International Journal of Drug Design and Discovery

Volume 2 ● *Issue* 1 ● *January – March* 2011. 369-374

Synthesis, Characterization and Biological Activity of novel 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4''-nitrophenyl amino)-1, 3-oxazolidines

T.Panneer Selvam[1]*, P. Ramu[2] and A. Siva Kumar[3]

[1]Department of Pharmaceutical Chemistry, D.C.R.M. Pharmacy College, Inkollu-523 167, Andhrapradesh, India

[2]Department of Biotechnology, IITMadras, Chennai-600 036, Tamilnadu, India

[3]School of Bio Science and Technology, VIT University, Vellore-632 014 Tamilnadu, India

ABSTRACT: Here, we describe the design, synthesis, and biological evaluation of oxazolidine derivatives. Oxazolidine ring is a reduced form of oxazole system; many of its derivatives possess interesting biological activities, such as anti-convulsant, anti-microbial, analgesic, anti-inflammatory and anti-tumor. In our study, the biological activity of synthesized novel 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4''-nitrophenyl amino)-1, 3-oxazolidines **6a-e** were characterized by antimicrobial screening against several gram-positive, gram negative bacteria and fungus. The purity of the synthesized compounds was characterized by means of IR, ^{1}H-NMR, mass spectral and elemental analysis. Antimicrobial screening for all the compounds exhibits characteristic microbial inhibition against *Bacillus lentus, Micrococcus luteus, Bacillus cereus, Staphylococcus albus, Escherichia coli, Klebsiella aerogenes, Salmonella paratyphi, Proteus vulgaris* and *Candida albicans.*

KEY WORDS: Oxazolidine; Anti-bacterial; Anti-fungal

Introduction

The clinical potential of microbial product as therapeutic agents was first investigated by Pasteur and Joubert, who recorded their observation and speculations in 1877. The golden age of antibiotics began with the production of penicillin in 1941. The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram positive bacteria [1-5]. The N-aryloxazolidine moiety is present in a number of compounds which show diverse biological activities. Some of these activities include monoamine oxidase (MAO) inhibition [6], GP IIb/IIIa antagonism [7], neuroleptic activity [8], and antibacterial activity [9]. Due to this medicinal importance, methods for generating libraries of these compounds have received significant interest [10]. The compound class is particularly well recognized for its activity against clinically important susceptible and resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus*, ancomycin-resistant *Enterococcus faecium* and penicillin-resistant *Streptococcus pneumoniae* [11]. N-aryloxazolidines are also being rediscovered for their activity against *Mycobacterium* sp [12]. In the latter case, the increased prevalence of drug resistant Mycobacterium tuberculosis combined with the rise of Mycobacterium avium complex (MAC) infections in immuno-compromised populations has placed renewed attention on the potential use of oxazolidines for the treatment of mycobacterial infections [13]. Recently, a new class of oxazolidines was described. These oxazolidines showed impressive antibacterial activity and were less prone to inhibit MAOs [14]. Our analogue-based design encompasses the synthesis of 3-benzyl-2-(4'-substituted phenyl)-4(5H)-(4''-nitrophenyl amino)-1, 3-oxazolidines **6a-e** derivatives to be tested for their *in vitro* antimicrobial properties against Gram positive and Gram negative bacteria and fungus.

* *For correspondence:* T. Panneer Selvam,

Tel.: +91 9393753210

E-mail: tpsphc@gmail.com

Materials and Methods

Materials

Synthetic starting material, reagents and solvents were of analytical reagent grade or of the highest quality commercially available and were purchased from Aldrich Chemical Co., Merck Chemical Co. and were dried when necessary.

The melting points were taken in open capillary tube and are uncorrected. IR spectra were recorded with KBr pellets (ABB Bomem FT-IR spectrometer MB 104 ABB Limited Bangaluru, India). Proton (^{1}H) NMR spectra (Bruker 400 NMR spectrometer Mumbai, India) were recorded with TMS as internal references. Mass spectral data were recorded with a quadrupol mass spectrometer (Shimadzu GC MS QP 5000, Chennai, India), and microanalyses were performed using a *vario EL* V300 *elemental* analyzer (*Elemental* Analysensysteme *GmbH* Chennai, India). The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF_{254}, 200 mesh) aluminium plates (E.Merck) using ethyl acetate: benzene (1:3) and visualized in UV chamber. IR, ^{1}H-NMR, mass spectral datas and elemental analyses were consistent with the assigned structures.

