



Larvicidal isoxazoles: Synthesis and their effective susceptibility towards *Aedes aegypti* larvae

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ABSTRACT

Twenty 3,5-disubstituted isoxazoles have been synthesized and tested against fourth instar *Aedes aegypti* larvae. In the synthesis of title compounds, modifications have been made in the C-5 side-chain with a view to test their larvicidal activity. These isoxazoles have been obtained by 1,3-dipolar cycloaddition of aryl nitrile oxides to terminal alkynes which furnished the desired products in 20% to 79% yields. A comparative study of the larvicidal activity between 3-(3-aryl-isoxazol-5-yl)-propan-1-ols and 3-(3-aryl-isoxazol-5-yl)-propionic acids clearly demonstrated that the latter compounds possess much better larvicidal activity than the former. We also tested two esters, viz., methyl 3-[3-(phenyl)-isoxazole-5-yl] propionate and methyl 3-[3-(4-chlorophenyl)-isoxazole-5-yl] propionate, where the latter presented an excellent larvicidal profile.

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1. Introduction

Dengue is an endemic disease that occurs in tropical and sub-tropical regions, transmitted by the female *Aedes aegypti* mosquito.¹ About eighty million people are infected per year by one of the four dengue virus types, which is becoming one of the world's major public health problems, since this mosquito is present in more than 100 countries.² A huge dengue epidemic was expected for 2012, once the major peaks of dengue activity are observed in a four-year interval (the last big epidemic outbreak in Brazil took place in 2008, with almost one million notified cases).³ Although vaccines are being developed to combat the infection caused by dengue viruses,⁴ the sole effective strategy to control the disease is the vector eradication, since the increase in the incidence of this illness is closely linked to *Aedes aegypti* mosquito dissemination.⁵

Aedes aegypti mosquito is quite adapted to domestic environment and makes use of clean water held in containers for reproduction, which is the fundamental cause of proliferation.^{4,6} Mosquito control campaigns encourage people to reduce water storages and to use water larvicides, aiming to control the adult mosquito population. These integrated approaches help to reduce mosquitoes and larvae population, which might decrease virus transmission.⁶

One of the most employed larvicides used for combating *A. aegypti* mosquitoes is Temephos[®].⁷ Although quite effective, this organophosphate insecticide is related to mosquito strains resis-

tance. About twelve years ago, this kind of resistance has been found in mosquitoes in Rio de Janeiro, RJ, Brazil.⁸ Temephos-induced resistance has also been reported by Indian researchers for different stages of *A. aegypti* mosquitoes.⁹ This way, the development of new, non-toxic and cheap substances capable of disrupting *A. aegypti* life cycle is an urgent need.^{10,11}

Our research group has been involved in the synthesis of larvicidal compounds after we discovered that 3-(3-aryl-1,2,4-oxadiazole-5-yl) propionic acids possess such activity.^{6,12} Due to the great effectiveness of the above-mentioned compounds against mosquito larvae (ca. 15 ppm) and their low genotoxicity in mammals, these products have been patented and are the first prototypes of synthetic larvicides containing the 1,2,4-oxadiazole nucleus.¹² The mechanism of action is not yet fully elucidated, but some experimental evidences show that this class of substances may act in the kynurenine pathway,^{13–18} the major catabolic pathway in insects, causing the death by apoptosis of neuronal cells.

Isoxazoles are five-membered heterocycles quite similar in structure to 1,2,4-oxadiazoles. Indeed, the difference lies in the replacement of N-4 atom of oxadiazole with a CH group to furnish an isoxazole.^{19,20} This latter heterocycle is present in a large number of pharmacologically active drugs, such as cefoxazole and oxacillin, two beta-lactam antibiotics; isoxicam, an oxycam anti-inflammatory agent and the antidepressant drug isocarboxazid, a monoamine oxidase (MAO) inhibitor.²¹ In 2011, an antiviral compound (NITD-982) containing an isoxazole-pyrazole core has been found to inhibit dengue virus by suppressing dihydroorotate dehydrogenase (DHODH), an enzyme required for pyrimidine biosynthesis in the host cell.²² Besides being highly active compounds

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in biological systems and very similar to 1,2,4-oxadiazoles in structure, isoxazole-containing molecules have not been used to control *A. aegypti* mosquito dissemination.

As indicated above, isoxazoles are highly effective in biological systems and are widely used as drugs.²³ In fact, this heterocycle is found even in some natural products such as ibotenic acid.²⁴ In addition, a number of drugs including the COX-2 inhibitor valdecoxib (Bextra®) belong to this class of compounds.

We are inspired by our past research work on 1,2,4-oxadiazoles and decided to investigate isoxazoles because of their similarities with the former except that isoxazoles have only one nitrogen atom in the five-membered ring instead of two atoms found in oxadiazoles.

Considering the above mentioned advantages of isoxazole-containing compounds and the increasing drug resistance of usual compounds towards *A. aegypti* larvae, we decided to synthesize 3-(3-aryl-isoxazol-5-yl)-propan-1-ols, 3-(3-aryl-isoxazol-5-yl)-propionic acids and two of their esters capable of inhibiting L4 stage larvae growth in aqueous media. To the best of our knowledge, no study has been reported employing such compounds against *A. aegypti* larvae.

2. Results and discussion

2.1. Chemistry

Earlier results of our research group showed that 3-(3-aryl-1,2,4-oxadiazole-5-yl) propionic acids had good yield in the synthesis, a very good larvicidal activity and no genotoxicity.⁶ However, no further studies were carried out to discover the pharmacophore, i.e. the minimal structure responsible for the biological activity. Then, with the goal of finding out the pharmacophore structure and, as a consequence, developing more potent larvicidal prototypes, we have synthesized *n*-propanols and propionic acids containing an isoxazole-5-yl ring attached at their C-3 atoms. This way, two parameters in the prototype structure could be

evaluated: the type of heterocycle in the structure and the presence or absence of a carbonyl group in the molecules.

