Improved Microwave-Mediated Synthesis of 3-(3-Aryl-1,2,4-oxadiazol-5-yl)propionic Acids and Their Larvicidal and Fungal Growth Inhibitory Properties

Ricardo Antonio Wanderley Neves Filho,[a] Cecília Aguiar da Silva,[a] Clécia Sipriano Borges da Silva,[a] Vanessa Passos Brustein,[b] Daniela Maria do Amaral Ferraz Navarro,*,[a] Fábio André Brayner dos Santos,[c,d] Luiz Carlos Alves,[c,d] Marília Gabriela dos Santos Cavalcanti,[c,d] Rajendra Mohan Srivastava,*,[a] and Maria das Graças Carneiro-Da-Cunha[b]

[a] *Departamento de Química Fundamental, Universidade Federal de Pernambuco; 50745–540 Recife, PE, Brazil:* [b] *Departamento de Bioquímica, Universidade Federal de Pernambuco; 50670–901 Recife, PE, Brazil:* [c] *Departamento de Parasitologia, Centro de Pesquisas Aggeu Magalhães (CPqAM/FIOCRUZ); 50.670–420 Recife, PE, Brazil: and* [d] *Laboratório de Imunopatologia Prof. Keizo Asami (LIKA) da Universidade Federal de Pernambuco; 50.670–420 Recife, PE, Brazil.* Received March 20, 2009; accepted May 7, 2009; published online May 14, 2009

The synthesis of 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids from arylamidoximes and succinic anhydride under focused microwave irradiation conditions is described. The new synthetic method furnished the desired products in 2—3 min and good yields. Furthermore, the previously complicated purification procedure has been simplified in a manner which is quick, eco-friendly and cost-effective. Larvicidal bioassay and fungal growth inhibitory tests were performed using several 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids. These acids presented strong larvicidal activity against L4 larvae of *Aedes aegypti*. The results suggest that larvicidal activity might be correlated with the presence of electron-withdrawing substituents in the *para* position of the phenyl ring except the fluorine atom. The alterations observed in the larvae spiracular valves of the siphon and anal papillae by 1,2,4-oxadiazoles in the larvicidal bioassay are responsible for larvae's death. Furthermore, all acids inhibited the fungal growth of five different types of fungi, *viz.*, *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *F. decemcellulare* and *F. lateritium* in a preliminary evaluation. Both of these activities are being disclosed for the first time for 1,2,4-oxadiazole-5-yl ring linked at C-3 of propionic acid.

Key words 1,2,4-oxadiazole; propionic acid; larvicide; *Aedes aegypti*; antifungal activity

Mosquitoes are vectors for a large number of diseases, including filariasis (transmitted by *Culex quinquefasciatus*), malaria (transmitted by *Anopheles gambiae*) dengue fever and yellow fever (transmitted by *Aedes aegypti*). Dengue fever may be as significant as diseases such as malaria and tuberculosis, posing economic burdens on the communities and governments. This disease occurs in tropical and subtropical areas due to the favorable environment these regions offer for the development of the transmitter mosquito of the arbovirus from the genus *Flavivirus*.[1] Dengue virus infection can cause the classic form of the disease, debilitating a worker for weeks, or the hemorrhagic form, which leads to death in many cases. Annual epidemics of dengue fever have been advancing in the Americas and to our knowledge there is no vaccine for this illness.[2]

One way to decrease the incidence of this disease is through the eradication of *Aedes aegypti*. Mosquito control involves integrated pest management programs that include larvicides, surveillance, source reduction, control of adult mosquitoes (adulticide), biological control and public awareness campaigns.[3,4] These integrated management actions focus on diminishing vector and larvicides are an important part of eliminating the mosquito in the larval stage. The use of a larvicide disrupts the evolutionary cycle of the mosquito vector, thereby promoting the interruption of virus transmission. The most often employed larvicide for *Aedes aegypti* are temephos and toxins of *Bacillus thuringiensis israelensis*.[5,6] Resistance is the ability of an insect population to tolerate a dose of insecticide that would cause death under normal circumstances. The literature has described the resistance of mosquitoes to temephos chemical compound.[7] Thus, the search for new larval insecticides has been the focus of a large number of publications addressing the use of essential oils[8—12] and plant extracts.[13—21]

Fungi are non-photosynthetic organisms which grow by forming a mass of entwined and branched filaments called micelia.[22] Many fungi cause diseases in plants leading to economical losses and health problems to the affected communities. The infection promoted by plant pathogens such as species of *Fusarium* do not cause only reduction in food quality, but also contamination with mycotoxins, which are hazardous to animal and human health.[23] In particular, fungi like *Fusarium oxyporum*, *F. solani* and *F. moniliforme*, besides attacking plants' roots also promote systemic infection in immunosuppressed patients.[22] Furthermore, *F. oxysporum* is a soilborne fungus that causes damage to a wide variety of crops and has also been reported as an opportunistic human pathogen.[24] In 2001 the antifungal properties of two 1,2,4-oxadiazoles have been described.[25] Although, these compounds inhibited mycelial growth of *Trychophyton mentagrophytes*, no evaluation has been done against *Fusarium* species.

