicitacao LI209-11
19.02.2014 UFPE

Sample Name:
Cl3sensitivity
Data Collected on:
Agilent300-vmmr300
Archive directory:

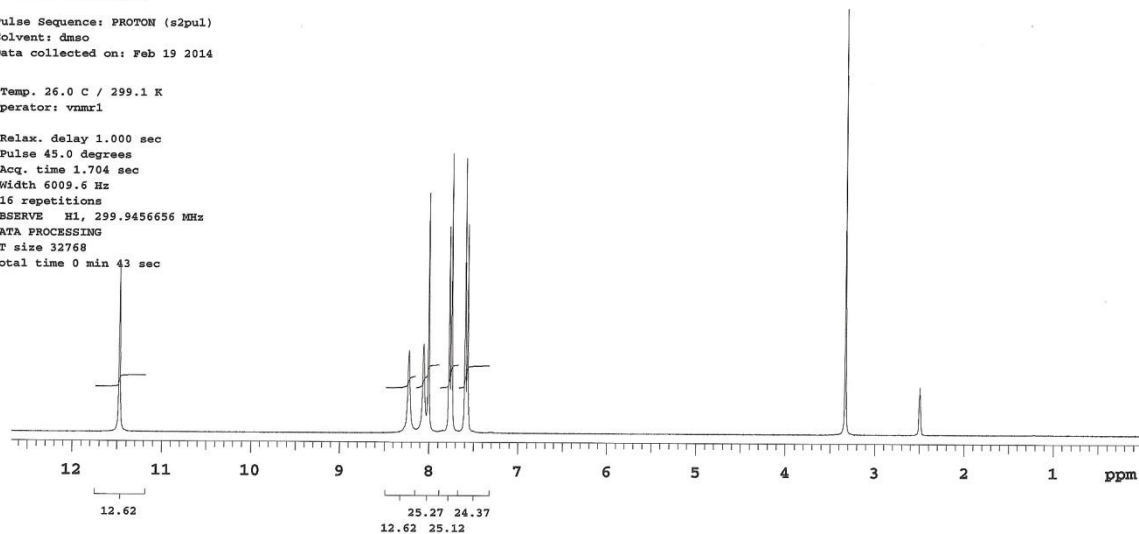
Sample directory:

FidFile: LI209_11.1h

Pulse Sequence: PROTON (s2pul)
Solvent: dmsc
Data collected on: Feb 19 2014

Temp. 26.0 C / 299.1 K
Operator: vmmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 6009.6 Hz
16 repetitions
OBSERVE H1, 299.9456656 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 43 sec



H1 - (comp. 9) 4-Bromobenzaldehyde thiosemicarbazone

Jose Wanderlan
Composto 10
Solicitacao LI209-14
19.02.2014 UFPE

Sample Name:
C13sensitivity
Data Collected on:
Agilent300-vnmr300
Archive directory:

