41

Egypt. J. Chem. **Vol. 60,** No.4, pp. 613 - 618 (2017)

Evaluation of Antibacterial and Antifungal Effects of Novel Hydroxamic Acids Linked-natural Amino Acids

Marzough Aziz Albalawi
Department of Chemistry, Alwajh College, University of Tabuk, KSA

A SERIES of new derivatives containing hydroxamic acids linked-amino acids has been synthesized and fully characterized by spectroscopic techniques including; ^{1}H, ^{13}C, DEPT 135 and HRMS.These new compounds were tested for their antibacterial and antifungal activities.

Keywords : Amino acid, Hydroxamic acid, Sulfonamides, Antibacterial and antifungal activities

Introduction

Since their discovery by Wahlroos and Virtanen (1) in 1959, and over the past decades, the chemistry and biochemistry of hydroxamic acids and their derivatives have attracted considerable attention, due to their pharmacological, toxicological and pathological properties. Recently, the compounds containing hydroxamic acid functionality have been reported to exhibit multifarious pharmacological effects including antioxidant [1], anticancer [2,3], antibacterial agents [4], antifungal [5], growth factors inhibitors [6], anti-melanogenic[7], antimalarial [8], enzyme inhibitors [9], rare earth mineral collectors [10]and reagents for solvent extraction and spectrophotometric determination of metals [11].A number of hydroxamic acid derivatives have also been found to inhibit several important enzymes in cellular systems, including FeII and MnII *E. coli* methionine aminopeptidase [12], γ-lactam based histone deacetylase(HDACs) [13] and matrix metalloproteinases [14, 15].Herein, we report the synthesis and characterization of new hydroxamic acids derivatives.The antibacterial and antifungal activities against some strains of bacteria and fungi were investigated.

Experimental

All reactions were performed under an argon atmosphere and were monitored by thin-layer chromatography (TLC) Merck 60 F-254 silica-gel plates (layer thickness 0.25 mm). Column chromatography was performed on silica gel (70–230 mesh) using ethyl acetate and cyclohexane mixture as eluents. Melting temperatures were determined on an Electrothermal 9002 apparatus and were reported uncorrected. NMR spectra were recorded on a Bruker AC-300 spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C). All chemical shifts were reported as δ values (ppm). relative to internal tetramethylsilane. High-resolution mass spectra (HRMS) were recorded on a matrix-assisted laser desorption=ionization time-of-flight (MALDI-TOF) Perspective Biosystems Voyager DE-STR instrument.Finally, the antibacterial and antifungal activities of the synthesized compounds were tested against *Pseudomonas aeruginosa* and various fungal strains at concentration of 10 mg/mL in methanol.