There are no reports in the literature about the larvicidal activity of 1,2,4-oxadiazole containing compounds, but one report cites the fungicidal property of 1,2,4-oxadiazoles.[25] This is an important class of heterocycle with a large number of biological activities. To date, four literature reviews have addressed the synthesis and biological studies on these

* To whom correspondence should be addressed. e-mail: rms_indu@yahoo.com

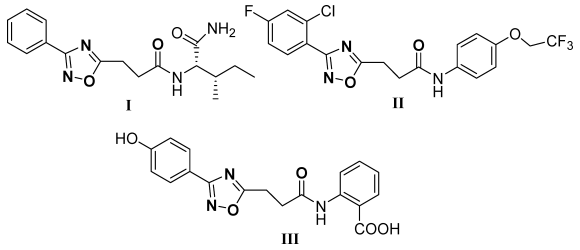

Fig. 1. 1,2,4-Oxadiazoles Possessing Biological Properties

rings.[26—29] Many molecules containing a 1,2,4-oxadiazole moiety exhibit interesting pharmacological properties, such as analgesic,[30] anti-inflammatory,[31] anti-convulsant,[32] anti-tumor,[33] anti-kinetoplastid,[34] HPTPβ inhibitors,[35] human βII-tryptase inhibitors[36] and selective $\alpha v\beta 3$ receptor antagonists.[37] 3-(3-Aryl-1,2,4-oxadiazol-5-yl)propionic acids have exhibited pharmaceutical properties with no mutagenic activity, as determined by the Ames and SOS Chromotest.[38] This makes such compounds an interesting area for further pesticide activity studies. Recently, these compounds have also been used as building blocks for the synthesis of peptidomimetics I,[38] cannabinoid receptor 2 (CB2) agonist II,[39] and niacin receptor agonist III (Fig. 1).[40]

The first synthesis of 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids was achieved by the reaction of amidoximes and succinic anhydride in refluxing toluene or 1,4-dioxane.[41] Later, these reagents were heated in a household microwave oven under solvent-free conditions for 10 min[42] with better yields in comparison with the solution method. It is now well established that focused microwave reactors are quite superior to the domestic ones due to their ability of focusing energy on the sample producing a faster heating of the reagents.[43] The oven which we used in this work is equipped with infrared and pressure sensors, which improves the reliability of the reaction.

The aim of the present study was to improve the synthesis of 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids by employing focused microwave irradiation (FMW) with a quick, eco-friendly and low-cost method of purification and to determine their larvicidal and fungicidal properties. Therefore, this work will be a novel addition in the literature and should be the subject of further research.

Results and Discussion

Chemistry Initially, we tried to repeat the reaction of arylamidoximes and succinic anhydride to synthesize 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids at 120 °C in a domestic microwave oven without any solvent. As reported in the literature, the above-cited acids were obtained in good yields in 10 min.[42]

Since these compounds are gaining more and more significance, we decided to improve their microwave-accelerated synthesis under solventless conditions. With this aim, we performed a few experiments using a specific focused microwave oven. First, the reaction was carried out at 120 °C using an equimolar quantity of amidoxime **1g** and succinic anhydride **2** for 2 min under stirring. This experiment did not produce any result. Next, we repeated the reaction under the same conditions as described but by adding 3 drops of DMF/mmol of **1** and increasing the time from 2 to 3 mins.

Table 1. Conditions for the F.MW. Reaction of **1g** and **2**

Entry	Temperature (°C)	Time (min)	Stirring	Yields (%)[b]
1[a]	120	2	On	N.R.
2	120	3	On	21
3	140	3	On	74
4[a]	140	3	On	25
5	140	2	On	76
6	140	2	Off	66
7[c]	140	3	On	77
8	150	1	On	62[d]

a) Experiment conducted without addition of DMF. *b*) Isolated yields. *c*) Experiment conducted at 10 mmol scale. *d*) The reaction mixture becomes a little red and we observed decomposition by TLC.