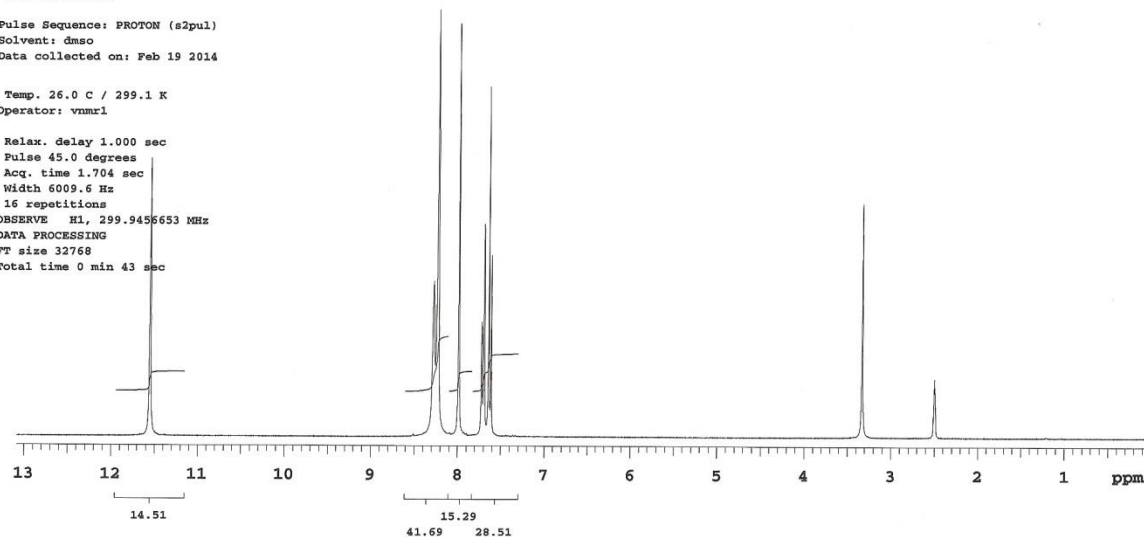
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmsd
Data collected on: Feb 19 2014

Temp. 26.0 C / 299.1 K
Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 6009.6 Hz
16 repetitions
OBSERVE H1, 299.9456653 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 43 sec



H1 - (comp. 10) 3,4-Dichlorobenzaldehyde thiosemicarbazone

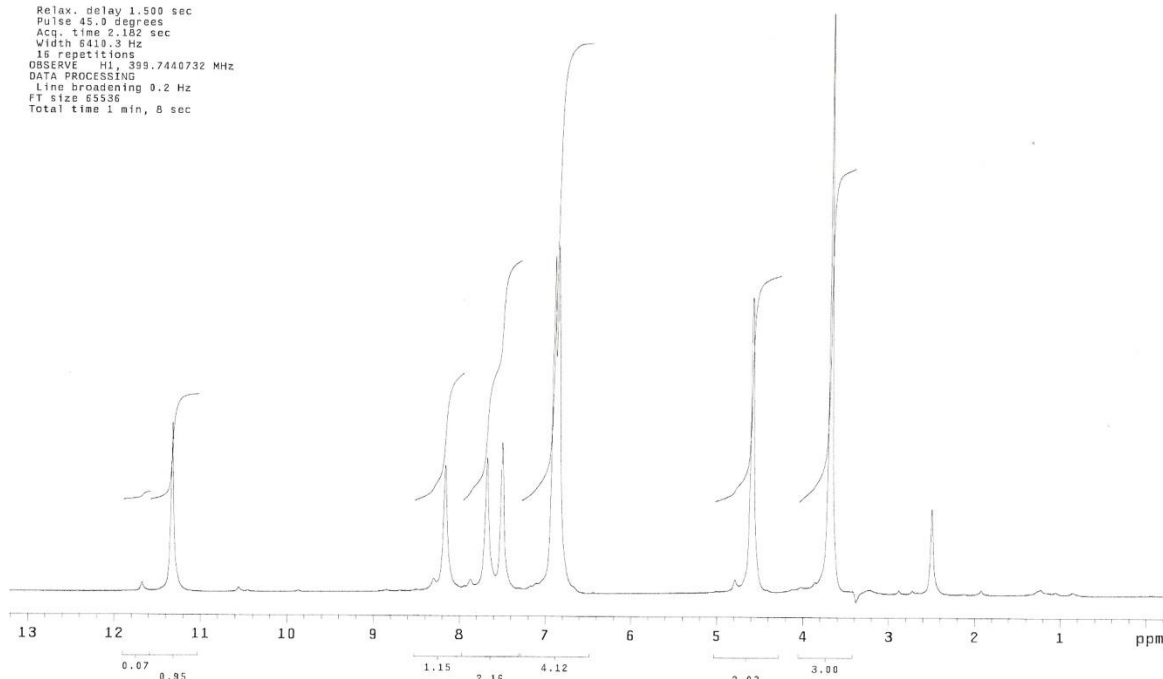
Wanderlan
Composto 11
Solicitacao N. I0617_9
05.07.10 UFPE