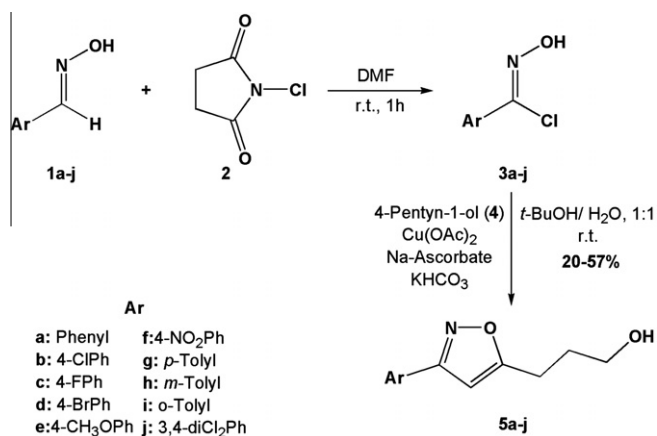
Prior to the synthesis of the isoxazoles, imidoyl chlorides have been prepared from corresponding aryloximes and N-chlorosuccinimide (NCS). The oximes were prepared from aromatic aldehydes and hydroxylamine.²⁰ Reaction of the imidoyl chlorides with an appropriate terminal alkyne (4-pentyn-1-ol, **4**, or sodium 4-pentynoate, **6**), using Cu(OAc)₂ and sodium ascorbate as catalyst (click conditions),²⁵ in the presence of base (KHCO₃), in 1:1 *tert*-butanol/water yielded the desired products in moderate to good yields (Schemes 1 and 2). Our efforts to synthesize the 3-[3-(3,4-dichlorophenyl)-isoxazol-5-yl]-propionic acid failed. Even after making several attempts to obtain this product, we had to abandon its preparation because of extensive decomposition and/or side products.

For all synthesized isoxazoles, only one regioisomer could be detected by ¹H NMR, because only one singlet was observed in the ¹H NMR spectra for C-4 ring proton between δ 6.16–6.85 ppm. According to earlier literature for the copper catalyzed isoxazole synthesis, it is believed that the obtained products are 3,5-disubstituted regioisomers.²⁵ We employed HMBC (Heteronuclear Multiple Bond Correlation) experiments to determine the regioisomers formed during the reaction. In the HMBC spectrum, C–H interactions up to three bonds can be detected. To illustrate how this magnetic resonance tool can be useful to differentiate the regioisomers (3,5 and 3,4-disubstituted isoxazoles), a HMBC experiment was performed for compound **7a** (Fig. 1). Thus, if an imaginary line is set in the carbon spectrum axis at 99.3 ppm, parallel to the hydrogen spectrum axis, it intersects three peaks. The intersection with the peak in 3.2 ppm in the hydrogen axis corresponds to a coupling between the methylene group adjacent to the isoxazole ring, showing a ³J_{CH} coupling. The other two interactions represent a doublet which shows a direct correlation between this carbon and the hydrogen attached to it (¹J_{CH} coupling). Another line parallel to hydrogen axis at 161.8 ppm intercepts two signals: one at 6.82 ppm representing a ²J_{CH} coupling and the other signal, at 8.00 ppm, for aromatic hydrogen, which confirms that the carbon signal at 161.8 ppm is due to C-3 of the isoxazole ring (³J_{CH} coupling). This piece of evidence provides strong support that compound **7a** is the 3,5-disubstituted regioisomer and not the 3,4-disubstituted one.

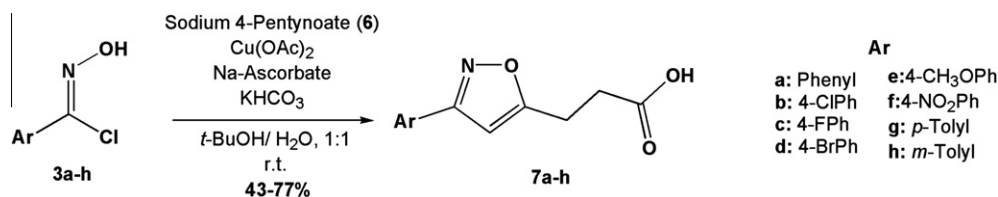
Previous results of our research group indicated that esters of propionic acids containing an oxadiazole nucleus possess more potent larvicidal activity than the parent acids.¹⁸ In order to verify the effectiveness of propionic acid esters containing an isoxazol-5-yl as substituent, we decided to prepare two such products. The acids **7a–b** were subjected to the classical Fisher esterification conditions, which furnished methyl esters in good yields (Scheme 3).

2.2. Bioassays

As mentioned earlier, we decided to synthesize these 3,5-disubstituted isoxazoles, which are similar to 1,2,4-oxadiazoles in structure, except for having CH function instead of N-4. This modification will show which core (1,2,4-oxadiazole or isoxazole)



Scheme 1. Synthesis of 3-(3-aryl-isoxazol-5-yl)-propan-1-ols (**5a–j**).



Scheme 2. Synthesis of 3-(3-aryl-isoxazol-5-yl)-propionic acids (**7a–h**).

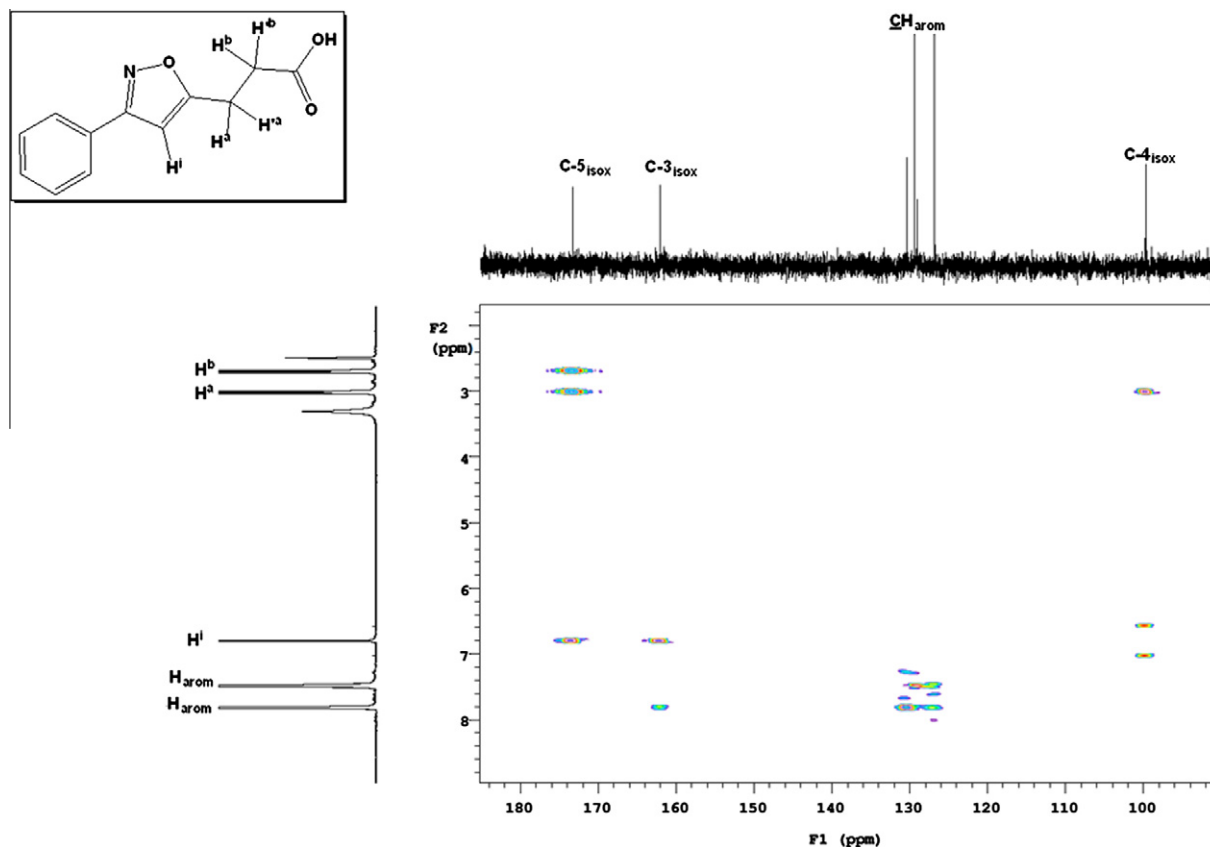
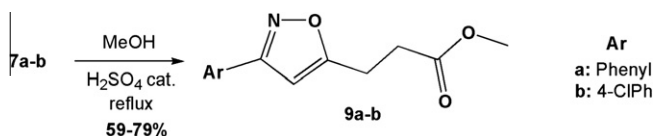