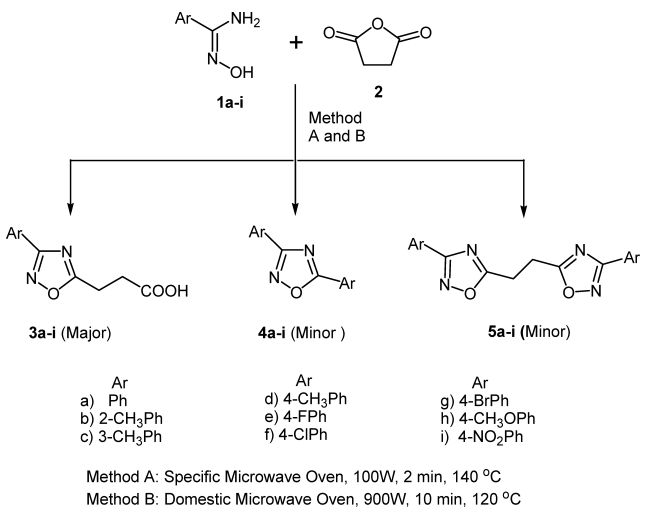

Chart 1

This experiment gave the acid in 21% yield. Encouraged with this, the temperature was raised to 140 °C while maintaining other conditions the same. Here, the result was satisfactory and the yield was 74%. In fact, the same result can be obtained in 2 min. It is noteworthy that unstirred reaction causes a decrease in yields (Table 1). On a 10 mmol scale, the reaction works well and yields 77% of the pure acid.

It is known that the microwave-induced reaction of a suitable arylamidoxime **1** with succinic anhydride **2** under solvent free conditions furnished the desired 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids **3a**—**i** as the major products along with two minor substances **4a**—**i** and **5a**—**i** (Chart 1). In fact, compounds **3a**—**i** can be crystallized and recrystallized to remove **4a**—**i** and **5a**—**i**, but it is tedious and the final yields of the desired acids are low as has been found earlier by our group. In order to get the best yield of these acids, we have chromatographed the crude mixture over silica gel. This way the yield was excellent,[41,42] but this procedure is expensive and time-consuming. In this work we isolated compounds **4a**—**i** and **5a**—**i**, respectively. The combined yields of these heterocycles were very low (*ca.* 10%). Their structures were verified using spectroscopic techniques (IR and NMR spectra) including the elemental analyses.

In the present article we wish to emphasize that the synthetic procedure has been scaled-up (from milligrams to a couple of grams quantity for both focused and unfocused microwave techniques) with remarkable results. In addition, we

have been able to eliminate the chromatographic purification procedure, thus getting rid of the expensive adsorbants and solvents. The new purification strategy involves the direct transformation of the carboxylic acids to their sodium salts, which become water soluble followed by the cautious addition of an aqueous citric acid solution to precipitate the desired acids. These acids are sufficiently pure and further crystallization becomes quite easy and the yields are high (Table 2).

The focused microwave (FMW) methodology followed by a practical procedure of purification provides the access to 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids **3a**—**i** with high purity in shorter reaction time and better yields in comparison with the ones published previously using household microwave oven or conventional heating (Table 2).

Larvicidal Bioassay According to Cheng *et al.*,[44] essential oils or plant extracts with LC_{50} values <100 ppm should be considered active in larvicidal bioassays. All compounds reported in the present work were tested against fourth instar *Aedes aegypti* larvae and exhibited larvicidal activity below 100 ppm (Table 3). 3-(3-Phenyl-1,2,4-oxadiazol-5-yl)propionic acid (**3a**) has been found to have the highest LC_{50} concentration among the compounds studied. The introduction of a substituent group in the *orto*, *meta* or *para* positions of the phenyl ring attached on C-3 of these 1,2,4-oxadiazoles led to a decrease in LC_{50} values in *Aedes aegypti* larvicidal bioassays. The acids containing $-CH_3$ (*meta*, **3c**) achieved a lower LC_{50} (63.8 ppm) value when compared to compounds containing the same substituent in the *ortho* (**3b**) ($LC_{50}=70.9$ ppm) and *para* (**3d**) ($LC_{50}=65.8$ ppm) positions. The MeO-substituent in the *para* position of the phenyl ring (**3d**) had the similar value of LC_{50} (71.5 ppm) as that of **3b**. The electron-withdrawing substituents-Cl (**3f**), -Br (**3g**) and NO_2 (**3i**) in the *para* position significantly decreased LC_{50} values. Substitution of the fluorine atom at *para* position in the phenyl ring (**3e**) was an exception in LC_{50} behavior, because of its resonance with the aromatic ring, thus achieving the highest value among electron-withdrawing groups. We observed a relationship between LC_{50} values and substituent electronegativity properties: LC_{50} F **3e**$>LC_{50}$ Cl **3f**$>LC_{50}$ Br **3g**. Halogenated compounds **3g** ($LC_{50}=15.2$ ppm) and **3f** ($LC_{50}=28.1$ ppm) may be considered highly active (LC_{50} values <50 ppm) and these LC_{50} values are similar to the activity of essential oils obtained from the leaf, stem and inflorescence of *Piper marginatum* ($LC_{50}=19.9$—23.8 ppm)[42] as well as the leaf of *Cryptomeria japonica* D. Don ($LC_{50}=37.6$ ppm).[45] Moreover the best LC_{50} values for 1,2,4-oxadiazole are comparable to the larvicidal effect of β-asarone, natural botanical insecticide, which have exhibited an LC_{100} of 16 ppm.[46,47]