File: exp

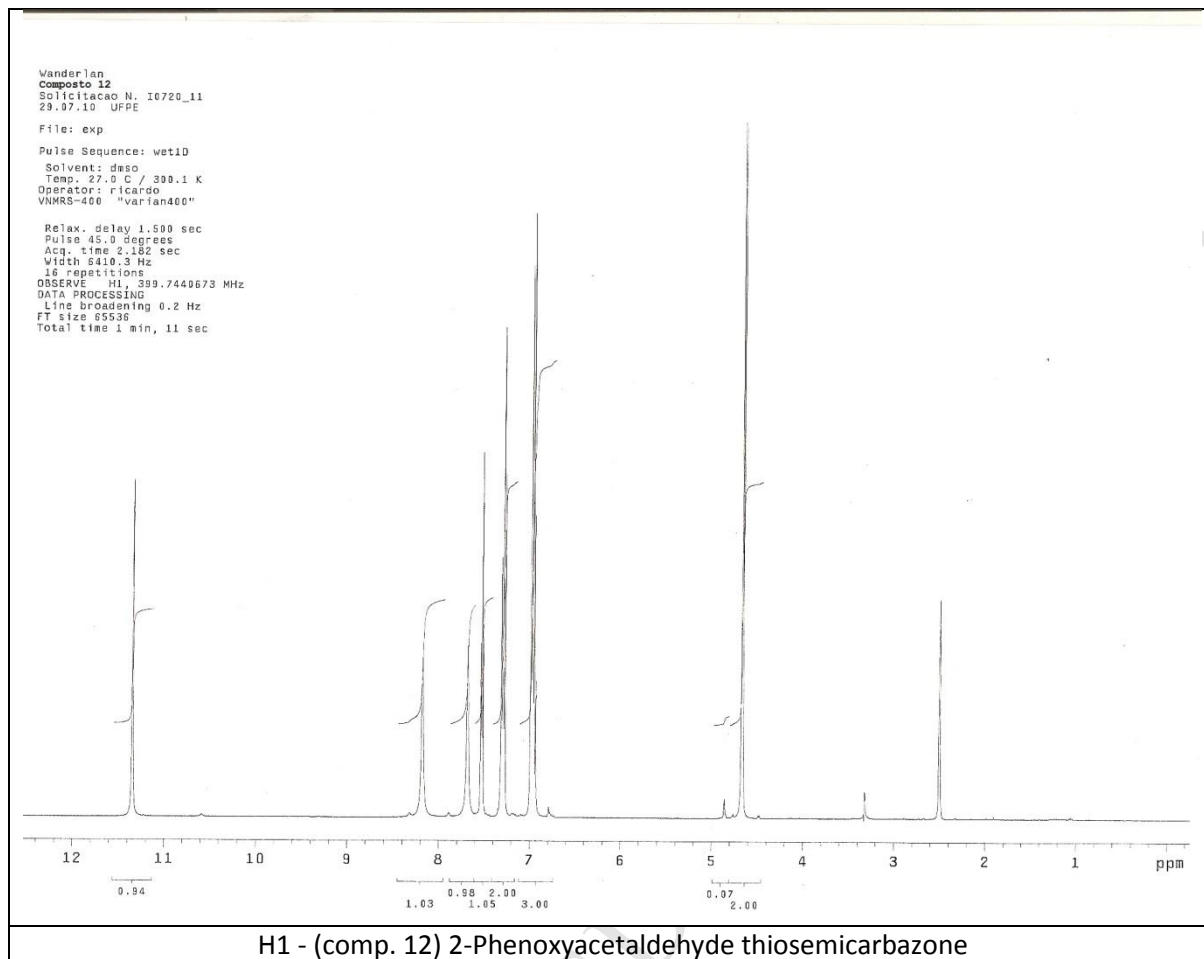
Pulse Sequence: wet10

Solvent: dmsd
Temp. 25.0 C / 298.1 K
Operator: ricardo
VNMRS-400 "varian400"

