Received Date : 21-Oct-2014
Revised Date : 13-Jan-2015
Accepted Date : 16-Jan-2015
Article type : Research Article

Novel oxazolidinone antibacterial analogues with a substituted ligustrazine C-ring unit

Yan Chen[+], Zhi-Xiong Ruan[+], Fang Wang, De-Sheng Huangfu, Ping-Hua Sun, Jing Lin, Wei-Min Chen[*]

College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China

[+]*These authors contributed equally.*

**Corresponding author. Tel.: +86 20 8522 4497; Fax: +86 20 8522 4766.*

E-mail address: twmchen@jnu.edu.cn

Abstract: A series of novel oxazolidinone compounds with a substituted ligustrazine C-ring unit and different substituted groups at the C-5 side chain were designed and synthesized by using linezolid as a lead and based on a scaffold hopping strategy. Their antibacterial and anti-inflammatory activities were evaluated. The results of *in vitro* antibacterial assays showed that all fourteen target compounds displayed potent activity against gram-positive pathogens, particularly **8b**, **13b**, **14a**, **14b**, **15a** and **15b**. Moreover, **14a** and **14b** exhibited significant inhibitory activities on the production of inflammatory mediators, including nitric oxide (NO), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Thus, these derivatives could serve as valuable candidates to develop anti-infective agents for the treatment of chronic wounds.

Keywords: Antibacterial; Anti-inflammatory; Linezolid; Ligustrazine; Oxazolidinone;

Introduction

The alarming increasing rate of bacterial pathogens resistant to existing antimicrobials has become a serious problem in current medicine. In particular, the emergence of drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *epidermis Staphylococcus* (MRSE) severely reduce the efficacy of existing drugs, which leads to the significantly extension of treatment time and the increase of mortality. Therefore, it is urgent to discover novel antibacterial agents that are effective against the resistance strains to overcome the growing problem of antibiotics resistance. A possible approach of solving this problem is to develop antibacterial drugs with novel structure and unique mechanism (1, 2).

Linezolid, which is the first oxazolidinone antibacterial agent (Figure 1), has shown promising effect for the treatment of multiple drug-resistant infections caused by gram-positive bacteria. Oxazolidinones are a new class of antibacterial agents, and their action sites are located inside the ribosomal 50S subunit near the peptidyl transferase center (PTC) and peptidyl site (P), overlapping with peptide acyl transferase inhibitor (such as clindamycin and chloramphenicol) loci (3). However, with excessive use of linezolid, it has raised serious concerns that the emergence of linezolid-resistant strains such as *Staphylococcus aureus* (4, 5) and *Enterococcus* (6) in the clinic. In the last few years, a variety of structural modifications of linezolid has been carried out towards broadening the antibacterial spectrum, re-establishing sensitivity to linezolid-resistant strains (7-10).

The infections caused by drug-resistant bacteria, whatever gram-positive or gram-negative bacterium, often lead to chronic inflammation, even aggravate diseases or induce permanent organ damage (11), and bacterial profile often observed in many chronic wounds, as an example, *Staphylococcus aureus* (in 93.5% of the investigated ulcers), *Enterococcus faecalis* (71.7%), *Pseudomonas aeruginosa* (52.2%), coagulase-negative *staphylococci* (45.7%), *Proteusspecies* (41.3%), and anaerobic bacteria (39.1%) were found in venous leg ulcers (12). Currently, to cure these chronic infection phlogosis such as venous leg ulcers, pressure ulcers, and diabetic foot ulcers is still with great difficulties (13, 14). Thus, there is an urgent need to develop drugs with both antibacterial and anti-inflammation effects to treat the chronic wounds.

Inflammation is very common and important basic pathological process and a fundamental defensive response of the immune system to pathogens or harmful irritants (15). The classical phenomena of inflammation are redness, swelling, heat, and pain (16). For example, excessive production of inflammatory mediators, such as nitric oxide (NO), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (17-19) may be observed after infection or injury, which have been implicated in many inflammatory diseases (20, 21).

Ligustrazine (tetramethylpyrazine, TMP; Figure 1) is one of the major efficient component from traditional Chinese medicine herb *Chuanxiong* (*Ligusticum wallichii Franchat*) (22). It has been found that ligustrazine shows a certain degree of anti-inflammatory effects both *in vivo* and *in vitro* (23-28). This encouraged us to design new compounds using ligustrazine moiety to hybridize with oxazolidinone for exploring their anti-inflammatory activity. Therefore, a series of novel oxazolidinone compounds with a substituted ligustrazine C-ring unit and different substituted groups at the C-5 side chain were designed and synthesized based on the template compound linezolid so as to obtain novel oxazolidinone anti-infective drugs with both antibacterial and anti-inflammatory activities.