The quantitative structure–activity relationship (QSAR) study of larvicidal activity of eight substituted benzoylphenylbiurets against the *Aedes aegypti* larvae demonstrated a presence of strong electron withdrawing and high lipophilicity of the *para*-substituent on the phenyl ring was required for high larvicidal effect of the biuret and urea compounds.[48] The relationship between mosquito repellent activity and chemical structure of 31 amide analogues of *N*,*N*-diethyl-*m*-toluamide and *N*,*N*-diethylphenylacetamide investigated by Katritzky *et al.*[49] demonstrated that the most active compounds seem to be amides with one substituent (CH_3 or Cl) in the aromatic ring. The data presented in these articles corroborate with the previous results. Our observations suggest that larvicidal activity might be correlated with the presence of electron-withdrawing substituents in the *para* position in the phenyl group.

Some 1,2,4-oxadiazoles have exhibited insecticidal and pesticide properties.[50,51] These properties have been reported for 1,3,4-oxadiazoles-containing compounds as well.[52—55] In order to demonstrate the importance of 1,2,4-oxadiazole ring on the larvicidal property we have tested the isomer of compound **3a**, 3-(3-phenyl-1,3,4-oxadiazol-5-yl)propionic acid **6**. This compound displayed no larvicidal effect even at 100 ppm. Thus, this lack of activity might be related with the no presence of 1,2,4-oxadizole ring.

Furthermore, these parameters were involved in explaining the relationship between larvicidal activity and chemical structure. All bioassay results discussed here can play an important role in larvicidal activity against *Aedes aegypti*.

Mode of Action The stage L4 mosquito larvae of the *Aedes aegypti* were exposed to 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids in the larvicide bioassays where considerable change in their movements has been observed followed by paralysis between 5 and 8 h after being placed in the test solution. Similarly, Chaithong *et al.*[56] found excitation, restlessness, tremors and convulsions, followed by paralysis during the first 2 h of exposure to ethanol extracts from three species of *Piper* [*P. logum* (fruit), *P. ribesoides* (wood) and *P. sarmentosum* (whole plant)] in CL_{99} concentrations. The number of dead and dying larvae increased significantly from 2 to 7 h after starting the bioassays, but death to all larvae only occurred after 24 h of exposure. The authors report that

Table 2. Synthesis of 3-(3-Aryl-1,2,4-oxadiazol-5-yl)propionic Acids **3a**—**i**

Compound	Yield (%) Method A	Yield (%) Method B	Yield (%) lit.[42]	mp (°C)	mp (°C) lit.[41,42]
3a	73	68.0	73.3	119—120	119—120
3b	60	57.0	25.0	101—102	102
3c	74	71.0	76.4	100—101	101—102
3d	76	68.0	67.7	144—145	145
3e	78	72.0	—	146—147	—
3f	89	84.0	83.1	153—154	153—154
3g	77	68.0	76.5	157	157—158
3h	82	71.0	—	138—139	138—139
3i	84	76.0	—	134—135	—

Table 3. Larvicidal Activity of 1,2,4-Oxadiazoles **3a**—**i** and 1,3,4-Oxadiazole **6**

Compound	LC_{50} (ppm)	S.D.	Concentration range
3a	98.6	2.9	70—130
3b	70.9	2.5	50—90
3c	63.8	2.9	50—90
3d	65.8	3.2	57—73
3e	81.2	1.3	70—95
3f	28.1	1.9	22—36
3g	15.2	0.8	13—25
3h	71.5	1.5	65—76
3i	50.5	2.4	47—60
6	N.A.[a]	—	>100

a) Non-active compound.