Figure 1. HMBC spectrum for compound 7a.



Scheme 3. Synthesis of methyl 3-[3-(aryl)-isoxazol-5-yl]-propionates (9a–b).

is more important for the maintenance or for the improvement of larvicidal activity. Larvicidal profile of compounds **5a–j** is described in Table 1. It can be observed that only compounds **5b**, **5d**, and **5j** showed significant larvicidal activity (LC_{50} below 100 ppm). Isoxazole **5j** having 3,4-dichlorophenyl substituent proved to be the most active in this series of compounds (LC_{50} = 17.2 ppm), indicating the significant contribution of two chlorine atoms as substituents in the phenyl ring.

Larvicidal profile for compounds **7a–h** is summarized in Table 2. In this series, only one compound (**7h**) has not shown significant larvicidal activity. As an example, for compound **7e**, the LC_{50} value

was 35.7 ppm. Just for comparison, the corresponding alcohol (**5e**), presented a LC_{50} value above 100 ppm.

Table 3 shows the observed LC_{50} values for 1,2,4-oxadiazole propionic acids (**8a–i**).⁶ These results clearly indicate that the replacement of the 1,2,4-oxadiazole nucleus with a isoxazole ring results in better larvicidal activity. It is interesting to note that the substitution of N-4 in 1,2,4-oxadiazole with a CH moiety caused a dramatic increase in larvicidal activity.

It was observed that the addition of electronegative atoms in the phenyl ring has some influence in improving the larvicidal activity (see LC_{50} values for **7b** (*p*-ClPh), **7c** (*p*-FPh) and **7d** (*p*-BrPh)). It can be noticed that, for these isoxazoles, the electronic effects are as important as the steric effects, since there is a huge difference in the larvicidal profile when **7a** (Ph) and **7c** (*p*-FPh) are compared. This way, the isoxazole containing the bromine atom, bulky and electronegative as a substituent in the aryl group, was the most active in this series (**7d**, LC_{50} = 13.9 ppm). The corresponding isoxazole alcohol (**5d**) did not show the same effect.

Table 1
Larvicidal activity for compounds **5a–j**

Compound	Ar	LC_{50} (ppm)	Standard error	Confidence interval (ppm)
5a	Phenyl	>100	—	—
5b	4-ClPh	28.9	±1.1	26.8–31.0
5c	4-FPh	>100	—	—
5d	4-BrPh	33.3	±0.7	32.0–34.7
5e	4-CH ₃ OPh	>100	—	—
5f	4-NO ₂ Ph	81.9	±1.6	78.7–85.1
5g	<i>p</i> -Tolyl	>100	—	—
5h	<i>m</i> -Tolyl	>100	—	—
5i	<i>o</i> -Tolyl	>100	—	—
5j	3,4-Cl ₂ Ph	17.2	±0.5	16.1–18.2

Table 2
Larvicidal activity for compounds **7a–h**

Compound	Ar	LC ₅₀ (ppm)	Standard error	Confidence interval (ppm)
7a	Phenyl	82.8	±1.5	79.9–85.7
7b	4-ClPh	37.7	±1.2	35.2–40.1
7c	4-FPh	33.7	±0.7	32.4–35.1
7d	4-BrPh	13.9	±1.0	12.0–15.7
7e	4-CH ₃ OPh	35.7	±0.5	34.7–36.6
7f	4-NO ₂ Ph	73.1	±1.4	70.4–75.8
7g	<i>p</i> -Tolyl	38.8	±0.7	37.4–40.2
7h	<i>m</i> -Tolyl	>100	—	—

Table 3
Larvicidal activity for 3-[3-(aryl)-1,2,4-oxadiazol-5-yl]-propionic acids (**8a–h**)

Compound	Ar	LC ₅₀ (ppm)	Standard error	Confidence interval (ppm)
8a	Phenyl	98.6	±2.9	95.7–101.5
8b	4-ClPh	28.1	±1.9	26.2–30.0
8c	4-FPh	81.2	±1.3	79.9–82.5
8d	4-BrPh	15.2	±0.8	14.4–16.0
8e	4-CH ₃ OPh	71.5	±1.5	70.0–73.0
8f	4-NO ₂ Ph	50.5	±2.4	48.1–52.9
8g	<i>p</i> -Tolyl	65.8	±3.2	62.6–69.0
8h	<i>m</i> -Tolyl	73.8	±2.9	70.9–76.7

Table 4
Larvicidal activity for compounds **9a–b**

Compound	Ar	LC ₅₀ (ppm)	Standard error	Confidence interval (ppm)
9a	Phenyl	13.2	±0.5	12.5–14.3
7a	Phenyl	82.8	±1.5	79.9–85.7
9b	4-ClPh	3.4	±0.1	3.2–3.6
7b	4-ClPh	37.7	±1.2	35.2–40.1

Our earlier research has shown that 3-(3-aryl-isoxazol-5-yl)-propionic acids are more effective than their corresponding oxadiazole derivatives (**8a–i**), because LC₅₀ values of the former are lower than the latter.⁶ For example, the LC₅₀ value of 3-[3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl] propionic acid (**8e**) is 81.2 ppm⁶ whereas 3-[3-(4-fluorophenyl)-isoxazol-5-yl] propionic acid (**7c**) has LC₅₀ = 33.7 ppm, clearly demonstrating the increase of larvicidal activity (See Tables 2 and 3). We can conclude that the substitution of nitrogen atom in the position 4 of the oxadiazole ring shows a positive effect in the larvicidal activity.