Relax. delay 1.500 sec
Pulse 45.0 degrees
Acq. time 2.182 sec
Width 6410.3 Hz
16 repetitions
OBSERVE H1, 399.7440732 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 65536
Total time 1 min, 8 sec



H1 - (comp. 11) 2-(4-Methoxyphenoxy)acetaldehyde thiosemicarbazone



Wanderlan
Composto 13
Solicitacao N. 10617_10
05.07.10 UFPE

File: exp

Pulse Sequence: wet10

Solvent: dmsd

Temp. 25.0 C / 298.1 K

Operator: ricardo

VNMRS-400 "varian400"

Relax. delay 1.500 sec

Pulse 45.0 degrees

Acq. time 2.162 sec

Width 6410.3 Hz

16 repetitions

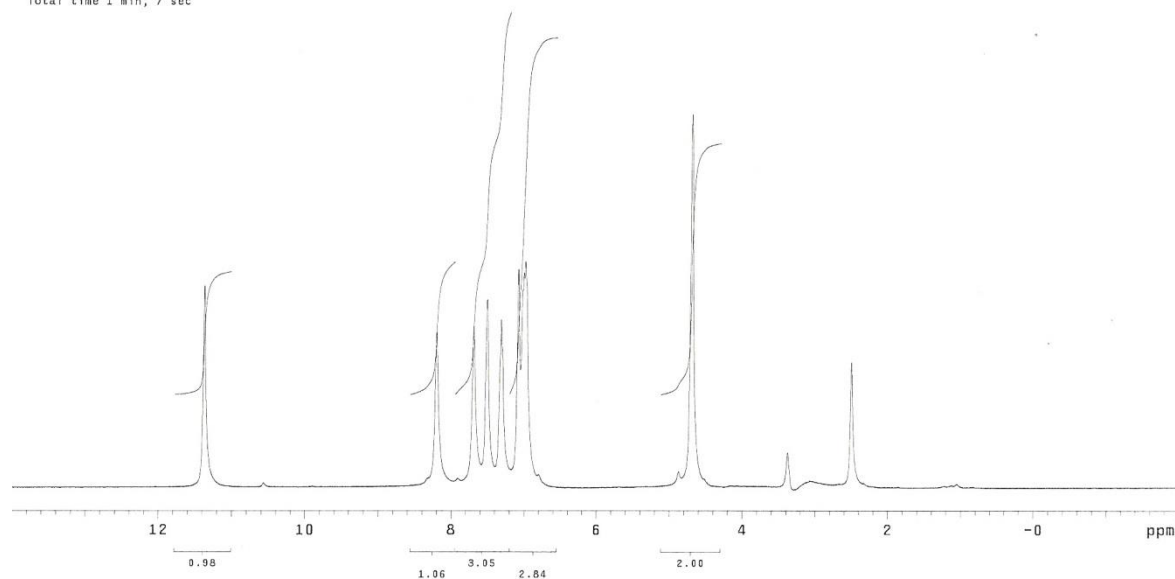
OBSERVE H1, 399.7440728 MHz

DATA PROCESSING

Line broadening 0.2 Hz

FT size 65536

Total time 1 min, 7 sec



H1 - (comp. 13) 2-(3-Chlorophenoxy)acetaldehyde thiosemicarbazone

Vanderlan
Composto 14
Solicitação N. 10617_14
30.05.10 UFPE

File: exp

Pulse Sequence: wet10

Solvent: dmsd

Temp. 25.0 C / 298.1 K

Operator: ricardo

VNMRS-400 "varian400"