General Procedures

The target novel oxazolidine derivatives **Scheme 1** were synthesized by previously reported method [15]. Accordingly, benzylamine **1** was treated with an equimolar amount of substituted benzaldehyde **2** and an hydroxy acetic acid **3** in dry toluene under reflux 24- 48 h to give 3-benzyl-2-(4'-substituted phenyl)-1,3-oxazolidine-4(5*H*)-one **4**, further its treat with thionyl chloride and DMF to get chloro derivative **5** 3-benzyl-2-(4'-substituted phenyl)-4(5H)-chloro-1,3-oxazolidine and then coupled with *p*-nitro anilines in DMF at 80^{0}C and quenched in ice-water to get the product were separated by filtration, vaccum dried and recrystallized from warm ethanol to yields 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines **6a-e**.

3-benzyl-2-(4'-hydroxy phenyl)-1,3-oxazolidine-4(5*H*)-one (4)

Yellow solid; Yield: 78%; mp. 183-185°C, IR : 3476 (O-H), 3096 (Ar-CH), 1728 (C=O), 1468 (C=C) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 9.84 (s, 1H, Ar-OH), 6.96-7.54 (m, 9H, Ar-H), 6.67 (s, 1H, -CH), 4.12-4.62 (m, 4H, 2 × CH_2); EI-MS (m/z, %): 269 [M]+; (Calcd for $C_{16}H_{15}NO_3$; 269.3). Anal. Calcd for $C_{16}H_{15}NO_3$, C, 71.36; H, 5.61; N, 5.20; Found: C, 71.41; H, 5.69; N, 5.27.

3-benzyl-2-(4'-hydroxy phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines (6a)

Pale yellow solid; Yield: 76%; mp. 156-158°C, IR : 3464 (O-H), 3027 (Ar-CH), 1494 (C=C), 1564 (N=O), 1306 (N-H bending), 3396 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 9.87 (s, 1H, Ar-OH), 6.76-7.27 (m, 13H, Ar-H), 6.31 (s, 2H, -CH), 7.21 (s, 1H, N-H), 3.44-3.67 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 391; (Calcd for $C_{22}H_{21}N_3O_4$; 391.42). Anal. Calcd for $C_{22}H_{21}N_3O_4$; C, 67.51; H, 5.41; N, 10.74; Found: C, 67.57; H, 5.44; N, 10.79.

3-benzyl-2-(4'-methoxy phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines (6b)

White solid; Yield: 89%; mp. 184-186°C, IR : 3026 (Ar-CH), 1524 (C=C), 1567 (N=O), 1316 (N-H bending), 3319 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.72-7.23 (m, 13H, Ar-H), 6.36 (s, 2H, -CH), 3.78 (s, 3H –OCH_3), 7.15 (s, 1H, N-H), 3.54-3.72 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 405; (Calcd for $C_{23}H_{23}N_3O_4$; 405.45). Anal. Calcd for $C_{23}H_{23}N_3O_4$; C, 68.13; H, 5.72; N, 10.36; Found: C, 68.19; H, 5.76; N, 10.31.

3-benzyl-2-(4'-methyl phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines (6c)