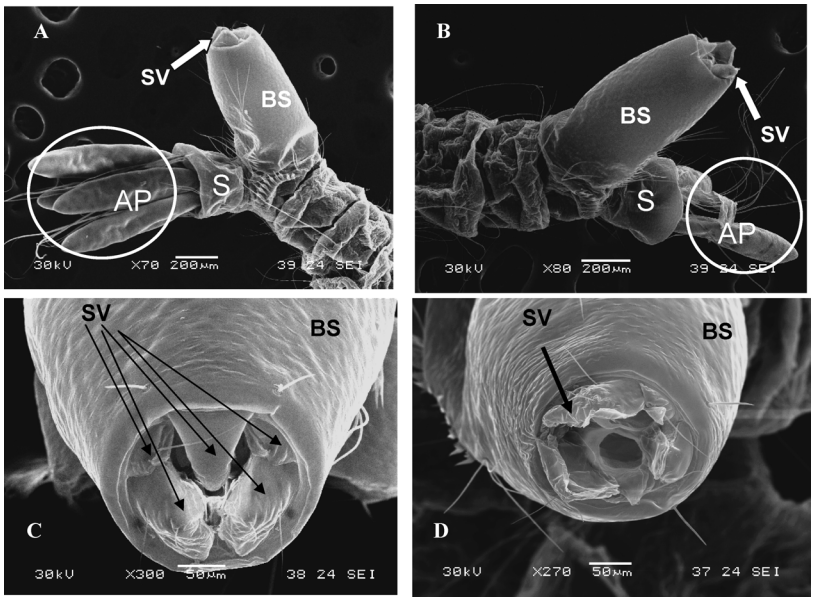

Fig. 2. (A, C) *Aedes aegypti* Larvae after 8 h of Exposure to Control 2 and (B, D) *Aedes aegypti* Larvae after 8 h of Exposure to **3g**
Observe larvae organs: breathing siphon (BS), spiracular valves (SV), anal papillae (AP), and saddle (S).

the symptoms that preceded larval death is correlated to the toxic effect that reaches the neuromuscular system of the larvae. The authors also report that scanning electron microscopy (SEM) revealed that only the anal papillae underwent morphological changes in the larvae treated with the plant extracts.

The *Piper* ethanol extracts treatment caused external destruction, with extensive damage and a shrunken cuticle of the anal papillae, organ responsible for regulation of electrolyte levels in larva body.

SEM of the L4 stage *Ae. aegypti* larvae exposed to distilled water (Control 1), co-solvent water solution (Control 2) and co-solvent water solution of 3-(3-*p*-bromophenyl-1,2,4-oxadiazol-5-yl)-propionic acid **3g** (treatment) revealed morphological changes in the anal papillae and the spiracular valves of the siphon. Scanning electron micrographs on the body of the larvae exposed to Controls 1 and 2 revealed no morphological changes in the breathing siphon, saddle, anal papillae, and thorax or abdomen segment.

When the larvae were submitted to the 1,2,4-oxadiazoles **3a**—**i** at concentrations capable of killing 50% or 100% of the larvae, there were alterations in the spiracular valves of the siphon (Figs. 2B, D) and a loss of anal papillae (Fig. 2B).

The larvae use a breathing siphon to obtain oxygen and must periodically come to the surface to do so. The tracheal system is correlated with breathing siphon and morphological alterations in the spiracular valves of the siphon may be responsible for the flooding of the tracheal system when the larvae submerge, thereby causing death. According to Corbet *et al.*,[57] physical flooding of the tracheal system in mosquito larvae can be promoted by oils and others surface films as larvicidal.

The loss of anal papillae also leads the larva to collapse due to the lack of one of the major sites of salt regulation in this species.[58,59] No other paper published thus far has determined morphological alterations using scanning electron microscopy on the spiracular valves of the siphon in mosquito larvae following treatment with potential larval insecticides.

Preliminary Antifungal Assay All synthesized compounds **3a**—**i** presented fungal growth inhibition for at least one of the tested species. Nevertheless, compounds **3h**, **3i**, **3f** and **3e** inhibited the growth of five microorganisms during 72 h (Fig. 3). Compound **3h** was the most effective against *F. decemcellulare* and *F. lateritium* exhibiting 52.8±3.3% and 45.8±1.6% inhibition, respectively. Product **3f** presented the best inhibitory effect against *F. solani* and *F. decemcellulare*, displaying respectively 59.7±2.2% and 66.0±0.0% of inhibition. For *F. moniliforme*, growth inhibition was observed at 48 h time for all the tested compounds. Compound **3a** was the most effective one against *F. moniliforme* exhibiting 52.8±2.8% of inhibition. Although all 1,2,4-oxadiazoles **3a**—**i** inhibited the growth of *F. moniliforme* for 48 h while **3a** was the only one capable to keep this inhibitory effect for 72 h (52.8±2.8%). This observation contradicts the expected results as antimicrobials usually show a greater killing effect over time. As experimental errors are not expected to have occurred (the applied procedure was repeated and was similar for all compounds), one possible explanation is that *F. moniliforme* became resistant to compounds **3b** to **3i** and the first 48 h corresponded to microbial growth lag phase. For fungus *F. lateritium* the greatest inhibition (43.0±1.6%) was found for acid **3e**.