Relax. delay 1.500 sec

Pulse 45.0 degrees

Acq. time 2.162 sec

Width 6410.3 Hz

15 repetitions

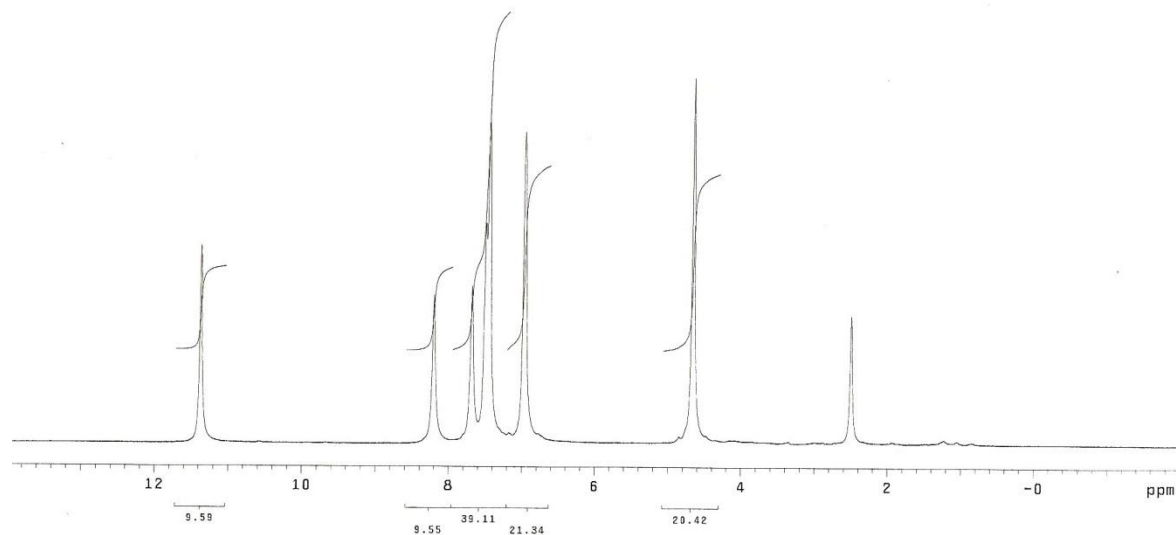
OBSERVE H1, 399.7440705 MHz

DATA PROCESSING

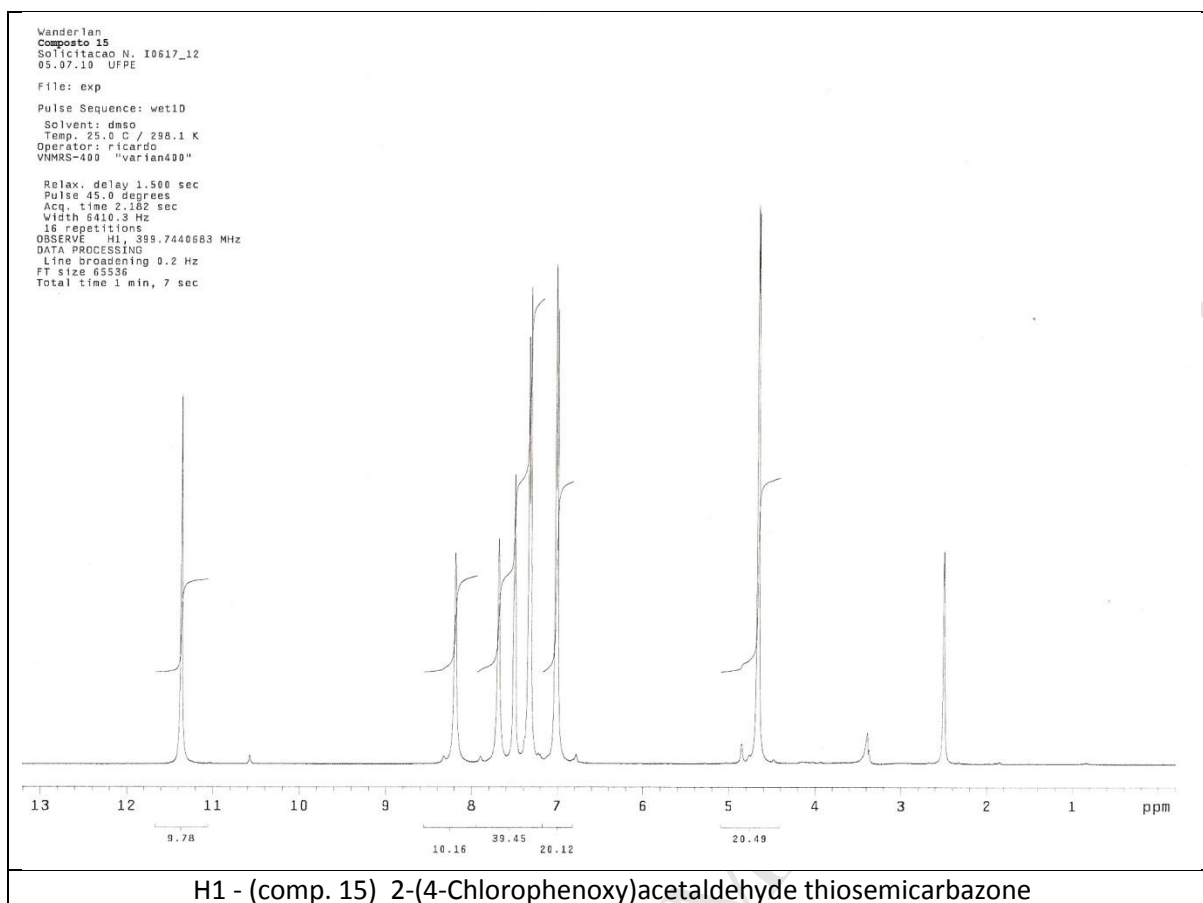
Line broadening 0.2 Hz

FT size 65536