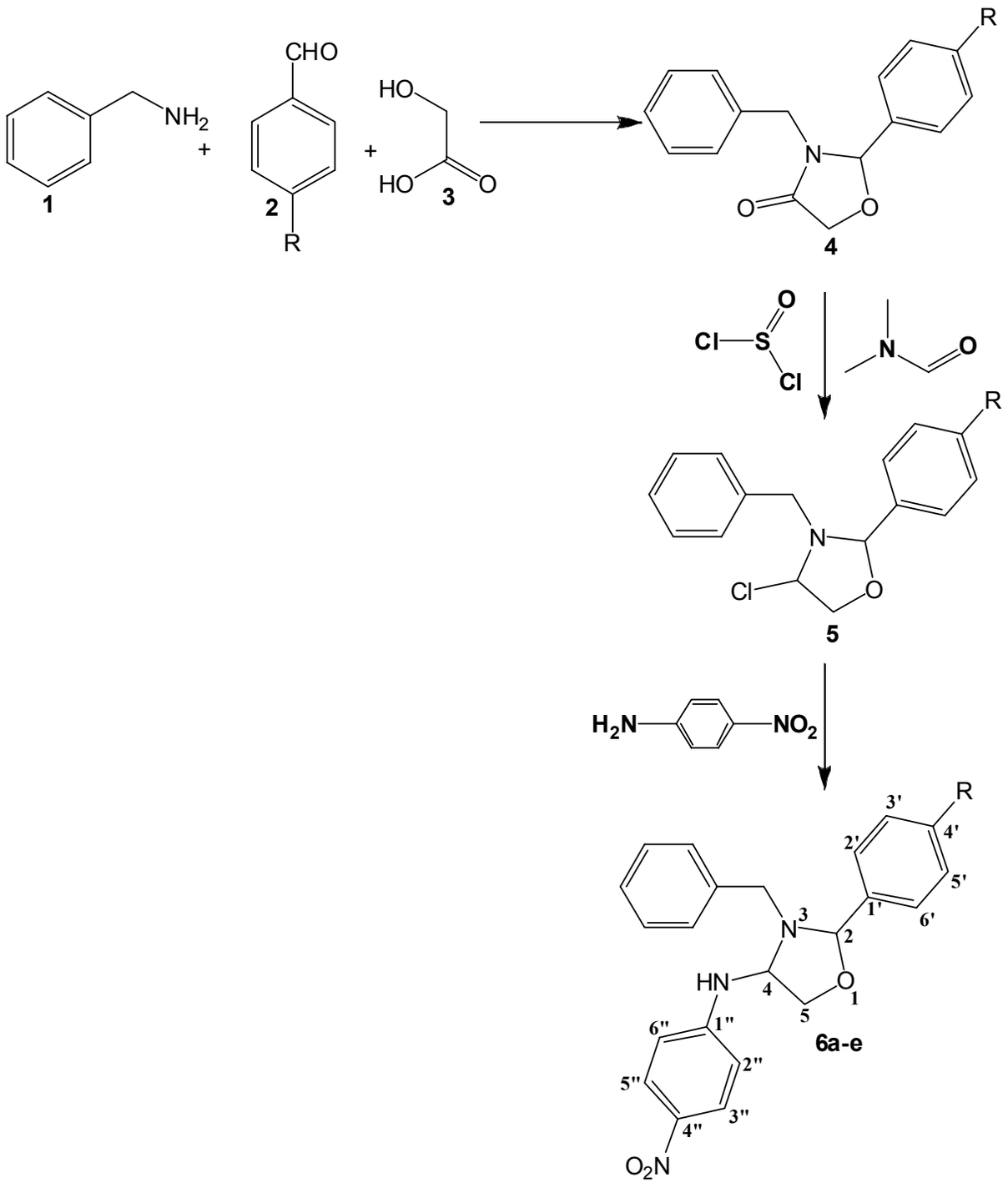
Paleyellow solid; Yield: 77%; mp. 170-123°C, IR : 3027 (Ar-CH), 1413 (C=C), 1570 (N=O), 1334 (N-H bending), 3313 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.62-7.18 (m, 13H, Ar-H), 6.29 (s, 2H, -CH), 3.69 (s, 3H –CH_3), 7.21 (s, 1H, N-H), 3.49-3.63 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 389; (Calcd for $C_{23}H_{23}N_3O_3$; 389.45). Anal. Calcd for $C_{23}H_{23}N_3O_3$; C, 70.93; H, 5.95; N, 10.79; Found: C, 70.95; H, 5.91; N, 10.83.