Materials and Methods

General synthetic procedure

All reagents and solvents used were purchased from common commercial of analytical grade. ^{1}H NMR and ^{13}C NMR were recorded on a 300 MHz spectrometer with TMS as the internal standard. Proton and carbon chemical shifts are expressed in parts per million (ppm) and coupling constants in Hz. Mass spectra (ESI-MS) and high resolution mass spectrometry (HRMS) were performed.

Detailed synthetic procedures and characterization data for the synthesized compounds are available in the Supporting Information (Appendix S1) of this manuscript.

Antibacterial assay

In this study, thirteen strains including nine different MRSA strains, *S.aureus* ATCC 25923, *S.epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922, were tested and stored at -80 ^{o}C. Tryptic soy broth (TSB) was prepared according to the manufacturer's instructions. After all bacterial were recovered, all strains were inoculated into 5 mL sterilized TSB, cultured at 37 ^{o}C for 16-18 h.

Minimum Inhibitory Concentration (MIC) was determined by the micro-broth dilution methodology, according to the guideline of the Clinical and Laboratory Standards Institute (CLSI) (29). Each tested compound was dissolved in a certain amount of dimethyl sulphoxide. Linezolid was chosen as a positive control drug. The bacteria solution was corrected to 0.5 McFarland standard (amount of bacteria about 108 colony-forming units/mL) using TSB. Then, it was diluted 1:100 with TSB broth and inoculated immediately. First, the concentration of each compound including linezolid was severally allocated to 256 μg/mL, 128 μg/mL, 64 μg/mL, 32 μg/mL, 16 μg/mL, 8 μg/mL, 4 μg/mL, 2 μg/mL and 1μg/mL. 100 μL different concentrations of each compound including linezolid was added into the holes of 1-12 rows of a 96-hole plate, then, 100 μL diluted bacterium solution was also added into each hole and tapped to mix sufficiently. The seventh line of the 96 well plate was used for the blank control group and the eighth line was used for the negative control (TSB broth 100 μL, diluted bacterium solution 100 μL). Finally, the plate was kept in incubator at 37 ^{o}C for 18-24 h. Meanwhile, each bacterium was repeated for three times.

Anti-inflammation assay

RAW264.7 murine macrophages were cultured in DMEM containing 10% new-born calf serum, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 ^{o}C in a 5% CO_2 humidified atmosphere.

Assay for NO production

RAW264.7 cells were inoculated at 10×10^4 cells per well in 96-well plate and cultured for 18 h. The concentration of each compound including the positive drugs (linezolid, TMP and indomethacin) was designed to 100 μM, 50 μM, 20 μM, 10 μM, 5 μM, 2 μM and 1 μM.

Then, the cells were pre-handled with different concentration compounds for 2 h and stimulated with LPS (100 ng/mL) for 24 h. Then, the Griess reagent was used to determine the NO produced in the culture medium, which was made up of 1% sulfanilamide and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in 5% phosphoric acid), that is to say, added 100 μL Griess reagent to 100 μL cultural supernatants and mixed sufficiently at room temperature. Then the plate was measured absorbance of the samples at 540 nm (OD_{540}) in a microplate reader (Bio-Rad Laboratories, CA, USA). Meanwhile, the results were evaluated from three independent experiments.

Cell Cytotoxicity

Cell cytotoxicity was evaluated by methyl thiazolyl tetrazolium (MTT) assay. RAW264.7 cells were inoculated at 8×10^3 cells per well in 96-well plate. The cells were treated with different compounds which were diluted in DEME for 48 h until the cells were cultured overnight. Then, 20 μL of 0.5 mg/mL MTT reagent was added into the each well and incubated for 4 h, followed by removing the cell supernatant and adding 150 μL DMSO to dissolve the formazan. The optical density was measured at 570 nm (OD_{570}). Cell viability was assessed from three independent experiments. The density of formazan formed in control group was set as 100% of viability.

Cell viability (%) = [compound (OD_{570})-blank (OD_{570})] / [control (OD_{570})-blank (OD_{570})] × 100%

Blank: fresh medium only

Control: the cells cultured with fresh medium only

Compound: treated with compounds

Measurement of IL-6 and TNF-α

RAW264.7 cells were inoculated at 5×10^5 cells per well in 24-well plate and pretreated with different concentrations (10 μM, 5 μM, 2.5 μM, 1.25 μM) of compounds, except TMP (200 μM to 20 μM) for 2 h, and then added LPS. The production of IL-6 and TNF-α was

stimulated by the addition of 100 ng/mL LPS and incubated for 24 h. The content of IL-6 and TNF-α in the supernatant were determined using the mouse ELISA kit (TNF-α; IL-6) which were operated according to the manufacturer's instructions.