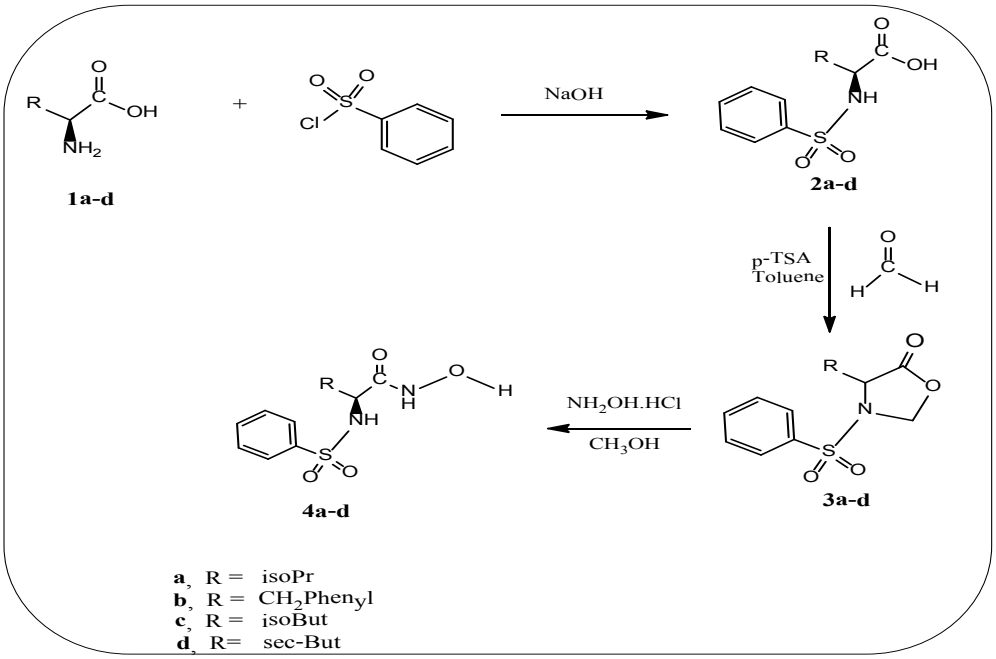
Results and Discussions

Chemistry of compounds

The starting materials (2a-d) were prepared [17]. Following a general procedure for the synthesis of N-arylsulfonyl-2-aminoacides,tosyl chloride (2.54 g, 13.3 mmol) was slowly added at 0 °C to 1.5 g (12.8 mmol) of (L) -valine dissolved in a solution of sodium hydroxide (2M, 6.41 ml) followed by the addition of di-isopropylethylamine (14.1 mmol, 2.24 ml) and acetone (6.41 ml). After 10 min, the ice bath is removed and the mixture is left stirring for 6 hr at room temperature. The reaction mixture is then washed with diethyl ether. The organic phase is extracted with an aqueous solution of NaOH (2M), then cooled to -10 ° C. and acidified to pH = 1 by the addition of concentrated HCl. This aqueous phase is extracted three times with ethyl acetate. The organic phase is washed with a saturated NaCl solution and dried over MgSO4. After evaporation of the solvent, the residue is recrystallized from ethyl acetate-petroleum ether in varying proportions.

Email: maalbalawi@ut.edu.sa.
DOI :10.21608/ejchem.2017.908.1042

In the present investigation, the synthetic approach was achieved in three steps as shown in Scheme 1. Initially, the commercially available amino acids (1a-d) have been protected with phenylsulfonyl chloride in the presence of one equivalent of sodium hydroxide to afford compounds (2a-d). When compounds (2a-d) were subjected to condensation with paraformaldehyde and p-toluene sulfonic acid as a catalyst gave the corresponding 1,3- oxazolidin-5-ones (3a-d). The cleavage of the latter derivatives with the hydroxylamine hydrochloride in methanol led to the formation of N -phenylsulfonyl -2-amino hydroxamic acids (4a-d) as shown in Scheme 1. In each step, the progress of the reaction was monitored by TLC (Thin layer chromatography). The 1H NMR spectra of hydroxamic acids under investigation showed the characteristic singlet of the proton of the hydroxyl group in the region δ 11-11.5 ppm. A broad signal appeared in the region δ 7.2-8.22 ppm which evidently belonged to the Ar-H and NH protons of the hydroxylamine unit. The 13C-NMR spectra exhibited an absorption signal due to carbonyl, C=O carbon nearby δ 165 ppm. The chemical shifts of the aromatic carbons appeared in the region δ 124-138 ppm. Beside these signals, a singlet near δ 21 ppm and a singlet at 171 ppm appeared which correspond to the carbon atom of alkyl group and carboxy group, respectively DEPT 135 spectrum indicated that the methylene carbon appeared at 24.45, 25.28 and 37.24, CH carbons were noticed in the region between 127.7ppm to 134.8ppm.



Scheme 1. The synthetic route for the synthesis of hydroxamic acids (4a-d)

Synthesis of compounds

The starting materials 2a-d were prepared [17].

Synthesis of N-phenylsulfonyl-L-valine (2a)

It was prepared from commercial L-valine and Para toluenesulfonylchloride,the recrystallization from ether afforded the product as a white solid (yield 80%);^{1}H NMR (300 MHz, methanol-d4) δ 0.90 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H), 2.21(m, 1H), 3.54 (d, J = 7.5 Hz, 1H), 7.62 -7.78(m, 7H).

Synthesis N-phenylsulfonyl-L-phenylalanine (2b)

It was prepared from L-phenylalanine; 1H NMR (300 MHz, methanol-d4) δ3.22 (d J = 14.7, 2H),4.87 (t,1H), 7.32-7.98 (m, 11H), 11.51 (s,1H).

Synthesis of N- phenylsulfonyl-L-leucine (2c)

It was prepared from commercial L-isoleucine;^{1}H NMR (300 MHz, methanol-d4) δ0.86 (d, J = 6.4 Hz , 6H), 1.68 (m, 1H) 2.16 (t, 2H), 3.81 (m, 1H), 7.61-7.87 (m, 6H), 11.55 (s,1H).

synthesis of N-phenylsulfonyl-L-isoleucine (2d)

It was Prepared from L-leucine;^{1}H NMR (300 MHz, methanol-*d*4) δ0.85 (m, 6H), 1.32

(q,2H),1.83 (m, 1H), 3.77 (d, *J* = 9.2,1H), 7.61-7.88 (m, 6H), 11.58 (s,1H).