3-benzyl-2-(4'-nitro phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines (6d)

Pale solid; Yield: 71%; mp. 181-183°C, IR : 3027 (Ar-CH), 1413 (C=C), 1546 (N=O), 1334 (N-H bending), 3313 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.79-7.33 (m, 13H, Ar-H), 6.21 (s, 2H, -CH), 7.27 (s, 1H, N-H), 3.46-3.78 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 420; (Calcd for $C_{22}H_{20}N_4O_5$; 420.42). Anal. Calcd for $C_{22}H_{20}N_4O_5$; C, 62.85; H, 4,79; N, 13.33; Found: C, 62.87; H, 4,75; N, 13.37.

3-benzyl-2-(4'-chloro phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines (6e)

Brown solid; Yield: 81%; mp. 184-186°C, IR : 3026 (Ar-CH), 1524 (C=C), 1532 (N=O), 1316 (N-H bending), 3319 (N-H stretching), 749 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.71-7.37 (m, 13H, Ar-H), 6.34 (s, 2H, -CH), 7.31 (s, 1H, N-H), 3.48-3.81 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 409; (Calcd for $C_{22}H_{20}ClN_3O_3$; 409.87). Anal. Calcd for $C_{22}H_{20}ClN_3O_3$; C, 64.47; H, 4.92; N, 10.25; Found: C, 64.43; H, 4.99; N, 10.29.


NH2
CHO
HO
HO
O
R
1
2
3
4
5
Cl
S
O
N
Cl
H2N
NO2
HN
6a-e
O2N
R = OH, OCH3, CH3, NO2, Cl

Scheme 1

Antimicrobial Screening

The biological evaluation of synthesized compound was performed using the disk diffusion method [16]. In the present study four gram-positive, four-gram negative, and one fungus were selected. The gram positive strains were *Bacillus lentus* (ATCC 155), *Bacillus cereus* (ATCC 11778), *Micrococcus luteus* (ATCC 4698), *Staphylococcus albus* (ATCC 9144); gram negative strains were *Escherichia coli* (ATCC 25922), *Klebsiella aerogenes* (ATCC 2853), *Salmonella paratyphi* (ATCC 11298), *Proteus vulgaris* (ATCC 9029) and fungus *Candida albicans* (ATCC 2091). The strain was confirmed for its purity and identity by the gram-staining method and it was further characterized by chemical reaction. The selected strains were preserved by periodical sub culturing on agar slant and storing them under frozen condition; for the study fresh 24 h broth cultures were used. Each bacterial and fungal pure culture was transferred into 100 ml of Muller Hinton nutrient broth and Sabouraud's dextrose broth, respectively. The inoculated broths were incubated at 37^0C for 24 h and 27^0C for 72 h for bacteria and fungus respectively. After incubation, inocula were standardized to 10^8 colony-forming units (CFU)/ml for bacteria and 10^6 CFU/ml for fungus by colony forming unit method. Muller Hinton agar media was prepared by using Beef infusion 300 g, Casein acid hydrolysation 17.5 g, starch 1.5 g, and agar 17 g. Accurately weighed quantities of these ingredients were suspended in 1,000 ml of distilled water. They were boiled to dissolve completely. The pH was adjusted to 7.3 ± 0.2 at 25^0C. It was then sterilized by autoclaving at 15 lbs pressure (121^0C for 15 minutes). The prepared Muller Hinton agar medium was transferred into sterile Petri plates; 200 µl of the standardized bacterial inoculums and fungus inoculum were spread on agar medium using sterile cotton swab. The synthesized product of oxazolidine derivatives were dissolved in suitable chloroform solvent to a final concentration of 50 µl of drug solution, each disk absorbed approximately 10 µl of the drug. The drug was impregnated on disk and placed on the inoculated agar medium. Ciprofloxacin and clotrimidazole were used as a standard for the antibacterial and antifungal activity respectively. All the bacterial Petri plates were kept in an incubator and the fungal Petri plate was kept at room temperature for approximately 18 h. Then the zones of inhibition were measured.