Finally, we evaluated the mortality of the larvae using two methyl esters of isoxazoles (**9a,b**) and compared their behavior with the parent acids **7a,b**. We found that **9a,b** are even more active than the acids, once the LC₅₀ values are 13.2 and 3.4 ppm, and the acids **7a,b** have the LC₅₀ values of 82.8 and 37.7 ppm, respectively (Table 4). We have chosen two compounds (**7a,b**) to be transformed into their esters to evaluate their behavior towards the mosquito larvae. Both acids have moderate activity, but when transformed into their methyl esters, they become good larvicidal candidates.

The larvicidal activity of the synthesized compounds is evaluated against fourth instar *A. aegypti* larvae in a small amount of water. Once *A. aegypti* larvae are immersed in water and since the larvae skin is lipophilic in nature, the drug penetration in their system is much easier compared to their predecessor acids, hence the esters are more active. This fact can be correlated to larvae lifetime when exposed to isoxazole containing acids and esters in their C-3 side chains. When exposed to acids, larvae die within 48 h, but when in contact with esters, larvae are almost all dead 24 h after the beginning of the test.

We suggest that the compounds described in this paper could act to inhibit the kynurenine pathway,^{13–17} the major catabolic

route in insects like *Aedes aegypti*. In this biochemical pathway, tryptophan is oxidized to kynurenic or xanturenic acids, which can then be oxidized to CO₂ and water or even be used in the biosynthesis of other substances.¹³ One of intermediates in this biochemical pathway is 3-hydroxy-kynurenine, harmful metabolite, which can disproportionate to nitrogen and oxygen reactive species.^{26,27} One enzyme in this pathway, the 3-hydroxy-kynurenine transaminase (3-HKT) mediates the conversion of 3-hydroxy-kynurenine to xanturenic acid.¹⁷ Once this enzyme is blocked, 3-hydroxy-kynurenine cannot be converted to xanturenic acid and this harmful substance can accumulate and cause neuronal cell apoptosis by inducing oxidative stress.^{28–31}

3-Hydroxy-kynurenine direct inhibition assays could not be performed to prove this hypothesis. But the HPLC (high performance liquid chromatography) assays,³² an indirect approach, can show if there is a decrease in the levels of xanturenic acid and an increase of 3-hydroxy-kynurenine in the insects when they get in contact with the larvicidal compounds synthesized in this work. In addition, molecular docking studies have shown that 3-(3-aryl-1,2,4-oxadiazole-5-yl) propionic acids do interact with 3-HKT. Such studies are being currently performed and the results will be published in the future.

3. Conclusions

In brief, we have accomplished the synthesis of twenty isoxazoles via copper catalyzed cycloaddition reaction between aryloximes and terminal alkynes, where sixteen are new (**5b, e–j; 7b–h; 9a–b**). All compounds presented as single 3,5-disubstituted regioisomers, as proven by HMBC experiments. Larvicidal data show that the replacement of the 1,2,4-oxadiazole moiety by a ring of isoxazole has a positive effect in larvae mortality. The propyl chain

present in the compounds does enhance the larvicidal activity, which can be correlated with an increase of lipophilicity of the terminal groups in the order: ester > acid > alcohol. The compounds with electronegative substituents in the phenyl ring exhibited the best activity. Methyl 3-[3-(4-chlorophenyl)isoxazol-5-yl] propionate (**9b**) presented an excellent larvicidal profile among the substances examined in this work.

4. Experimental section

4.1. General methods

All commercially available reagents were used without any further purification and the reactions were monitored by TLC analysis with TLC plates containing GF₂₅₄ (E. Merck). Melting points were determined on a Büchi apparatus and are uncorrected. Column chromatography was performed on Silica Gel 60 (70–230 mesh, E. Merck). NMR spectra were recorded with a Varian Unity Plus 300 MHz or a Varian UNMRS 400 MHz spectrometer. Infrared spectra were obtained on a Bruker IFS66 FT-IR, using KBr pellets. Elemental analysis was performed with a Carlo Erba instrument model E-1110. Exact mass measurements of the molecular ions were obtained on a Shimadzu LC/MS-IT-TOF Elettrospray. Aryloximes **1a–j** were prepared according to known literature procedures.²⁵ Compounds **5a**, **5c**³³ and **7a**³⁴ had their physical-chemical data compared to earlier literature for these substances.

4.2. Synthesis of 3-(3-aryl-isoxazol-5-yl)-propan-1-ols (**5a–j**)

To a solution of an appropriate aryloxime **1a–j** (4.0 mmol) in 20 mL of dimethylformamide (DMF), 4.8 mmol (0.64 g) of N-chlorosuccinimide (NCS) was added dropwise in 30 min. The resulting solution was stirred at room temperature until TLC analysis confirmed the disappearance of the starting oxime. The mixture was diluted with water (20 mL) and ethyl acetate (20 mL). The organic layer was separated, and the aqueous phase was extracted again with AcOEt (20 mL). Drying of the combined organic layers over anhydrous Na₂SO₄ and solvent removal in vacuo furnished the crude imidoyl chloride which was used in the next step without further purification (**3a–j**). 4-Pentyn-1-ol (4, 0.4 g, 4.8 mmol) and the imidoyl chloride (**3a–j**, 4.0 mmol) were suspended in 1:1 mixture of *tert*-butanol and water (10 mL). To this solution, a mixture of Cu(OAc)₂ (40 mg, 0.2 mmol) and sodium ascorbate (120 mg, 0.6 mmol) was added followed by the dropwise addition of an aqueous solution of KHCO₃ (18.0 mmol) to it. The contents were allowed to stir at room temperature until TLC analysis indicated the complete consumption of imidoyl chloride. The mixture was then diluted with water (10 mL) and AcOEt (10 mL). The organic layer was separated, and the water phase was extracted with AcOEt (2 × 10 mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The residue was chromatographed over silica gel using cyclohexane/EtOAc as eluant (1:1, v/v), crystallized from chloroform and hexane, which after work-up furnished the desired 3-(3-aryl-isoxazol-5-yl)-propan-1-ol.

4.2.1. 3-(3-Phenyl-isoxazol-5-yl)-propan-1-ol (**5a**)

Colorless crystals; yield: 54%; *R*_f: 0.40 (hexanes/AcOEt, 1:1 v/v); mp: 49–51 °C. ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 1.80 (s, 1H, OH); 2.01 (q, 2H, *J* 7.5 Hz, 6.3 Hz, CH₂); 2.93 (t, 2H, *J* 7.5 Hz, CH₂); 3.75 (t, 2H, *J* 6.3 Hz, CH₂OH); 6.33 (s, 1H, H_{isox}); 7.42–7.46 (m, 3H, H_{arom}); 7.78 (dd, 2H, *J* 9.0 Hz, 2.1 Hz, H_{arom}). ¹³C NMR (CDCl₃, 75 MHz) δ : 23.2; 30.3; 61.5; 99.2; 126.7; 128.8; 129.1; 129.9; 162.4; 173.4. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3259 (O–H); 3124 (C–H); 1610 (C=N); 1471 (N–O). Anal. Calcd for C₁₂H₁₃NO₂ (C, H, N): C, 70.92%; H, 6.45%; N, 6.89%. Found: C, 71.19%; H, 6.69%; N, 6.99%.