Results and discussion

Design of compounds

In order to develop novel anti-infective agents that are effective against the resistance strains, drug designs were carried out based on linezolid (Figure 2). Firstly based on a scaffold hopping strategy, the C-ring unit of linezolid was replaced with ligustrazine fragment (3,5,6-trimethyl-pyrazin-2-ylmethoxy group) which has been reported to show anti-inflammatory activity. Meanwhile, further modifications of the new skeleton compound were carried out at C-5 side chain, since the reported structural modifications of oxazolidinone compounds were mainly concentrated in the C-5 side chain and the C-ring unit (30-34) to optimize their biological activities. It has been reported that the introducing of acetamide group (3, 35, 36) or triazole group (37-40) at the C-5 position in oxazolidinone compounds shows good antibacterial activities, thus acetamide group or triazole group were chosen for the derivatization of C-5 side chain. Additionally, a growing body of evidence suggests that the introduction of halogen group in the anti-bacterial agents could improve their biological activities (41), thus, F atom and Cl atom were introduced to some designed compound.

Synthesis

The synthetic route of the novel oxazolidinone compounds is depicted in Scheme 1. Ligustrazine (TMP) was used as a starting material. It was firstly brominated to produce TMP-Br, which was then reacted with *p*-nitrophenol or 2-fluoro-4-nitrophenol to give the intermediate **A1** and **B1**. After a few reaction steps including reduction of the nitro group, carbamate protection of amino group with benzyl chloroformate and a ring closure using (*R*)-glycidyl butyrate and LiHMDS, the 5-hydroxymethyl oxazolidinone derivatives **1a** and **1b** were obtained.

Fluoromethyl oxazolidinones **2a** and **2b** were prepared from the reaction of **1a** and **1b** with diethylaminosulphurtrifluoride (DAST) as fluorinating agent. After the hydroxyl groups of **1a** and **1b** were converted into the corresponding mesylate group using mesyl chloride, the resulting compounds **3a** and **3b** were reacted with a variety of nucleophiles to yield the 5-substituted oxazolidinone derivatives. Replacement of methanesulfonate group in compounds **3a** and **3b** with a methoxy group was easily accomplished to get methoxy methyl oxazolidinones **4a** and **4b** using sodium methoxide in methanol at room temperature. Dimethylamino-substituted methyl oxazolidinones **5a** and **5b** was obtained from the reaction of **3a** and **3b** with dimethylamine hydrochloride under K_2CO_3 in DMF. To prepare compounds containing 1,2,4-triazole and 1,2,3-triazole moieties, unsubstituted triazole compounds **6a**, **6b**, **7a**, **7b, 8a** and **8b** were prepared according to the literature (42). The substituted triazole compounds **10a**, **10b**, **11a** and **11b** were easily synthesized using 1,3-dipolar cycloaddition condition (40) on azides **9a** and **9b**, which were obtained from reacting **3a** and **3b** with sodium azide in DMF. Finally, several 5-substituted amide compounds (**13a**-**17a** and **13b**-**17b**) were synthesized by acylation of compounds **12a** and **12b**, which were prepared from reduction of **9a** and **9b** in Pd/C under hydrogen at room temperature in THF.

Antibacterial activity

The *in vitro* antibacterial activity of compounds **1a-17a** and **1b-17b** were evaluated and the results were summarized in Table 1. Compounds **1b, 8a, 8b, 10b, 13a-17a and 13b-17b** showed potential antibacterial activities against gram-positive bacteria, while they were inactive against gram-negative bacteria. Meanwhile, compounds 1**a-7a, 10a, 11a, 2b-7b and 11b** showed no antimicrobial activity which were not listed in Table 1. Among these active compounds, **8b**, **13b, 14a, 14b, 15a** and **15b** which were listed and bolded in Table 1, exhibited potent antibacterial activities against gram-positive bacteria. **13b, 14b and 15b** showed the most prominent activities against *S. epidermidis*. **15b** showed the best activity against *S. aureus* which was comparable to linezolid. **14b** and **15b** also showed potent activities against *Enterococcus faecalis*. Moreover, **13b, 14b** and **15b** exhibited almost the same activities against nine different MRSA strains. It is obvious that compounds **14b** and

15b provided significant activities against resistant strains, which were equivalent to that of linezolid.