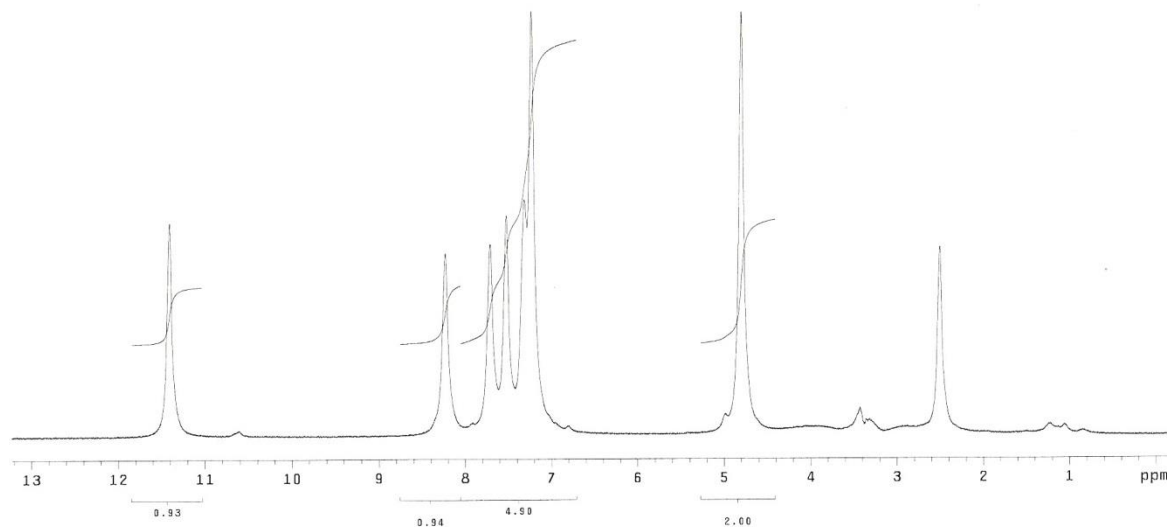
Total time 1 min, 8 sec



H1 - (comp. 14) 2-(4-Bromophenoxy)acetaldehyde thiosemicarbazone

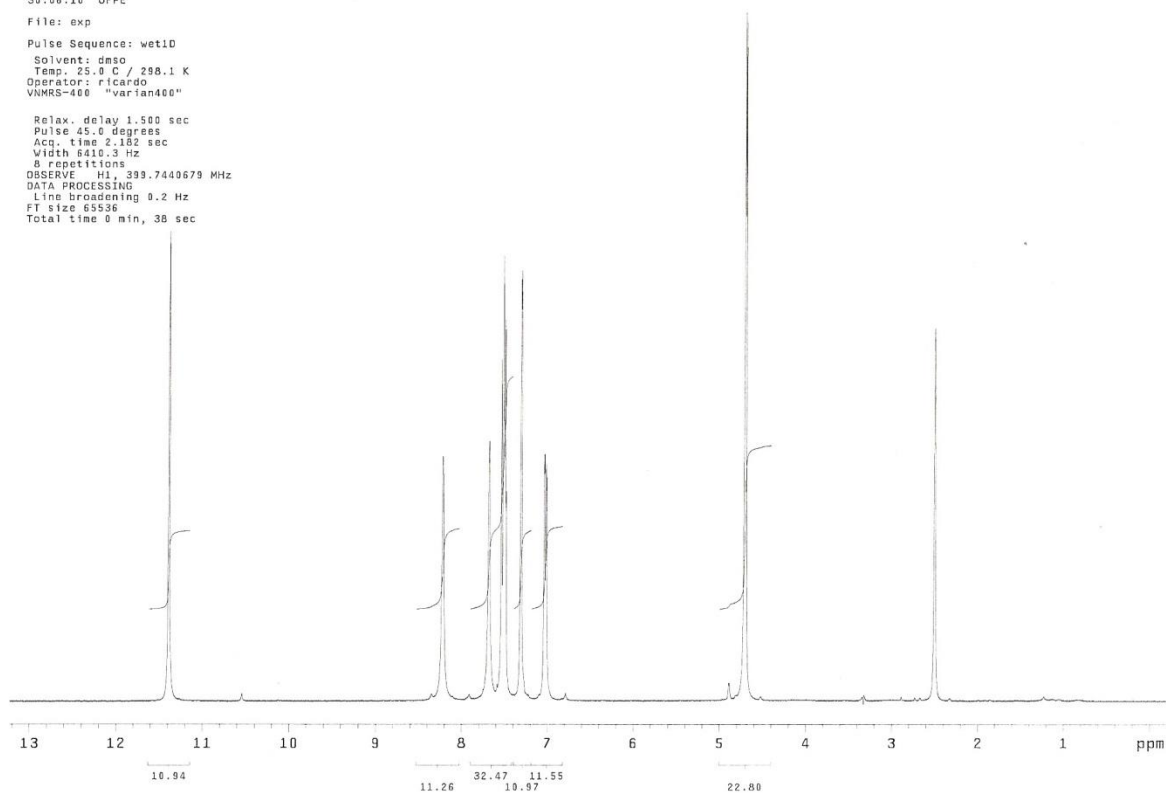


Wanderlan
Composto 16
Solicitacao N. I0617_11
05.07.10 UFPE
File: exp
Pulse Sequence: wet1D
Solvent: dmsd
Temp. 25.0 C / 298.1 K
Operator: ricardo
VNMR-400 "varian400"
Relax. delay 1.500 sec
Pulse 45.0 degrees
Acq. time 2.182 sec
Width 6410.3 Hz
16 repetitions
OBSERVE H1, 399.7440746 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 65536
Total time 1 min, 7 sec