4.1.2. 3-[3-(4-Chlorophenyl)-isoxazol-5-yl]-propan-1-ol (**5b**)

Colorless crystals; yield: 20%; *R*_f: 0.74 (hexanes/AcOEt, 1:1 v/v); mp: 71–72 °C. ¹H NMR (CDCl₃, 300 MHz) δ : 1.99 (q, 2H, *J* 7.5 Hz, 6.3 Hz, CH₂); 2.08 (s, 1H, OH); 2.91 (t, 2H, *J* 7.5 Hz, CH₂); 3.73 (t, 2H, *J* 6.3 Hz, CH₂OH); 6.29 (s, 1H, H_{isox}); 7.40 (d, 2H, *J* 6.9 Hz, H_{arom}); 7.70 (d, 2H, *J* 6.9 Hz, H_{arom}). ¹³C NMR (CDCl₃, 75 MHz) δ : 23.1; 30.2; 61.3; 99.0; 127.6; 127.9; 128.8; 129.1; 135.8; 161.4; 173.8. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3260 (O–H); 2939, 2873 (C–H); 1658 (C=N); 1430 (N–O). ESI-HRMS *m/z*: 305.9989 (Calcd for C₁₁H₁₂BrN₃O₂Na [M+Na]⁺: 304.0061).

4.2.3. 3-[3-(4-Fluorophenyl)-isoxazol-5-yl]-propan-1-ol (**5c**)

Colorless crystals; yield: 33%; *R*_f: 0.40 (hexanes/AcOEt, 1:1 v/v); mp: 74–75 °C. ¹H NMR (DMSO, 400 MHz) δ (ppm): 1.81 (q, 2H, *J* 7.6 Hz, 6.0 Hz, CH₂); 2.81 (t, 2H, *J* 7.6 Hz, CH₂); 3.35 (s, 1H, OH); 3.47 (t, 2H, *J* 6.0 Hz, CH₂OH); 6.77 (s, 1H, H_{isox}); 7.30 (dd, 2H, *J* 8.8 Hz, 6.8 Hz, H_{arom}); 7.87 (dd, 2H, *J* 8.8 Hz, 6.8 Hz, H_{arom}). ¹³C NMR (DMSO, 100 MHz) δ : 22.8; 30.3; 59.6; 99.2; 115.9; 116.1; 125.4; 125.5; 128.7; 128.8; 160.9; 161.8; 164.3; 174.2. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3252 (O–H); 2932, 2870 (C–H); 1613, 1594 (C=N); 1434 (N–O); 1243 (C–F). Anal. Calcd for C₁₂H₁₂FNO₂ (C, H, N): C, 65.15%; H, 5.47%; N, 6.33%. Found: C, 65.35%; H, 5.82%; N, 6.39%.

4.2.4. 3-[3-(4-Bromophenyl)-isoxazol-5-yl]-propan-1-ol (**5d**)

Colorless crystals; yield: 20%; *R*_f: 0.74 (hexanes/AcOEt, 1:1 v/v); mp: 71–72 °C. ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 1.68 (s, 1H, OH); 2.00 (q, 2H, *J* 6.3 Hz, CH₂); 2.93 (t, 2H, *J* 7.5 Hz, CH₂); 3.74 (t, 2H, *J* 6.3 Hz, CH₂); 6.30 (s, 1H, H_{isox}); 7.57 (dd, 2H, *J* 8.7 Hz, 2.1 Hz, H_{arom}); 7.65 (dd, 2H, *J* 8.7 Hz, 2.1 Hz, H_{arom}). ¹³C NMR (CDCl₃, 75 MHz) δ : 23.2; 30.2; 61.5; 99.0; 124.1; 128.2; 132.1; 153.5; 161.5; 173.8. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3252 (O–H); 2931 (C–H); 1613 (C=N); 1434 (N–O). Anal. Calcd for C₁₂H₁₂BrNO₂ (C, H, N): C, 51.09%; H, 4.29%; N, 4.96%. Found: C, 51.42%; H, 4.37%; N, 4.97%.

4.2.5. 3-[3-(4-Methoxyphenyl)-isoxazol-5-yl]-propan-1-ol (**5e**)

Colorless crystals; yield: 20%; *R*_f: 0.60 (hexanes/AcOEt, 1:1 v/v); mp: 60–62 °C. ¹H NMR (CDCl₃, 300 MHz) δ : 1.94–2.03 (m, 2H, CH₂); 2.89 (t, 2H, *J* 7.5 Hz, CH₂); 3.72 (t, 2H, *J* 6.3 Hz, CH₂); 3.83 (s, 3H, OCH₃); 3.93 (s, 1H, OH); 6.26 (s, 1H, H_{isox}); 6.94 (dd, 2H, *J* 9.0 Hz, 2.1 Hz, H_{arom}); 7.70 (dd, 2H, *J* 9.0 Hz, 2.1 Hz, H_{arom}). ¹³C NMR (CDCl₃, 75 MHz) δ : 23.1; 30.3; 55.3; 61.4; 98.9; 112.0; 114.2; 121.6; 126.2; 128.0; 160.8; 173.2. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3257 (O–H); 3123 (C–H); 1610 (C=N); 1431 (N–O). ESI-HRMS *m/z*: 256.1184 (Calcd for C₁₃H₁₅NO₃Na [M+Na]⁺: 256.0950).

4.2.6. 3-[3-(4-Nitrophenyl)-isoxazol-5-yl]-propan-1-ol (**5f**)

Yellow crystals; yield: 44%; *R*_f: 0.63 (hexanes/AcOEt, 1:1 v/v); mp: 80–82 °C. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 1.67 (s, 1H, OH); 2.03 (q, 2H, *J* 7.6 Hz, 6.4 Hz, CH₂); 2.97 (t, 2H, *J* 7.6 Hz, CH₂); 3.76 (t, 2H, *J* 6.4 Hz, CH₂OH); 6.41 (s, 1H, H_{isox}); 7.96 (dd, 2H, *J* 8.8 Hz, 2.0 Hz, H_{arom}); 8.30 (dd, 2H, *J* 8.8 Hz, 2.0 Hz, H_{arom}). ¹³C NMR (CDCl₃, 100 MHz) δ : 23.2; 30.2; 61.4; 99.3; 124.1; 127.6; 135.4; 148.6; 160.6; 174.6. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3462 (O–H); 3358 (C–H); 1662 (C=N); 1516 (N–O). Anal. Calcd for C₁₂H₁₂N₂O₄ (C, H, N): C, 58.06%; H, 4.87%; N, 11.29%. Found: C, 58.17%; H, 5.23%; N, 11.30%.