Inhibition of NO production in (LPS)-stimulated RAW264.7 cells

Nitric oxide (NO) is an important indicator of inflammatory determination, which is one of cytokines produced by active cells in the inflammation process. Excessive production of NO was needed to be connected with the pathogenesis of inflammatory diseases, and it is reported that NO inhibitors may provide the possibility of new therapeutic method for the inflammatory diseases (43). Meanwhile, it has been reported that TMP shows anti-inflammatory effects (44, 45). The NO levels were decreased after treatment with 10 μM or 100 μM TMP in UVA treated keratinocyte monoculture cells or melanoma/keratinocyte co-culture cells, respectively (45). Meanwhile, TMP could also decrease the UV-induced increase of cytokines such as TNF-α (45).

So all synthesized compounds were tested for their inhibitory activities against lipopolysaccharide (LPS)-induced NO release in RAW264.7 cells (LPS treatment caused significant changes in cell morphologies, which indicated that inflammation could be induced by LPS). Thus, linezolid, ligustrazine and indomethacin (Figure 1) were chosen as positive controls. The results show that most of the novel oxazolidinone compounds did not show the NO inhibitory activity. While, as shown in Table 2, compounds **14a** and **14b** (Figure 1) displayed improved NO inhibitory activity compared to linezolid, TMP and indomethacin.

Cytotoxicity in RAW264.7 cells

To confirm whether the NO inhibitory activities of **14a** and **14b** at the concentration of 10 μM were related to their effects on the cell viability, their cytotoxicities in RAW264.7 cells were examined by methyl thiazolyl tetrazolium (MTT) assay. As shown in Table 3 and Figure 3, all the agents (linezolid, TMP, indomethacin, **14a** and **14b**) at the concentration we used here had no obviously cytotoxicity in RAW264.7 cell, the relative cell viabilities of the treated cells were all more than 90%. Besides, it was shown that TMP did not induce significant cell death and with no obvious cytotoxicity to cells even up to 200 μM. These results indicated that the NO inhibitory effects of **14a** and **14b** were likely to be attributed to

the interaction of the substituent group of these two compounds with their specific target. These non-toxic concentrations were further used in subsequent experiment processes.

Inhibition of IL-6 and TNF-α production in RAW264.7 cells

IL-6 and TNF-α are two other critical pro-inflammatory cytokines in inflammatory diseases, and it is widely accepted that the inhibition of their generation would be an effective method for treatment of inflammation (46). Therefore, the inhibitory effects of **14a** and **14b** on IL-6 and TNF-α production were investigated. Linezolid, TMP and indomethacin were also used as positive reference drugs. As shown in Table 4, both **14a** and **14b** showed better inhibitory effects on the LPS-induced production of IL-6 and TNF-α than the positive control drugs (linezolid and indomethacin) at both concentrations of 10 μM and 5 μM. Meanwhile, TMP could decrease the LPS-induced TNF-α and IL-6 production but in a weak degree in a concentration-dependent manner (200 μM to 20 μM). It is worth noting that compound **14b** exhibited significant improvement on inhibition of IL-6 production compared to control drugs (Figure 4).

Taken together, these results suggest that there is a feeble anti-inflammatory activity of TMP and indomethacin in the LPS-induced RAW264.7. However, compounds **14a** and **14b** with TMP unit exhibit significant anti-inflammatory activities while linezolid does not show. Besides, **14a** and **14b** possess potent antibacterial activities which are equivalent to linezolid. These results suggest that the combination of ligustrazine moiety not only make the novel oxazolidinone derivatives (especially **14a** and **14b**) keep the antibacterial activities as linezolid, but also significantly enhance their anti-inflammatory activities.

Additionally, some preliminary structure-activity relationship (SAR) of the novel oxazolidinone could be summarized: (a) introducing fluorine into molecule would increase its antibacterial and anti-inflammatory activities, such as compounds **14a** and **14b**; (b) the volume of the chlorine substituent of acetyl group exerted influences on the antibacterial and anti-inflammatory activities. Apparently, monochloro and dichloroacetamide derivatives (compounds **14a**, **14b** ,**15a** and **15b**) showed better antibacterial activities, and monochloro

derivatives with TMP(compounds **14a** and **14b**) also exhibited better anti-inflammatory activities than linezolid; (c) different N-substituted triazole exerted different antibacterial activities (compounds **8a** and **8b** compared to **6a-7a** and **6b-7b**); (d) the introduction of a furan ring could exhibited potent antibacterial activities, such as compounds **17a** and **17b**.