General procedure for synthesis ofsubstituted 1,3-oxazolidin -5-ones

The reactions were performed according to the literature [18] and following the general procedure, 10 mmoles ofN-phenylsulfonyl-L-amino acid, 2 g of p-formaldehyde and 200 mg of toluene are added to a single-necked round bottom flask equipped with a condenser and a Dean-Stark assembly containing (50 ml) of p-toluenesulfonic acid. The reaction mixture was refluxed for 4 hr, to form a water toluene emulsion. After cooling, the reaction mixture is washed with an aqueous solution of $NaHCO_3$ (1M) to remove the remaining starting acid, then dried over Na_2SO_4 and evaporated, the residue obtained is recrystallized from ethyl acetate-petroleum ether with various proportions.

Synthesis of product 4-isopropyl-3-(phenylsulfonyl) oxazolidin-5-one(3a)

The mixture of compound 2a, p-formaldehyde, Toluene and p-toluensulfonic acid as catalyst afforded the compound 3a.yield :65%NMR ^{1}H: (300 MHz, $CDCl_3$)δ:0.98 (d, 6H),2.00 (q, 1H), 3.62 (dd, J = 14.8, 1H), 7.42-7.9(m, 7H).^{13}C: (75MHz, $CDCl_3$) δ: 18.42, 18.46, 31.04, 62.76 (C-N),94.11(CH_2-O), 127.28-136.00 (6 C aromatic), 171.19 (CO).

Synthesis of product 4-benzyl-3-(phenylsulfonyl) oxazolidin-5-one(3b)

The compound 3bwas prepared as the same procedure as 3a which was obtained as white solid. Yield :70%.NMR ^{1}H: (300 MHz, $CDCl_3$) δ:3.31 (d, 2H, J=5.6), 4.45 (t, 1H), 5.65 (dd, J = 9.2, 2H), 7.22- 7.94 (m, 10H).^{13}C: (75MHz, $CDCl_3$) δ: 37.24, 58.54, 79.83 (CH_2-O), 127.49-136.13 (12 C aromatic), 171.39(CO);DEPT 135: (300 MHz, $CDCl_3$)δ:

Synthesis of product 4-isobutyl-3-(phenylsulfonyl) oxazolidin-5-one (3c)

White solid. Yield :80%. NMR ^{1}H: (300 MHz, $CDCl_3$)δ: 0.93 (m, 6H, $2CH_3$),1.71 (m, 1H), 2.00(m, 1H), 4.45 (t, 1H), 5.31 (d, J = 14.6, 1H), 5.7 (d, J = 14.4, 1H), 7.61- 7.92 (m, 5H).
^{13}C: (75MHz, $CDCl_3$) δ: 21.43, 22.75, 24.45, 38.44, 56.20, 78.76(CH_2-O), 127.64-135.98 (6 C aromatic), 172.249(CO).

Synthesis of product 4-sec-butyl-3-(phenylsulfonyl) oxazolidin-5-one(3d)

White solid: Yield 54%. NMR ^{1}H: (300 MHz, $CDCl_3$)δ: 1 (m, 6H, $2CH_3$),1.44 (m, 2H), 2.22 (m, 1H), 4.46 (d, J = 14.6, 1H), 5.32 (d, J = 14.6, 1H), 5.6 (d, J = 14.6, 1H) 7.61- 7.95 (m, 5H).^{13}C: (75MHz, $CDCl_3$) δ:11.46, 14.93, 25.28, 37.75, 61.76, 79.56(CH_2-O), 127.63-135.95 (6 C aromatic), 171.03(CO).

General procedure for synthesis ofsubstituted hydroxamic acid

Following the general procedure, substitueted 1,3- oxazolidin -5-ones (2 mmoles) are dissolved in 5 ml of methanol, 3 mmoles of hydroxylamine hydrochloride and 3 mmoles of triethylamine. The mixture is stirred at room temperature for 12 hr. After evaporation under reduced pressure of methanol, 10 ml of ethyl acetate was added to precipitate the ammonium salt. After filtration, the ethyl acetate is evaporated and the resulting oil is purified by column chromatography.