Results and discussion

Chemistry

The synthesized series of heterocycles, **6a-e** by the reaction of **5** with appropriate *p* –nitro aniline in the presence of DMF as presented in **Scheme 1**. The IR, ^{1}H-NMR, mass spectroscopy and elemental analysis for the new compound is in accordance with the assigned structures. The IR spectra of compounds **4** showed stretching bands of keto group at 1728 cm^{-1}. In **5**, stretching bands of chloro group at 749 cm^{-1} is evidence to conversion of oxazolidinone. The title compounds **6a-e** stretching and bending NH bands appear at 3300-3400 cm^{-1}, 1300-1350 cm^{-1} respectively. The recorded IR spectrum of representative compounds **6a-e** showed missing of chloro group bands. This clearly envisages that the chloro group of **5** is converted into secondary NH. The proton magnetic resonance spectra of oxazolidine and their corresponding derivatives have been recorded in $CDCl_3$. In this **6a-e** NH signal of 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines moiety appear at 7.26 (s), 7.15 (s), 7.21 (s), 7.27 (s), 7.34 (s), ppm respectively. The position and presence of NH signal in the ^{1}H-NMR spectra of final compounds conforms the secondary NH proton in oxazolidine moiety. This clearly envisages that oxazolidine-4(5*H*)-one moiety involve in 4(5*H*)-chloro-1,3-oxazolidine and further (4"-nitrophenyl amino)-1,3-oxazolidines formation. All these observed facts clearly demonstrate that the 4^{th} position of keto group in oxazolidine ring is converted into secondary amino group as indicated in **scheme 1** and conforms the proposed structure (**6a -e**).

Antimicrobial

The antimicrobial screening of all the compounds showed an excellent zone of inhibition against both gram-positive and gram-negative bacteria than standard ciprofloxacin. Similarly, the zone of inhibition on the fungal strain showed a stronger activity than the standard clotrimidazole. New derivatives of 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines **6a-e** series exhibits stronger inhibition on gram-negative *Escherichia coli* compared with other bacterial strains. On the other hand, *Candida albicans* zone was highly inhibited by title compounds **6a-e**, which proves the efficiency of antifungal activity than anti-bacterial activity. The range of inhibition on *Staphylococcus albus* comparatively smaller than other bacterial species. Discussing the antimicrobial activity against individual organisms, it was clear that all the compounds have significant inhibitions. It was found that the *Escherichia coli* and *Candida albicans* were highly susceptible to killing by the synthesized 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines **6a-e** derivatives. The observed data on the anti-microbial activity of the synthesized compounds and standard drugs are given in **Table 1**.