Conclusions

In this study, a series of novel oxazolidinone derivatives with a substituted ligustrazine C-ring unit were synthesized by using linezolid as a lead and based on a scaffold hopping strategy. Meanwhile their antibacterial and anti-inflammatory activities were evaluated. Most of these compounds showed significant antibacterial activities against gram-positive pathogens. It's worth noting that two promising compounds **14a** and **14b** with ligustrazine fragment replaced C-ring and chloroacetamide group at the C-5 side chain, not only showed potent antibacterial activity, but also exhibited significant anti-inflammatory activity. And these results revealed that combination of the structural features with anti-inflammatory fragment into the potential anti-bacterial agents is likely to be a valuable strategy to obtain drug with both anti-bacterial and anti-inflammatory effects for the treatment of chronic wounds.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (No.81072554).

Conflict of interest

The authors have declared no conflict of interest.

References and notes

1. Michalska K., Karpiuk I., Król M., Tyski S. (2013) Recent development of potent analogues of oxazolidinone antibacterial agents. Bioorg Med Chem; 21: 577-591.
2. Yang Y., You Q.D. (2010) Advances in the Researches on SARs and Structure

Modification of Oxazolidinone Antibacterial Agents. Prog Pharm Sci; 34: 481-490.

3. Barbachyn M.R., Ford C.W. (2003) Oxazolidinone structure–activity relationships leading to linezolid. Angew Chem Int Ed; 42: 2010-2023.
4. Tsiodras S., Gold H.S., Sakoulas G., Eliopoulos G.M., Wennersten C., Venkataraman L., Moellering R.C., Ferraro M.J.(2001) Linezolid resistance in a clinical isolate of Staphylococcus aureus. Lancet; 358: 207-208.
5. Toh S.M., Xiong L., Arias C.A., Villegas M.V., Lolans K., Quinn J., Mankin A.S. (2007) Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant Staphylococcus aureus resistant to the synthetic antibiotic linezolid. Mol Microbial; 64: 1506-1514.
6. Gonzales R.D., Schreckenberger P.C., Graham M.B., Kelkar S., DenBesten K., Quinn J.P. (2001) Infections due to vancomycin-resistant Enterococcus faecium resistant to linezolid. Lancet; 357: 1179.
7. Brickner S.J., Hutchinson D.K., Barbachyn M.R., Manninen P.R., Ulanowicz D.A., Garmon S.A., Grega K.C., Hendges S.K., Toops D.S., Ford C.W. (1996) Synthesis and antibacterial activity of U-100592 and U-100766, two oxazolidinone antibacterial agents for the potential treatment of multidrug-resistant gram-positive bacterial infections. J Med Chem; 39: 673-679.
8. Das B., Rudra S., Yadav A., Ray A., Rao A., Srinivas A., Soni A., Saini S., Shukla S., Pandya M. (2005) Synthesis and SAR of novel oxazolidinones: discovery of ranbezolid. Bioorg Med Chem Lett; 15: 4261-4267.
9. Kalia V., Miglani R., Purnapatre K.P., Mathur T., Singhal S., Khan S., Voleti S.R., Upadhyay D.J., Saini K.S., Rattan A. (2009) Mode of action of ranbezolid against staphylococci and structural modeling studies of its interaction with ribosomes. Antimicrob Agents Chemother; 53: 1427-1433.
10. Skripkin E., McConnell T.S., DeVito J., Lawrence L., Ippolito J.A., Duffy E.M., Sutcliffe J., Franceschi F. (2008) Rχ-01, a new family of oxazolidinones that overcome ribosome-based linezolid resistance. Antimicrob Agents Chemother; 52: 3550-3557.
11. Medzhitov R. (2008) Origin and physiological roles of inflammation. Nature; 454: 428-435.