Synthesis of product (S)-N-hydroxy-3-methyl-2-(phenylsulfonamido)butanamide (4a)

White solid;m.p. 119–121 °C;Yield 62.5%. ^{1}H-NMR ($CDCl_3$) δ: 0.8 (m, 6H, $2CH_3$), 2 (m, 1H), 7.43-8. 22 (m, 7H); 11.3 (s, 1H, OH);^{13}C-NMR($CDCl_3$) δ: 16.71, 17.48, 31.37, 61.12(2 C), 127.21(2 C), 127.28, 132.75, 139.38, 171.09(CO). DEPT 135 δ: 18.42, 18.46, 31.4, 62.76, 127.64, 128.21, 128.93, 129.89, 134.31. (MALDI-TOF) calculated for$C_{15}H_{16}N_2O_4S[M]^+$: $C_{11}H_{16}N_2O_4S$ $[M]^+$: 272.08308 Found: 272.08221.

Synthesis of product(S)-N-hydroxy-3-phenyl-2-(phenylsulfonamido)propanamide (4b)

NMR ^{1}H: (300 MHz, $CDCl_3$)δ: 3.25(dd, J=14.8, J=5.6 Hz,2H), 4.13 (t, 1H), 7.20- 8.16 (m, 12H),11.35 (s, 1H, OH). ^{13}C: (75MHz, $CDCl_3$) δ: 37.30(CH_2), 57.23, 126.81-138.16 (12 C aromatic), 171.40(CO). DEPT 135 δ: 37.24(CH_2), 58.54, 127.49, 127.73, 128.83, 129.89, 129.94,134.26. HRMS (MALDI-TOF) calculated for$C_{15}H_{16}N_2O_4S[M]^+$: 320.08307 Found: 305.08162.

Synthesis of product (S)-N-hydroxy-4-methyl-2-(phenylsulfonamido)pentanamide (4c)

A yellowish solid, m.p: 153–156 °C;Yield 61.8%; ^{1}H NMR (300 MHz, $CDCl_3$)δ: 0.98 (d, 6H,$2CH_3$),1.5 (m, 1H), 1.89 (m, 1H), 3.88 (t, 1H), 7.56 - 8.22 (m, 7H),11.16 (s, 1H, OH); ^{13}C: (75MHz, $CDCl_3$) δ: 21.45, 21.98, 24.45, 38.44, 56.20, 124.70-128.9 (5C), 173.12(CO).DEPT 135 δ: 21.43, 22.75, 24.45(CH_2), 38.44,56.20, 127.46, 128.21, 129.9, 134.36. HRMS (MALDI-TOF) calculated for$C_{12}H_{18}N_2O_4S[M]^+$: 286.09761Found: 286.09218.

Synthesis of product (2S)-N-hydroxy-3-methyl-2-(phenylsulfonamido)pentanamide(4d)

White solid: m.p:161-164 °C.Yield 54%. NMR ^{1}H:(300 MHz, $CDCl_3$)δ: 0.93 (d, 6H, $2CH_3$),1.55 (m, 2H), 2.12 (m, 1H), 3.78 (d, J = 14.5, 1H), 7.61- 8.11 (m, 5H).^{13}C: (75MHz, $CDCl_3$) δ: 11.78, 14.16, 26.4, 34.28, 62.3 , 125.21-138.8 (C aromatic), 172.82(CO). DEPT 135 δ:11.46, 14.83, 25.28 (CH_2), 37.75, 127.63,129.89, 134.3. HRMS (MALDI-TOF) calculated for$C_{12}H_{18}N_2O_4S[M]^+$: 286.09872Found: 286.09213.

Biological evaluations

The synthesized compounds were tested for their antibacterial and antifungal activities against *Pseudomonas aeruginosa* and different fungal strains at concentration of 10 mg/ml in methanol. The yeasts were *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* and *Candida albicans* ATCC 2091 (Table 1). The zones of inhibition were clear with distinct borders, the diameters of inhibition were variable from 12 and 22 mm depending on the strain tested. The inhibition is noted positive when it is greater than 1 mm [16], (Fig. 1). The hydroxamic acid derivatives 4a-dwith differentsubstituents were able to inhibit the bacterial and fungal growth, The most promising hydroxamic acid derivative evaluatedwas 4b which showed the most inhibiting activity against the bacteria Pseudomonas aeruginosa. Compound 4aexhibited a very significantantifungal activity against *Candida parapsilosis* and *Candida albicans*. In a similar work, novel hydroxamic acid [19-20] has been synthesized and evaluated for its antimicrobial and antifungal activity using the Agar diffusion method. Results showed a narrow spectrum of antimicrobial activity. Minimal bactericidal concentrations of these substances are significantly higher than MMC of most antimicrobial drugs for human treatment.