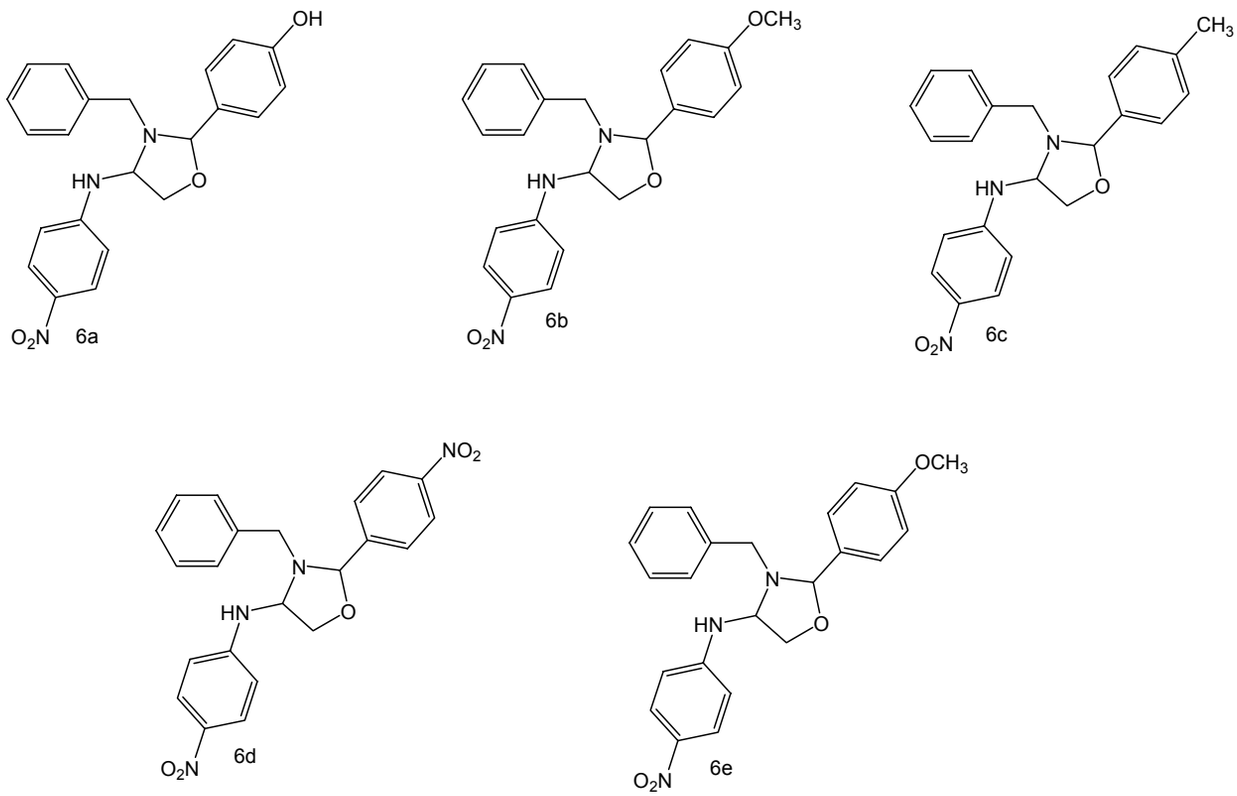


Fig. 1 Schematic structure for the synthesized oxazolidine derivatives

Table 1 Diameter of zone of inhibition by individual compounds against gram-positive, gram-negative bacteria, and fungus

Zone of inhibition in mm							
Organism	**Standard[a]**	**6a**	**6b**	**6c**	**6d**	**6e**	**Solvent[b]**
Gram +ve bacteria							
Bacillus lentus	8	16	17	16	16	17	5
Micrococcus luteus	7	15	14	13	11	15	2
Bacillus cereus	8	11	13	13	14	16	2
Staphylococcus albus	7	12	12	11	9	15	3
Gram -ve bacteria							
Escherichia coli	15	20	18	17	20	18	3
Klebsiella aerogenes	9	16	15	15	14	16	4
Salmonella paratyphi	7	15	14	14	15	14	3
Proteus vulgaris	7	16	15	16	15	17	3
Fungus							
Candida albicans	13	21	21	19	24	20	2

[a]Standard ciprofloxacin for bacteria, clotrimidazole for fungal

[b]Chloroform

References

[1] Tenover, F.C.; McDonald, L.C. Curr. Opin. Infect. Dis. 2005, 18, 300.

[2] Pfeltz, R.F.; Wilkinson, B.J. Curr. Drug Targets Infect. Disord. 2004, 4, 273.

[3] Roberts, M.C. Curr. Drug Targets Infect. Disord. 2004, 4, 207.

[4] Dessen, A.; Di Guilmi, A.M.; Ernet, T.V.; Dideberg, O. Curr. Drug Targets Infect.Disord. 2001, 1, 63.

[5] Muroi, H.; Nihei, K.; Tsujimoto, K.; Kubo, I. Bioorg. Med. Chem. 2004, 12, 583.

[6] (a) Kan, J. P.; Malnoe, A.; Strolin Benedetti, M. J. Pharm. Pharmacol. 1978, 30, 190. (b) Moureau, F.; Wouters, J.; Vercauteren, D. P.; Collin, S.; Evrard, G.; Durant, F.; Ducrey, F.; Koenig, J. J.; Jarreau, F. X. Eur. J. Med. Chem. 1992, 27, 939. (c) Curet, O.; Damoiseau, G.; Aubin, N.; Sontag, N.; Rovei, V.; Jarreau, F. X. J.Pharm. Exp. Ther. 1996, 277, 253. (d) Ramsay, R. R.; Gravestock, M. B. Mini-Rev.Med. Chem. 2003, 3, 129.

[7] Gante, J.; Juraszyk, H.; Raddatz, P.; Wurziger, H.; Bernotat-Danielowski, S.; Melzer, G.; Rippmann, F. Lett. Pep. Sci. 1995, 2, 135.