Interestingly, **3i** was not the most active of the series, though it contains the NO_2 group. Nitro compounds are well known for its well related toxicity in live organisms.[59] Many fungicides assign their inhibitory effect to the presence of this group.[60] Our results provide an evidence that the toxicity of compounds **3a**—**i** is not due to the presence of NO_2 moiety. Therefore, these compounds can be used against these fungi without adverse effect provoked by the nitro group.

Conclusion

In summary, we have succeeded in scaling-up the microwave-induced synthesis of a series of nine 3-(3-aryl-1,2,4-

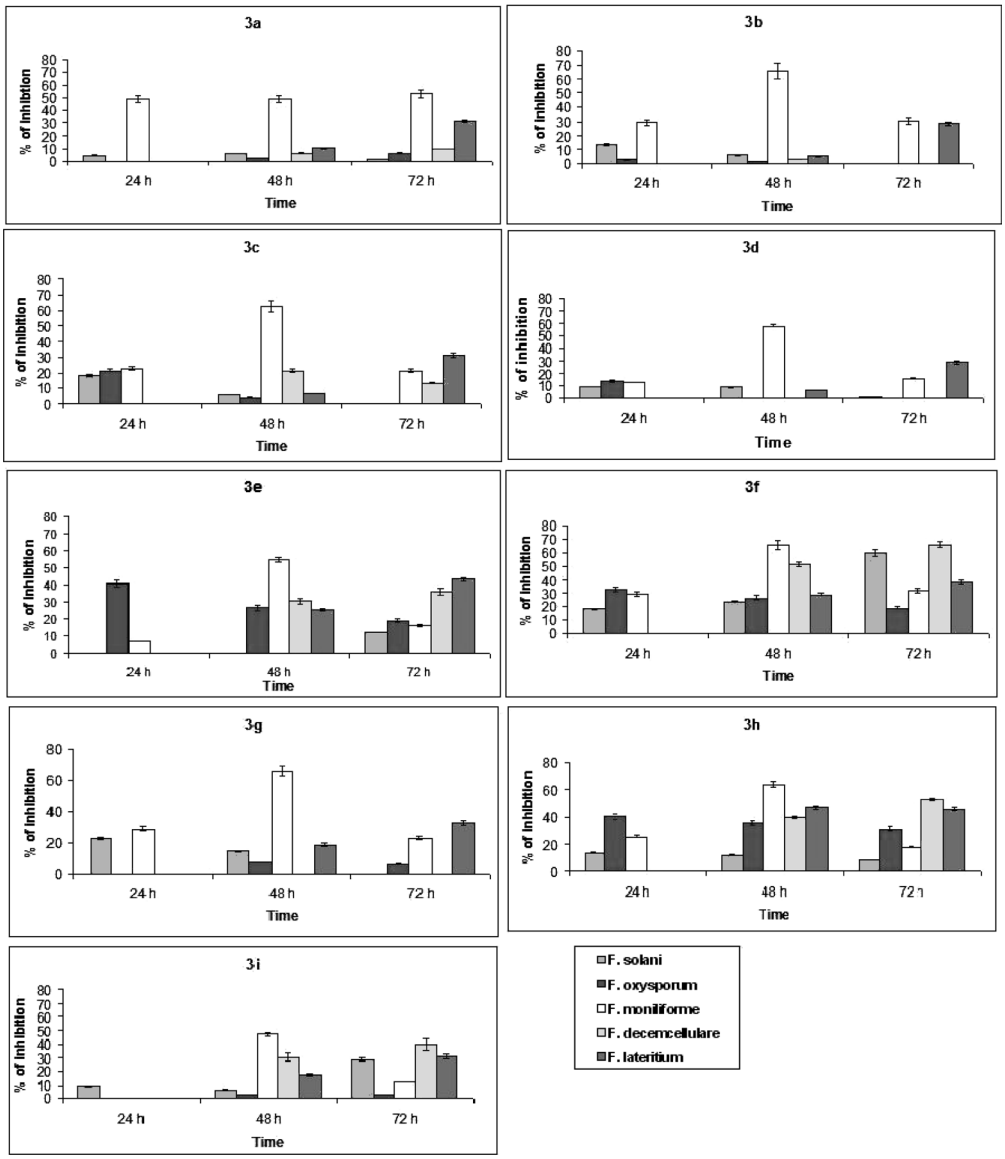

Fig. 3. Fungal Growth Inhibition from Compounds **3a**—**i**

oxadiazol-5-yl)propionic acids. A new synthetic method followed by an eco-friendly purification strategy allows us to get the target compounds in good yields after simple work-up. All compounds presented strong larvicidal activity against L4 larvae of *Aedes aegypti*. The death of larvae may be provoked by alterations in spiracular valves of the breath siphon and loss of anal papillae as revealed by SEM studies. Finally the 1,2,4-oxadiazole-based propionic acids **3a**—**i** inhibited the fungal growth of five different species of *Fusarium*.