12. Gjødsbøl K., Christensen J.J., Karlsmark T., Jørgensen B., Klein B.M., Krogfelt K.A. (2006) Multiple bacterial species reside in chronic wounds: a longitudinal study. Int Wound J; 3: 225-231.
13. Thomas B., Klaus K.M., Peter Ø.J., Kit G.M., Richard P., Karen K., Niels H., Michael G. (2008) Why chronic wounds will not heal: a novel hypothesis. Wound Rep Reg; 16: 2-10.
14. Garth A.J., Ellen S., Randall W., Elinor D.P., Patrick S., Jennifer S., John W.C., Philip S.S. (2008) Biofilms in chronic wounds. Wound Rep Reg; 16: 37-44.
15. Medzhitov R. (2010) Inflammation 2010: new adventures of an old flame. Cell; 140: 771–776.
16. Corriveau C.C., Danner R.L. (1993) Endotoxin as a therapeutic target in septic shock. Infect Agents Dis; 2: 35–43.
17. Baumann H., Gauldie J. (1994) The acute phase response. Immunol Today; 15: 74–80.
18. Lawrence T., Willoughby D.A., Gilroy D.W. (2002) Anti-inflammatory lipid mediators and insights into the resolution of inflammation. Nat Rev Immunol; 2: 787–795.
19. Kubes P., Cafferty D.M. (2000) Nitric oxide and intestinal inflammation. Am J Med; 109: 150–158.
20. Park H.Y., Kim G.Y., Hyun J.W., Hwang H.J., Kim N.D., Kim B.W., Choi Y.H. (2012) 7,8-Dihydroxyflavone exhibits anti-inflammatory properties by downregulating the NF-κB and MAPK signaling pathways in lipopolysaccharide-treated RAW264.7 cells. Int J Mol Med; 29: 1146-1152.
21. MacMicking J., Xie Q.W., Nathan C. (1997) Nitric oxide and macrophage function. Annu Rev Immunol; 15: 323–350.
22. Cheng X.C., Liu X.Y., Xu W.F., Guo X.L., Ou Y. (2007) Design, synthesis, and biological activities of novel Ligustrazine derivatives. Bioorg Med Chem; 15: 3315-3320.
23. Ozaki Y. (1992) Anti-inflammatory effect of tetramethyl-pyrazine and ferulic acid. Chem Pharm Bull; 40: 954-956.
24. Hu J.Z., Huang J.H., Xiao Z.M., Li J.H., Li X.M., Lu H.B. (2013) Tetramethylpyrazine accelerates the function recovery of traumatic spinal cord in rat model by attenuating

inflammation. J Neurol Sci; 324: 94-99.

25. Wu H.J., Hao J., Wang S.Q., Jin B.L., Chen X.B. (2012) Protective effects of ligustrazine on TNF-α-induced endothelial dysfunction. Eur J Pharmacol; 674: 365-369.
26. Kao T.K., Chang C.Y., Ou Y.C., Chen W.Y., Kuan Y.H., Pan H.C., Liao S.L., Li G.Z., Chen C.J. (2013) Tetramethylpyrazine reduces cellular inflammatory response following permanent focal cerebral ischemia in rats. Exp Neurol; 247: 188-201.
27. Gao Y., Xu C.S., Liang S.D., Zhang A., Mu S.N., Wang Y.X., Wan F. (2008) Effect of tetramethylpyrazine on primary afferent transmission mediated by P2X3 receptor in neuropathic pain states. Brain Res Bull; 77: 27-32.
28. Wu H.Y., Wei C.Y., Xu Q., Wang S.W. (2005) Anti-inflammatory and profibrinolytic effect of tetramethylpyrazine in acute coronary syndromes. J Geriatr Cardiol; 2: 233-235.
29. Wayne P.A. (2009) Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. CLSI document M100-S19; 29: 1-135.
30. Pandit N., Singla R.K., Shrivastava B. (2012) Current Updates on Oxazolidinone and Its Significance. Intl J Med Chem; 2012: 1-24.
31. Hutchinson D.K., (2003) Oxazolidinone Antibacterial Agents: A Critical Review. Curr Top Med Chem; 3: 1021-1042.
32. Gravestock M.B. (2005) Recent developments in the discovery of novel oxazolidinoneantibacterials. Curr Opin Drug Dis Dev; 8: 469-477.
33. Renslo A.R., Luehr G.W., Gordeev M.F. (2006) Synthesis and biological activities of new 1α,25-dihydroxy-19-norvitamin D3 analogs with modifications in both the A-ring and the side chain. Bioorg Med Chem; 14: 4227-4240.
34. Poce G., Zappia G., Porretta G.C., Botta B., Biava M. (2008) New oxazolidinone derivatives as antibacterial agents with improved activity. Informa Healthcare. 18: 97-121.
35. Gregory W.A., Brittelli D.R., Wang C., Wuonola M.A., Ripley R.J., Eustice D.C., Eberly V.S., Slee A.M., Forbes M., Bartholomew P. (1989) Antibacterials. Synthesis and structure-activity studies of 3-aryl-2-oxooxazolidines. 1. The B group. J Med Chem; 32: 1673-1681.
36. Renslo A.R., Luehr G.W., Lam S., Westlund N.E., Gómez M., Hackbarth C.J., Patel D.V.,

Gordeev M.F. (2006) Synthesis and structure-activity studies of antibacterial oxazolidinones containing dihydrothiopyran or dihydrothiazine C-rings. Bioorg Med Chem Lett; 16: 3475-3478.