TABLE 1. Antibacterial and antifungal activities of 4a-d Diffusion zone in mm

Bacteria	VL2 (4a)	PHA2 (4b)	IS2(4c)	IL2(4d)
Pseudomonas aeruginosa Yeast	16	21	17	18
Candida krusei ATCC 6258	12	7	7	8
Candida parapsilosis ATCC 22019	14	7	8	8
Candida tropicalis	7	7	7	7
Candida albicans ATCC 2091	15	7	12	14

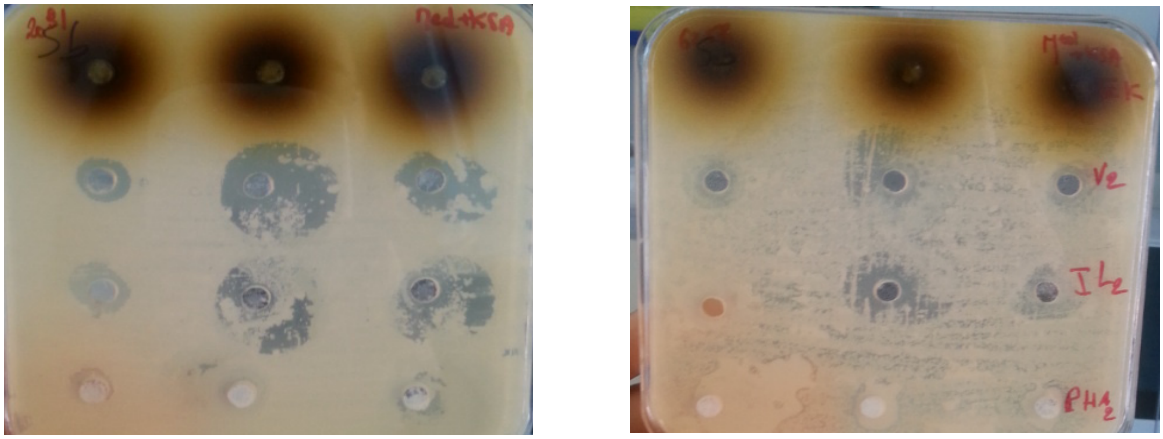


Fig. 1. Compound 4a and 4bagainst Candida parapsilosisand Candida albican

Conclusion

We have developed a facile method to synthesize different amino acid derived hydroxamic acid . The reaction offers convenience, mild conditions and excellent yields. These compounds were evaluated for their antibacterial and antifungal activity against some strains of fungi and bacteria. All tests proved that compounds 4a-dshowedexcellent antibacterial and antifungal activity. Compound 4b, was shown to be more effective in inhibiting the bacteria *Pseudomonas aeruginosa.* As for compound 4a against *Candida parapsilosis* and *Candida albicans* it showed a very significant antifungal activity.

Acknowledgment: I would like to acknowledge Professor Hassan Alhazmi ,God′s mercy, to Department of Chemistry , King Saud University.