Experimental

General Experimental Procedures All reagents were obtained from commercial sources and used without further purification. Infrared spectra were recorded on a Perkin-Elmer model 283 spectrometer in KBr discs. ^{1}H-NMR spectra were obtained with a Varian 300-MHz instrument using tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were recorded on a Varian 75-MHz spectrometer. Elemental analysis was performed with a Carlo Erba instrument model E-1110. The microwave experiments were conducted in a CEM-Discover microwave reactor or in a Sanyo domestic microwave oven model EM-300B (220v/650W/2450 MHz).

The 3-(3-phenyl-1,3,4-oxadiazol-5-yl)-propionic acid **6** was synthesized following the procedure reported earlier.[61]

Synthesis. Experimental Procedure Method A: A suitable amidoxime **1a**—**i** (1.0 mmol) was allowed to react with succinic anhydride **2** (1.2 mmol) in a 10 ml sealed vessel followed by microwave irradiation in a CEM-Discover microwave reactor.[62,63] The crude product was dissolved in ethyl acetate (20 ml) and then a concentrated solution of $NaHCO_3$ (50 ml) was added, the mixture was stirred in a 100 ml round botton flask overnight. The aqueous layer was separated and then a concentrated solution of citric acid was added until total precipitation of the desired compounds **3a**—**i**. The obtained product was dissolved in chloroform, dried over anhydrous Na_2SO_4 and re-crystallized in above mentioned solvent to afford pure compounds **3a**—**i**.

Method B: A suitable amidoxime **1a**—**i** (22.0 mmol) was allowed to react with succinic anhydride **2** (22.2 mmol) for 10 min under solvent free conditions in a domestic microwave oven.[41,42] The crude product was dissolved in ethyl acetate (30 ml), then a concentrated aqueous solution of $NaHCO_3$ (35 ml) was added to it, and the mixture was stirred overnight. The aqueous layer was separated followed by the addition of concentrated aqueous citric acid solution until total precipitation of the desired compound **3a**—**i** occurred. The precipitate was dissolved in chloroform, dried over anhydrous Na_2SO_4 and re-crystallized from above mentioned solvent to afford pure compounds **3a**—**i**.

3-(3-Phenyl-1,2,4-oxadiazol-5-yl)-propionic Acid (**3a**): Colorless crystals from chloroform, mp 119—120 °C (Method A: 73%; Method B: 68%); lit.[42]

mp 119—120 °C. (73.3%) IR and ^{1}H-NMR spectra gave the same absorptions as reported earlier.[41]

3-(3-*o*-Tolyl-1,2,4-oxadiazol-5-yl)-propionic Acid (**3b**): Colorless crystals from chloroform, mp 101—102 °C (Method A: 60%; Method B: 57%); lit.[42] mp 102 °C. (25.0%) IR and ^{1}H-NMR spectra agreed with the proposed structure.[41]

3-(3-*m*-Tolyl-1,2,4-oxadiazol-5-yl)-propionic Acid (**3c**): Colorless crystals from chloroform, mp 100—101 °C (Method A: 74%; Method B: 71%); lit.[42] mp 101—102 °C. (76.4%) IR and ^{1}H-NMR spectra gave the same absorptions as reported earlier.[41]

3-(3-*p*-Tolyl-1,2,4-oxadiazol-5-yl)-propionic Acid (**3d**): Colorless crystals from chloroform, mp 144—145 °C (Method A: 76%; Method B: 68.0%); lit.[41] mp 145 °C. (67.7%) IR and ^{1}H-NMR spectra gave the same absorptions as reported earlier.[41]

3-[3-(4-Fluorophenyl)-1,2,4-oxadiazol-5-yl]-propionic Acid (**3e**): Colorless crystals from chloroform, mp 146—147 °C. (Method A: 78%; Method B: 72.0%) IR (KBr) cm^{-1}: 2929, 1707. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.00 (2H, t, J=7.2 Hz), 3.25 (2H, t, J=7.2 Hz), 7.52 (2H, m), 8.05 (2H, m). *Anal.* Calcd for $C_{11}H_9FN_2O_3$: C, 55.93; N, 11.86; H, 3.84. Found: C, 55.80; N, 12.05; H, 3.66.