4.2.7. 3-(3-*p*-Tolyl-isoxazol-5-yl)-propan-1-ol (**5g**)

Colorless crystals; yield: 52%; *R*_f: 0.78 (hexanes/AcOEt, 1:1 v/v); mp: 48–50 °C. ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 1.59 (s, 1H, OH); 2.01 (q, 2H, *J* 7.5 Hz, 6.0 Hz, CH₂); 2.39 (s, 3H, CH₃Ph); 2.92 (t, 2H, *J* 7.5 Hz, CH₂); 3.75 (t, 2H, *J* 6.0 Hz, CH₂); 6.30 (s, 1H, H_{isox}); 7.25 (dd, 2H, *J* 8.1 Hz, 0.6 Hz, H_{arom}); 7.68 (dd, 2H, *J* 8.1 Hz, 0.6 Hz, H_{arom}). ¹³C NMR (DMSO, 100 MHz) δ : 21.3; 23.2; 30.3; 61.5; 99.0; 126.3;

126.6; 129.5; 139.9; 162.3; 173.3. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3264 (O–H); 2931, 2872 (C–H); 1608 (C=N); 1433 (N–O). ESI-HRMS m/z : 240.0951 (Calcd for $\text{C}_{13}\text{H}_{15}\text{NO}_2\text{Na}$ $[\text{M}+\text{Na}]^+$: 240.1000).

4.2.8. 3-(3-*m*-Tolyl-isoxazol-5-yl)-propan-1-ol (5h)

Yellow oil; yield: 57%; R_f : 0.73 (hexanes/AcOEt, 1:1 v/v). ^1H NMR (CDCl_3 , 300 MHz) δ : 1.95–2.04 (m, 3H, CH_2 , OH); 2.39 (s, 3H, CH_3); 2.91 (t, 2H, J 7.5 Hz, CH_2); 3.73 (t, 2H, J 6.3 Hz, CH_2); 6.30 (s, 1H, H_{isox}); 7.22–7.35 (m, 2H, H_{arom}); 7.54–7.61 (m, 2H, H_{arom}). ^{13}C NMR (CDCl_3 , 75 MHz) δ : 21.3; 23.1; 30.2; 61.4; 99.2; 123.8; 127.3; 128.7; 129.0; 130.6; 138.5; 162.5; 173.4. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3285 (O–H); 2940, 2872 (C–H); 1604 (C=N); 1420 (N–O). ESI-HRMS m/z : 256.0904 (Calcd for $\text{C}_{13}\text{H}_{15}\text{NO}_2\text{K}$ $[\text{M}+\text{K}]^+$: 256.0740).

4.2.9. 3-(3-*o*-Tolyl-isoxazol-5-yl)-propan-1-ol (5i)

Yellow oil; yield: 22%; R_f : 0.48 (hexanes/AcOEt, 1:1 v/v). ^1H NMR (CDCl_3 , 300 MHz) δ : 1.98 (q, 2H, J 7.5 Hz, 6.3 Hz, CH_2); 2.22 (s, 1H, OH); 2.43 (s, 3H, CH_3Ph); 2.89 (t, 2H, J 7.5 Hz, CH_2); 3.71 (t, 2H, J 6.3 Hz, CH_2); 6.16 (s, 1H, H_{isox}); 7.20–7.30 (m, 3H, H_{arom}); 7.2 (bd, 1H, J 7.2 Hz, H_{arom}). ^{13}C NMR (CDCl_3 , 75 MHz) δ : 21.0; 23.1; 30.2; 61.4; 101.8; 125.9; 128.9; 129.3; 130.9; 136.7; 163.0; 172.5. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3383 (O–H); 2951, 2876 (C–H); 1597 (C=N); 1403 (N–O). ESI-HRMS m/z : 240.0927 (Calcd for $\text{C}_{13}\text{H}_{15}\text{NO}_2\text{Na}$ $[\text{M}+\text{Na}]^+$: 240.1000).

4.2.10. 3-[3-(3,4-Dichlorophenyl)-isoxazol-5-yl]-propan-1-ol (5j)

Colorless oil; yield: 27%; R_f : 0.70 (hexanes/AcOEt, 1:1 v/v). ^1H NMR (CDCl_3 , 300 MHz) δ (ppm): 1.86 (s, 1H, OH); 2.01 (q, 2H, J 7.5 Hz, 6.3 Hz, CH_2); 2.93 (t, 2H, J 7.5 Hz, CH_2); 3.74 (t, 2H, J 6.3 Hz, CH_2); 6.30 (s, 1H, H_{isox}); 7.51 (d, 1H, J 8.4 Hz, H_{arom}); 7.61 (d, 1H, J 8.4 Hz, H_{arom}); 7.86 (s, 1H, H_{arom}). ^{13}C NMR (CDCl_3 , 75 MHz) δ (ppm): 23.2; 30.2; 61.4; 99.0; 125.8; 128.5; 129.1; 130.9; 133.1; 133.9; 160.5; 174.2. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3382 (O–H); 2938 (C–H); 1604 (C=N); 1421 (N–O). Anal. Calcd for $\text{C}_{12}\text{H}_{11}\text{Cl}_2\text{NO}_2$ (C, H, N): C, 52.96%; H, 4.07%; N, 5.15%. Found: C, 52.65%; H, 4.06%; N, 5.07%.

4.3. Synthesis of 3-(3-aryl-isoxazol-5-yl)-propionic acids (7a–h)

The imidoyl chlorides were prepared as described for the synthesis of **5a–j**. Sodium 4-pentynoate (6, 0.58 g, 4.8 mmol) and the appropriated imidoyl chloride (**3a–h**, 4.0 mmol) were suspended in 1:1 mixture of *tert*-butanol and water (10 mL). To this solution was added a mixture of $\text{Cu}(\text{OAc})_2$ (40 mg, 0.2 mmol) and sodium ascorbate (120 mg, 0.6 mmol). Finally, an aqueous solution of potassium hydrogen carbonate (1.8 g, 18.0 mmol) was added dropwise to the reaction mixture. The contents were allowed to stir at room temperature until TLC analysis indicated complete consumption of the imidoyl chloride. To the reaction contents were added 10 mL of an aqueous potassium bicarbonate solution and 10 mL of AcOEt and this resulting mixture was allowed to stir overnight. The phases were separated and the aqueous phase was neutralized with AcOEt (2×10 mL). Drying the combined organic layers over anhydrous Na_2SO_4 , filtration and solvent evaporation under reduced pressure left the product as a solid which could be crystallized from cold ethanol in all cases.