37. Gravestock M.B., Acton D.G., Betts M.J., Dennis M., Hatter G., Gregor A., Swain M.L., Wilson R.G., Woods L., Wookey A. (2003) New classes of antibacterial oxazolidinones with C-5, methylene O-Linked heterocyclic side chains. Bioorg Med Chem Lett; 13: 4179-4186.
38. Reck F., Zhou F., Girardot M., Kern G., Eyerman C.J., Hales N.J., Ramsay R.R., Gravestock M.B. (2005) Identification of 4-substituted 1,2,3-triazoles as novel oxazolidinone antibacterial agents with reduced activity against monoamine oxidase A. J Med Chem; 48: 499-506.
39. Hauck S.I., Cederberg C., Doucette A., Grosser L., Hales N.J., Poon G., Gravestock M.B. (2007) New carbon-linked azole oxazolidinones with improved potency and pharmacokinetics. Bioorg Med Chem Lett; 17: 337-340.
40. Ebnera D.C., Culhanea J.C., Winkelmana T.N., Hausteinb M.D., Ditty J.L., Ippoliti J.T. (2008) Synthesis of novel oxazolidinone antimicrobial agents. Bioorg Med Chem; 16: 2651-2656.
41. Ahmad S.S., Nevien A.S. (2009) Hydrazonoyl halides: their versatile biological activities. Open Bioactive Compd J; 2: 8-16.
42. Im W.B., Choi S.H., Park J.Y., Choi S.H., Finn J., Yoon S.H. (2011) Discovery of torezolid as a novel 5-hydroxymethyl-oxazolidinone antibacterial agent. Eur J Med Chem; 46: 1027-1039.
43. Vallance P. (2003) Nitric oxide: therapeutic opportunities. Fund Clin Pharmacol; 17: 1-10.
44. Li X.Y., He J.L., Liu H.T., Li W.M., Yu C. (2009) Tetramethylpyrazine suppresses interleukin-8 expression in LPS-stimulated human umbilical vein endothelial cell by blocking ERK, p38 and nulear factor-κB signaling pathways. J Ethnopharmacol; 125: 83-89.
45. Yeom G.G.M., Min S., Kim S.Y. (2014) 2,3,5,6-Tetramethylpyrazine of Ephedra sinica regulates melanogenesis and inflammation in a UVA-induced melanoma/keratinocytes

co-culture system. Int Immunopharmacol; 18: 262-269.

46. Dinarello C.A. (2000) Proinflammatory cytokines. Chest; 118: 503-508.
47. Yang C.Y., Huang X.M. (1980) Synthesis of 2-bromomethyl-3,5,6-trimethyl pyrazine and its deriatives. J Fudan University (Nat Sci); 4: 390-394.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Experimental details and characterization for the synthesized compounds.

Figure Captions

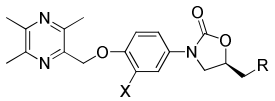
Figure 1. Chemical structures of linezolid, ligustrazine, indomethacin, **14a** and **14b**.

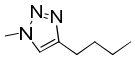
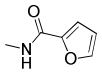
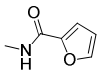
Figure 2. Design strategy for oxazolidinone derivatives

Figure 3. Effects of compounds **14a, 14b**, linezolid and indomethacin on the viability of RAW264.7 cells at the concentration of 10 μM, except TMP of 200 μM. Data were presented as means ± SD (n=3). CK was indicated that the cells were cultured with fresh medium only.

Figure 4. The effects of compounds **14a**, **14b**, linezolid, TMP and indomethacin on the LPS-induced production of IL-6 in RAW264.7 cells. RAW264.7 cells were treated with **14a**, **14b**, linezolid, indomethacin (at the concentrations of 10 μM, 5 μM, 2.5 μM, 1.25 μM), TMP (at the concentrations of 200 μM, 100μM, 50 μM and 20μM) and LPS (100 ng/mL) for 24 h. Data were presented as means ± SD (n=3). $^{*}P < 0.05$, $^{**}P < 0.01$ versus the LPS (treated with LPS only) group.