3-[3-(4-Chlorophenyl)-1,2,4-oxadiazol-5-yl]-propionic Acid (**3f**): Colorless crystals from chloroform, mp 153—154 °C (Method A: 89%; Method B: 84.0%); lit.[41] mp 153—154 °C. (83.1%) IR and ^{1}H-NMR spectra gave the same absorptions as reported earlier.[41]

3-[3-(4-Bromophenyl)-1,2,4-oxadiazol-5-yl]-propionic Acid (**3g**): Colorless crystals from chloroform, mp 157 °C (Method A: 77%; Method B: 68.0%); lit.[41] mp 157—158 °C. (76.5%) IR and ^{1}H-NMR spectra gave the same absorptions as reported earlier.[41]

3-[3-(4-Methoxyphenyl)-1,2,4-oxadiazol-5-yl]-propionic Acid (**3h**): Colorless crystals from chloroform, mp 138—139 °C (Method A: 82%; Method B: 71.0%); lit.[41] mp 138—139 °C. IR and ^{1}H-NMR spectra gave the same absorptions as reported earlier.[41]

3-[3-(4-Nitrophenyl)-1,2,4-oxadiazol-5-yl]-propionic Acid (**3i**): Yellow crystals from chloroform, mp 134—135 °C (Method A: 84%; Method B: 76.0%); IR (KBr) cm^{-1}: 3099 1718. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.04 (2H, t, J=7.2 Hz), 3.29 (2H, t, J=7.2 Hz), 8.27 (2H, d, J=8.7 Hz), 8.32 (2H, d, J=8.7 Hz). *Anal.* Calcd for $C_{11}H_9N_3O_5$: C, 50.20; N, 15.96; H, 3.45. Found: C, 49.95% N, 16.01% H, 3.26%.

Larvicidal Bioassay The larvicidal activity of 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids were evaluated using an adaptation of the method recommended by the World Health Organization.[64,65] A stock solution (150 ppm) was prepared by diluting 0.0750 g of 33-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acids in 7 ml of ethanol (analytical grade) or 20 drops of Tween 80, and completing to a volume of 500 ml with distilled water. In order to test the effect of the 1,2,4-oxadiazoles on the survival of larvae, fourth instar *Ae. aegypti* were added to the beakers (20 larvae per beaker) containing the 3-(3-aryl-1,2,4-oxadiazol-5-yl)propionic acid solutions in a range of concentrations obtained by appropriate dilution of the stock solution with distilled water. Four replicate assays were carried out for every sample concentration. A negative control was included for each assay, using distilled water containing the same amount of co-solvent as the test sample. Mortality of the larvae was determined after 48 h of incubation at 28±2 °C. Larvae were considered dead when not responding to stimuli or not rising to the surface of the solution. Lethal concentration LC_{50} values were calculated through probity analysis using the Status Plus 2006 software program.

Scanning Electron Microscopy The larvae were placed in a glutaraldehyde (2.5%) and *p*-formaldehyde (4%) solution with a sodium cacodylate buffer (0.1 M), pH 7.2, for 12 h at room temperature. The larvae were washed with the buffer solution and fixed in OsO_4 (1%) in the same buffer solution for 1 h. The larvae were dehydrated in an ethanol series, submitted to critical point drying in carbon dioxide. The fixed larvae were coated with gold using a sputter coater and observed under a scanning electron microscope (JEOL-JSM 5600LV).

Antifungal Activity *Fusarium solani* (URM-2480), *F. oxysporum* (URM-2489), *F. moniliforme* (URM-3226), *F. decemcellulare* (URM-3006) and *F. lateritium* (URM-2491) were obtained from Culture Collections of Mycology Department from Federal University of Pernambuco, Brazil.

Fungi were grown at 28 °C on Potato Dextrose Agar (PDA) plates for an 8-to-15-d period until the surface of plates were completely covered by them. Afterwards, fungal mycelium disks with 0.6 mm diameter were removed from peripheral part of the colonies. To test the inhibitory effect of each compound, 50 μl of the compound aqueous solution at a concentration of 2 μg/ml were placed in the centre of each PDA plate and spreaded. This was followed by the placement of the mycelium disk in the centre of each plate. Ethyleneglicol was used as negative control, and the plates were incubated at 28 °C for 72 h. All experiments were performed in triplicate. Antifungal activity was determined by the reduction of fungus growth diameter (ϕ) and the percentage of inhibition (I%) was calculated as follows:

$$I\% = \frac{\phi \text{ of negative control} - \phi \text{ of sample}}{\phi \text{ of negative control}} \times 100 \quad (1)$$

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