4.3.1. 3-(3-Phenyl-isoxazol-5-yl)-propionic acid (7a)

Colorless crystals; yield: 63%; R_f : 0.32 (chloroform/methanol, 9:1 v/v); mp: 148–150 °C. ^1H NMR (CDCl_3 , 300 MHz) δ (ppm): 2.85 (t, 2H, J 7.5 Hz, CH_2); 3.16 (t, 2H, J 7.5 Hz, CH_2); 6.37 (s, 1H, H_{isox}); 7.45 (m, 3H, H_{arom}); 7.78 (m, 2H, H_{arom}). ^{13}C NMR (CDCl_3 , 75 MHz) δ (ppm): 21.8; 31.1; 99.3; 126.5; 128.7; 129.0; 130.0;

161.8; 172.9; 173.0. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3116 (C–H); 3048–2934 (O–H); 1695 (C=O); 1601 (C=N). Anal. Calcd for $\text{C}_{12}\text{H}_{11}\text{NO}_3$ (C, H, N): C, 66.35%; H, 5.10%; N, 6.45%. Found: C, 66.12%; H, 5.60%; N, 6.63%.

4.3.2. 3-[3-(4-Chlorophenyl)-isoxazol-5-yl]-propionic acid (7b)

Colorless crystals; yield: 50%; R_f : 0.30 (chloroform/methanol, 9:1 v/v); mp: 189–190 °C. ^1H NMR (CDCl_3 , 300 MHz) δ (ppm): 2.70 (t, 2H, J 7.2 Hz, CH_2); 3.02 (t, 2H, J 7.2 Hz, CH_2); 6.85 (s, 1H, H_{isox}); 7.57 (dd, 2H, J 8.7 Hz, 1.8 Hz, H_{arom}); 7.87 (dd, 2H, J 8.7 Hz, 1.8 Hz, H_{arom}). ^{13}C NMR (CDCl_3 , 75 MHz) δ (ppm): 22.8; 32.1; 100.5; 128.6; 129.3; 130.2; 135.8; 161.9; 174.0; 174.4. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3120 (C–H); 2935 (O–H); 1706 (C=O), 1602 (C=N). Anal. Calcd for $\text{C}_{12}\text{H}_{10}\text{ClNO}_3$ (C, H, N): C, 57.27%; H, 4.01%; N, 5.57%. Found: C, 57.08%; H, 4.51%; N, 5.46%.

4.3.3. 3-[3-(4-Fluorophenyl)-isoxazol-5-yl]-propionic acid (7c)

Colorless crystals; yield: 77%; R_f : 0.37 (chloroform/methanol, 9:1 v/v); mp: 165–168 °C. ^1H NMR (CDCl_3 , 300 MHz) δ (ppm): 2.70 (t, 2H, J 7.2 Hz, CH_2); 3.01 (t, 2H, J 7.2 Hz, CH_2); 3.34 (s, 1H, OH); 6.84 (s, 1H, H_{isox}); 7.35 (dd, 2H, J 8.4 Hz, 2.4 Hz, H_{arom}); 7.89 (dd, 2H, J 8.4 Hz, 2.4 Hz, H_{arom}). ^{13}C NMR (CDCl_3 , 75 MHz) δ (ppm): 22.5; 31.8; 100.1; 116.7; 117.0; 126.0; 129.4; 129.6; 161.6; 165.4; 173.8. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3115 (C–H); 2936 (O–H); 1696 (C=O); 1607 (C=N). ESI-HRMS m/z : 234.0501 (Calcd for $\text{C}_{12}\text{H}_9\text{FNO}_3$ $[\text{M}-\text{H}]^+$: 234.0567).

4.3.4. 3-[3-(4-Bromophenyl)-isoxazol-5-yl]-propionic acid (7d)

Colorless crystals; yield: 45%; R_f : 0.17 (chloroform/methanol, 9:1 v/v); mp: 152–153 °C. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ (ppm): 2.70 (t, 2H, J 7.2 Hz, CH_2); 3.02 (t, 2H, J 7.2 Hz, CH_2); 6.84 (s, 1H, H_{isox}); 7.69 (dd, 2H, J 8.8 Hz, 2.4 Hz, H_{arom}); 7.81 (dd, 2H, J 8.8 Hz, 2.4 Hz, H_{arom}). ^{13}C NMR (CDCl_3 , 100 MHz) δ (ppm): 21.7; 31.1; 99.3; 123.4; 127.9; 128.5; 132.1; 160.9; 166.9; 173.3. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3119 (C–H); 2934 (O–H); 1706 (C=O); 1600 (C=N). ESI-HRMS m/z : 293.9715 (Calcd for $\text{C}_{12}\text{H}_9\text{BrNO}_3$ $[\text{M}-\text{H}]^+$: 293.9766).

4.3.5. 3-[3-(4-Methoxyphenyl)-isoxazol-5-yl]-propionic acid (7e)

Colorless crystals; yield: 43%; R_f : 0.14 (chloroform/methanol, 9:1 v/v); mp: 159–161 °C. ^1H NMR (DMSO , 400 MHz) δ (ppm): 2.69 (t, 2H, J 7.2 Hz, CH_2); 3.00 (t, 2H, J 6.8 Hz, CH_2); 3.81 (s, 3H, OCH_3); 6.73 (s, 1H, H_{isox}); 7.04 (d, 2H, J 8.4 Hz, H_{arom}); 7.76 (d, 2H, J 8.4 Hz, H_{arom}). ^{13}C NMR (DMSO , 100 MHz) δ (ppm): 21.8; 31.1; 55.2; 56.3; 99.1; 113.2; 114.4; 121.1; 126.6; 127.7; 127.9; 160.6; 161.4; 172.6; 173.0. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 2839 (C–H); 1696 (C=O); 1612 (C=N); 1435 (N–O). ESI-HRMS m/z : 248.0860 (Calcd for $\text{C}_{13}\text{H}_{14}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 248.0923).

4.3.6. 3-[3-(4-Nitrophenyl)-isoxazol-5-yl]-propionic acid (7f)

Yellow oil; yield: 46%; R_f : 0.38 (chloroform/methanol, 9:1 v/v); ^1H NMR (CDCl_3 , 300 MHz) δ (ppm): 2.97 (t, 2H, J 7.2 Hz, CH_2); 3.76 (t, 2H, J 6.3 Hz, CH_2); 6.41 (s, 1H, H_{isox}); 7.96 (dd, 2H, J 8.7 Hz, 1.8 Hz, H_{arom}); 8.30 (dd, 2H, J 8.7 Hz, 1.8 Hz, H_{arom}). ^{13}C NMR (CDCl_3 , 75 MHz) δ (ppm): 23.2; 30.2; 61.4; 99.3; 124.1; 127.5; 135.3; 148.5; 160.6; 174.6. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3133 (C–H); 2874 (O–H); 1706 (C=O), 1602 (C=N). ESI-HRMS m/z : 263.0668 (Calcd for $\text{C}_{12}\text{H}_{11}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$: 263.0690).