References

1. Christian, M., Benjamin, W., Alexander, K., Andreas, K. and Franz, J.M.A., Perfluorinated hydroxamic acids are potent and selectiveinhibitors of HDAC-like enzymes from*Pseudomonas*. Bioorganic & Medicinal Chemistry Letters. **27**, 1508–1512 (2017)
2. Pal, D. K. and Saha, S., Hydroxamic acid A novel molecule for anticancer therapy. *J. Adv. Pharm. Tech. Res.,* **3**, 92-99 (2012).
3. Tardibono Jr, L. P. and Miller, M. J., Synthesis and evaluation of novelhydroxamicacids as potentantibacterial and antifungal agents. *Org. Lett.,* **11**, 1575-1578 (2009).
4. Jahangirian, H.,Haron, J.,Silong, S.,Yusof, N. A., Shameli, K., Eissazadeh, S., Moghaddam, R. R.,Mahdavi, B. and Jafarzade, M., *J. Med. P. Res.,***5**, 4826-4831 (2011).
5. Hector, R. B. and Lazo, W., Antialgal and antifungal activity of natural hydroxamic acids and related compounds. *J. Agric. Food Chem.,* **44**, 1569-1571 (1996).
6. Nakagawa, E., Amano, T., Hirai, N. and Iwamura, H., Non-induced cyclic hydroxamic acids in wheatduringjuvenile stage of growth. *Phytochemistry,***38**, 1349-1354 (1995).
7. Rho, H. S., Baek, H. S., Ahn, S. M.,Yoo, J. W., Kim, D. H. and Kim, H. G., *Bull. Korean Chem. Soc.,* **30,** 475-482 (2009).
8. Holland, K. P. Elford, H. L. Bracchi,V. Annis, C. G. Schuster, S. M. and Chakrabarti., D. Antimalarialactivities of polyhydroxyphenyl and hydroxamicacidderivatives. *Antimicrob. Agent. Chemother.,***42**, 2456-2458 (1998).
9. Dankwardt, S. M.,Martin, R. L.,Chan, C .S.,Van Wart, H. E.,Walker, K. A. M.,Delaet, N. G. and Robinson, L. A.,Amino acid derived sulfonamide hydroxamates as inhibitors of procollagen c-proteinase: solid-phase synthesis of ornithine analogues. *Bioorg. Med. Chem. Lett.,* **II**, 1465-1468 (2001).
10. Agrawal, Y. K. and Kaur, H., Poly (styrene-p-hydroxamic acids): synthesis, and ion exchange separation of rare earths. React. Funct. Polym., **39**, 155-164 (1999).
11. Agrawal, Y. K. and Patel, S. A.,Hydroxamic acids: Reagents for the solvent extraction and spectrophotometric determination of metals. *Rev. Anal. Chem.,* **4**, 237-276 (1980).
12. Huguet, F., Melet, A., Sousa, R. A., Lieutaud, A., Chevalier, J., Maigre, L., Deschamps, P.,Tomas, A. and Leulliot, N., Hydroxamicacids as potentinhibitors of Fe(II) and Mn(II) E. coli methionineaminopeptidase : biologicalactivities and X-ray structures of oxazolehydroxamate-EcMetAP-Mn complexes.*Chem.Med. Chem.,* **7**, 1020-1030 (2012).
13. Lee, C., Choi, E., Cho, M., Lee, B., Oh, S. J., Park, S. K.,Lee, K. H.,Kim , M. and Han, G.,Photoinduced DNA cleavage by anthracene based hydroxamic acids. Bioorg. Med. *Chem. Lett.,* **22**, 4189-4192 (2012).
14. Santos, M. A., Marques, S. M., Tuccinardi, T., Carelli, P., Panelli, L. and Rossello, A., Design, synthesis and molecularmodellingstudy of iminodiacetylmonohydroxamicacidderivatives as MMP inhibitors. *Bioorg. Med. Chem.,***14**, 7539- 7550(2006).
15. Levin , J. I.,The Design and Synthesis of Aryl Hydroxamic Acid Inhibitors of MMPs and TACE. *Curr. Top. Med. Chem.,* **4**, 1289-1310 (2004).
16. Schillinger, U. and Luke, F.K.,Antibacterial activity of Lactobacillus sake isolated from meat. *Appl. Environ . Microbiol,* **55**, 1901–1906 (1989).
17. Malkov, A. V.,Bourhani, Z. and Kočovský, P., Amino acid-derived hydroxamic acids as chiral ligands in the vanadium catalysed epoxidation. *Org. Biomol. Chem.,* **3**, 3194-3200 (2005).

18. Pasha, M.A., Jayashankara, V.P., Venugopala, K.N., and Rao, G.K., Zinc Oxide (ZnO): an efficient catalyst for the synthesis of 4-arylmethylidene-2-phenyl-5-(4H)-oxazolones having antimicrobial activity. Journal of Pharmacology and Toxicology, 2, 264- 270 (2007).

19. Stjepan, P., Branka, Z. and Ivan, B., Antimicrobial activity of some hydroxamic acids. *Acta Pharm.,* **55**, 401-408 (2005).

20. Eleni, P. and Dimitra, J.H.L., Acetic and Hydroxamic Acids as Novel Lipoxygenase Inhibitors. *Medicinal Chemistry,* **3**,251-64 (2006).

(*Received:* 12 / 4 / 2017;
accepted: 12 / 7 / 2017)