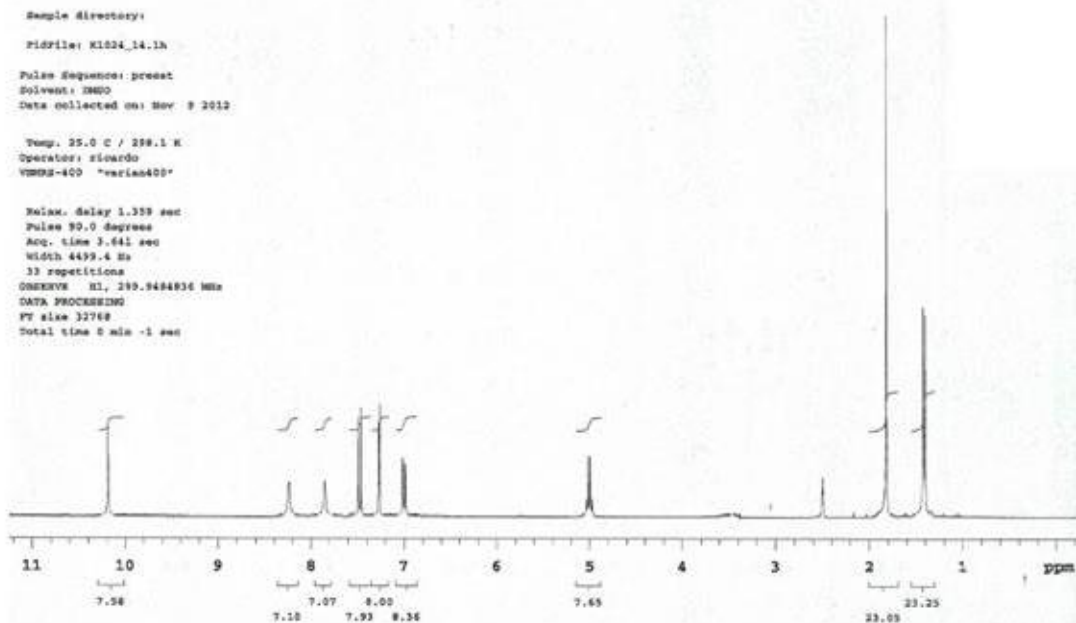
H1 - (comp. 16) 2-(2,3-Dichlorophenoxy)acetaldehyde thiosemicarbazone

Vanderlan
 Composto 17
 Sollicitacao N. 10617_13
 30.06.10 UFPE
 File: exp
 Pulse Sequence: wet10
 Solvent: dms
 Temp: 25.0 C / 298.1 K
 Operator: ricardo
 VNMR-400 "varian400"
 Relax. delay 1.500 sec
 Pulse 45.0 degrees
 Acq. time 2.182 sec
 Width 6410.3 Hz
 8 repetitions
 OBSERVE H1, 399.7440679 MHz
 DATA PROCESSING
 Line broadening 0.2 Hz
 FT size 65536
 Total time 0 min, 38 sec



H1 - (comp. 17) 2-(3,4-Dichlorophenoxy)acetaldehyde thiosemicarbazone

Ann Osuna
 Annotas DT 28
 Sollicitacao N. K1024_14
 09.11.12 UFPE
 Sample Name:
 Archive Directory:
 Sample Directory:
 FidFile: K1024_14.1h
 Pulse Sequence: preset
 Solvent: DMSO
 Data collected on: Nov 9 2012
 Temp: 25.0 C / 298.1 K
 Operator: ricardo
 VNMR-400 "varian400"
 Relax. delay 1.359 sec
 Pulse 90.0 degrees
 Acq. time 3.641 sec
 Width 6499.4 Hz
 33 repetitions
 OBSERVE H1, 299.9484836 MHz
 DATA PROCESSING
 FT size 32768
 Total time 0 min -1 sec



H1 - (comp. 18) 3-(3,4-Dichlorophenoxy)butan-2-one thiosemicarbazone

ACCEPTED MANUSCRIPT