4.3.7. 3-(3-*p*-Tolyl-isoxazol-5-yl)-propionic acid (7g)

Colorless crystals; yield: 48%; R_f : 0.36 (chloroform/methanol, 9:1 v/v); mp: 168–169 °C. ^1H NMR (CDCl_3 , 300 MHz) δ (ppm): 2.39 (s, 3H, CH_3); 2.84 (t, 2H, J 7.3 Hz, CH_2); 3.14 (t, 2H, J 7.3 Hz, CH_2); 6.33 (s, 1H, H_{isox}); 7.25 (d, 2H, J 8.4 Hz, H_{arom}); 7.67 (d, 2H, J 8.4 Hz, H_{arom}). ^{13}C NMR (CDCl_3 , 75 MHz) δ (ppm): 21.4; 21.9;

31.3; 45.7; 99.5; 126.1; 126.6; 129.6; 162.4; 176.0. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3122 (C–H); 2944 (O–H); 1696 (C=O); 1602 (C=N). ESI-HRMS m/z : 230.0768 (Calcd for $\text{C}_{13}\text{H}_{12}\text{NO}_3$ $[\text{M} - \text{H}]^+$: 230.0817).

4.3.8. 3-(3-*m*-Tolyl-isoxazol-5-yl)-propionic acid (7h)

Colorless crystals; yield: 52%; R_f : 0.19 (chloroform: methanol, 9:1 v/v); mp: 148–150 °C. ^1H NMR (DMSO, 400 MHz) δ (ppm): 2.71 (t, 2H, J 7.2 Hz, CH_2); 3.02 (t, 2H, J 7.2 Hz, CH_2); 3.33 (s, 3H, CH_3); 6.81 (s, 1H, H_{isox}); 7.50 (d, H, J 5.2 Hz, H_{arom}); 7.83 (dd, H, J 5.2 Hz, 2.4 Hz, H_{arom}). ^{13}C NMR (DMSO, 100 MHz) δ (ppm): 21.8; 31.1; 99.3; 126.4; 128.7; 129.0; 130.0; 161.8; 172.9; 173.0. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3125 (C–H); 2907 (O–H); 1703 (C=O), 1607 (C=N). ESI-HRMS m/z : 230.0768 (Calcd for $\text{C}_{13}\text{H}_{12}\text{NO}_3$ $[\text{M} - \text{H}]^+$: 230.0801).

4.4. Synthesis of methyl esters of 3-[3-(aryl)-isoxazol-5-yl]-propionic acids (9a–b)

To an appropriate solution of the 3-[3-(aryl)-isoxazol-5-yl]-propionic acid (5 mmol) in 40 mL of methanol, five drops of concentrated sulfuric acid were added followed by reflux under stirring until TLC analysis indicated the total consumption of the starting acid. Dilution of the contents with water (20 mL) followed by solvent removal under reduced pressure gave the crude product. Extraction of the residue with AcOEt (2×20 mL) and drying solvent over anhydrous Na_2SO_4 , pursued by solvent removal in vacuo furnished the crude product. Crystallization from methanol/water provided the desired methyl 3-[3-(aryl)-isoxazol-5-yl]-propionate.

4.4.1. Methyl 3-[3-(phenyl)-isoxazol-5-yl]-propionate (9a)

Colorless crystals; yield: 60%; R_f : 0.85 (chloroform/ethyl acetate, 7:3 v/v); mp: 94–95 °C. ^1H NMR (DMSO- d_6 , 400 MHz) δ (ppm): 2.80 (t, 2H, J 7.2 Hz, CH_2); 3.07 (t, 2H, J 7.2 Hz, CH_2); 3.63 (s, 3H, CH_3); 6.82 (s, 1H, H_{isox}); 7.50 (m, 3H, H_{arom}); 7.83 (dd, 2H, J 8.0 Hz, 2.0 Hz, H_{arom}). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 21.6; 30.8; 51.5; 99.4; 126.4; 128.7; 129.0; 130.0; 161.8; 171.9; 172.6. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3119 (C–H); 1733 (C=O); 1600 (C=N); 1444 (N–O). ESI-HRMS m/z : 254.0779 (Calcd for $\text{C}_{13}\text{H}_{13}\text{NNaO}_3$ $[\text{M} + \text{Na}]^+$: 254.0793).

4.4.2. Methyl 3-[3-(4-chlorophenyl)isoxazol-5-yl] propionate (9b)

Colorless crystals; yield: 80%; R_f : 0.94 (chloroform/ethyl acetate, 7:3 v/v); mp: 103–104 °C. ^1H NMR (CD_3OD , 400 MHz) δ (ppm): 2.77 (t, 2H, J 7.2 Hz, CH_2); 3.12 (t, 2H, J 7.2 Hz, CH_2); 3.66 (s, 3H, CH_3); 6.58 (s, 1H, H_{isox}); 7.45 (dd, 3H, J 8.4 Hz, 2.0 Hz, H_{arom}); 7.76 (dd, 2H, J 8.4 Hz, 2.0 Hz, H_{arom}). ^{13}C NMR (CD_3OD , 100 MHz) δ : 23.0; 32.3; 52.3; 100.4; 129.1; 129.3; 130.2; 137.1; 162.9; 173.9; 174.4. I.R. (KBr pellet) $\nu_{\max}/\text{cm}^{-1}$: 3117 (C–H); 1732 (C=O); 1605 (C=N); 1439 (N–O). Anal. Calcd for $\text{C}_{13}\text{H}_{12}\text{ClNO}_3$ (C, H, N): C, 58.77%; H, 4.55%; N, 5.27%. Found: C, 59.09%; H, 4.65%; N, 5.16%.

4.5. Larvicidal bioassay

The larvicidal activity of the synthesized drugs was evaluated using an adaptation of the method recommended by the World Health Organization.^{35,36} A stock solution (100 ppm) was prepared by diluting 0.005 g of test-compounds in 0.7 mL of ethanol, dimethylsulfoxide or acetone (analytical grade) or 2 drops of Tween 80, and completing to a volume of 50 mL with distilled water. In order to test the effect of the compounds on the larvae survival, fourth instar *A. aegypti* were added to the beakers (20 larvae per beaker) containing the synthesized compounds 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 for each assay, using distilled water containing the same amount of co-solvent as the test sample has been used. Mortality of the larvae was determined after 48 h of incubation at 28 ± 2 °C. Larvae were considered dead when no response to stimuli or not rising to the solution surface was observed. Lethal concentration (LC_{50}) values were calculated through probit analysis using the Status Plus 2006 software program.³⁶

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2012.12.006>.

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