[8] (a) Aschwanden, W.; Kyburz, E.; Schoenholzer, P. Helv. Chim. Acta. 1976, 59, 1245. (b) Pruecher, H.; Gottschlich, R.; Haase, A.; Stohrer, M.; Seyfried, C. Bioorg. Med. Chem. Lett. 1992, 2, 165.

[9] (a) Slee, A.M.; Wuonola, M.A.; McRipley, R.J.; Zajac, I.; Zawada, M.J.; Bartholomew, P.T.; Gregory, W.A.; Forbes, M. Antimicrob. Agents Chemother. 1987, 31, 1791. (b) Gregory, W.A.; Brittelli, D.R.; Wang, C.L.; Wuonola, M.A.; McRipley, R.J.; Eustice, D.C.; Eberly, V.S.; Bartholomew, P.T.; Slee, A.M.; Forbes, M. J. Med. Chem. 1989, 32, 1673. (c) Barbachyn, M.R.; Ford, C.W. Angew. Chem. Int. Ed. 2003, 42, 2010; (d) Zappia, G.; Menendez, P.; Delle Monache, G.; Misiti, D.; Nevola, L.; Botta, B. Mini-Rev. Med. Chem. 2007, 7, 389.

[10] (a) Dyen, M.E.; Swern, D. Chem. Rev. 1967, 67, 197; (b) Ager, D.J.; Prakash, I.;

Schaad, D.R. Aldrichim. Acta. 1997, 30, 3. (c) Zappia, G.; Gacs-Baitz, E.; Delle Monache, G.; Misiti, D.; Nevola, L.; Botta, B. Curr. Org. Syn. 2007, 4, 81.

[11] (a) Clemett, D.; Markham, A. Drugs 2000, 59, 815. (b) Perry, C.M.; Javis, B. Drugs 2001, 61, 525. (c) Norrby, R. Exp. Opin. Pharmacother. 2001, 2, 293. (d) Stevens, D.L.; Herr, D.; Lampiris, H.; Hunt, J.L.; Batts, D.H.; Hafkin, B. Clin. Infect. Dis. 2002, 34, 1481.

[12] (a) Ashtekar, D.R.; Costa-Periera, R.; Shrinivasan, T.; Iyyer, R.; Vishvanathan, N.; Rittel, W.S. Diagn. Microbiol. Infect. Dis. 1991, 14, 465. (b) Cynamon, M.H.; Klemens, S.P.; Sharpe, C.A.; Chase, S. Antimicrob. Agents Chemother. 1999, 43, 189. (c) Wallace, R.J., Jr.; Brown-Elliott, B.A.; Ward, S. C.; Crist, C.J.; Mann, L.B.; Wilson, R.W. Antimicrob. Agents Chemother. 2001, 45, 764.

[13] Sood, R.; Bhadauriya, T.; Rao, M.; Gautam, R.; Barman, S.; Malhotra, T.K.; Upadhyay, D.J.; Rattan, A. Infect. Dis. Drug Targets. 2006, 6, 343.

[14] (a) Phillips, O.A.; Udo, E.E.; Ali, A.A. M.; Al-Hassawi, N. Bioorg. Med. Chem. 2003, 11, 35. (b) Reck, F.; Zhou, F.; Girardot, M.; Kern, G.; Eyermann, C.J.; Hales, N.J.; Ramsay, R.R.; Gravestock, M.B. J. Med. Chem. 2005, 48, 499. (c) Hauck, S. I.; Cederberg, C.; Doucette, A.; Grosser, L.; Hales, N.J.; Poon, G.; Gravestock, M. B. Bioorg. Med. Chem. Lett. 2007, 17, 337. (d) Phillips, O.A.; Udo, E. E.; Ali, A.A. M.; Samuel, S. M. Eur. J. Med. Chem. 2007, 42, 214.

[15] Zarghi, A.; Najafnia, L.; Daraie, B.; Dadrass, O.G.; Hedayati, M. Bioorg Med Chem Lett. 2007, 17, 5634–5637.

[16] Rameshkumar, N.; Ashokkumar, M.; Subramaniyan, E.H.; Ilavarasan, R.; Sridhar, S.K. Eur. J. Med. Chem. 2003, 38, 1001–1004.