Table 1. Antibacterial activity *in vitro* of the synthesized compounds



Compd	X	R	MIC (μg/mL)												
			MRSA									SA[j]	SE[k]	EF[m]	EC[n]
			a	b	c	d	e	f	g	h	i				
1b	-F	-OH	64	64	64	64	64	64	64	64	64	64	128	64	>16
8a	-H		32	32	32	32	64	64	32	32	32	64	64	128	>16
8b	**-F**		8	4	8	4	8	8	8	8	8	8	8	32	>16
10b	-F		128	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	>16
13a	-H	$-NHCOCH_3$	16	8	16	8	16	16	16	16	16	16	16	32	>16
13b	**-F**	**$-NHCOCH_3$**	4	4	4	4	4	4	4	4	4	8	4	16	>16
14a	**-H**	**$-NHCOCH_2Cl$**	8	4	8	8	8	8	8	8	8	8	8	16	>16
14b	**-F**	**$-NHCOCH_2Cl$**	4	2	2	2	4	2	4	4	2	8	4	8	>16
15a	**-H**	**$-NHCOCHCl_2$**	4	4	8	4	8	4	8	8	4	8	8	16	>16
15b	**-F**	**$-NHCOCHCl_2$**	4	2	2	2	4	4	4	4	2	4	4	8	>16
16a	-H	$-NHCOCH_2OCH_3$	64	64	128	128	Y	128	128	128	128	Y	128	Y	>16
16b	-F	$-NHCOCH_2OCH_3$	32	32	64	32	64	64	32	32	64	128	32	128	>16
17a	-H		64	64	64	128	128	128	64	64	64	128	64	Y	>16
17b	-F		32	32	32	32	32	32	32	32	32	32	16	128	>16
Linezolid			1	1	1	1	1	1	1	1	1	4	2	2	>16

Y: >128 μg/mL.

MRSA: Methicillin-resistant *Staphylococcus aureus* (a: ATCC 43300; b: ATCC 60202; c: ATCC 51345; d: ATCC 510019; e: ATCC 52056; f: ATCC 52351-2; g: ATCC 51599-2; h: ATCC 51033; i: ATCC 405055).

[g]SA: *Staphylococcus aureus* (ATCC 25923). [k]SE: *Staphylococcus epidermidis* (ATCC 12228).

[m]EF: *Enterococcus faecalis* (ATCC 29212). [n]EC: *E. coli* (ATCC 25922).

Table 2. The inhibitory effects of the synthesized compounds on NO production in LPS-stimulated RAW264.7 cells.

Compounds	Linezolid	TMP	Indomethacin	**14a**	**14b**
IC_{50} (μM)[a]	>100	>100	>100	7.21±0.97	3.45±0.37

[a]Results were showed as means ± SD (n=4) of at least three independent experiments.

Table 3. Effects of compounds on the viability of RAW264.7 cells

Compounds	Concentrations (μM)	Cell viability (%)[a]
CK[b]		100±3.2
Linezolid	10	107.3±2.9
Indomethacin	10	108.9±5.1
TMP	200	97.5±1.1
14a	10	95.5±0.8
14b	10	94.1±1.3

[a]Results were expressed as means ± SD (n=3) of three independent experiments.

[b]CK: Control group (the cells cultured with fresh medium only).

Table 4. The inhibitory effects of linezolid, TMP, indomethacin, **14a** and **14b** on LPS-induced IL-6 and TNF-α production in RAW264.7 cells.

TNF-α (pg/mL)[a]				
Compounds	Concentrations (μM)			
	10	5	2.5	1.25
Blank	58.31±7.2			
LPS	8756.7±167.4			
LPS+Linezolid	8702.7±88.1	8827.9±11.9	8888.7±16.2	8974.4±27.4
LPS+Indomethacin	8588.6±134.5	8636.8±154.6	8670.7±130.5	8778.5±127.7
LPS+**14a**	8487.6±18.8	8578.1±67.5	8667.4±155.7	8777.6±188.8
LPS+**14b**	8180.2±33.7**	8559.2±116.2	8672.2±18.1	8756.2±52.2
LPS+TMP	200	100	50	20
	7968.6±405.4*	8782.2±257.6	9208.7±216.8	9814.6±338.8

Compounds	IL-6 (pg/mL)[a]			
	Concentrations (μM)			
	10	5	2.5	1.25
Blank	45.2±5.9			
LPS	5750.9±75.4			
LPS+Linezolid	5694..7±261.5	5829.5±419.6	5985.5±540.3	6398.3±378.2
LPS+Indomethacin	5564.5±468.0	5987.4±682.3	6116.5±680.1	6307.9±554.6
LPS+**14a**	4907.8±152.4**	5688.7±364.6	5715.7±310.5	5907.7±448.5
LPS+**14b**	3823.2±273.3**	5103.7±79.7**	5911.7±477.5	6203.7±594.1
LPS+TMP	200	100	50	20
	5062±229.2**	5508.5±363.3	5657.3±173.4	5952.6±146.5

*P < 0.05, **P < 0.01 versus the LPS (treated with LPS only) group.

[a]Results were showed as means ± SD (n=3) of three independent experiments.

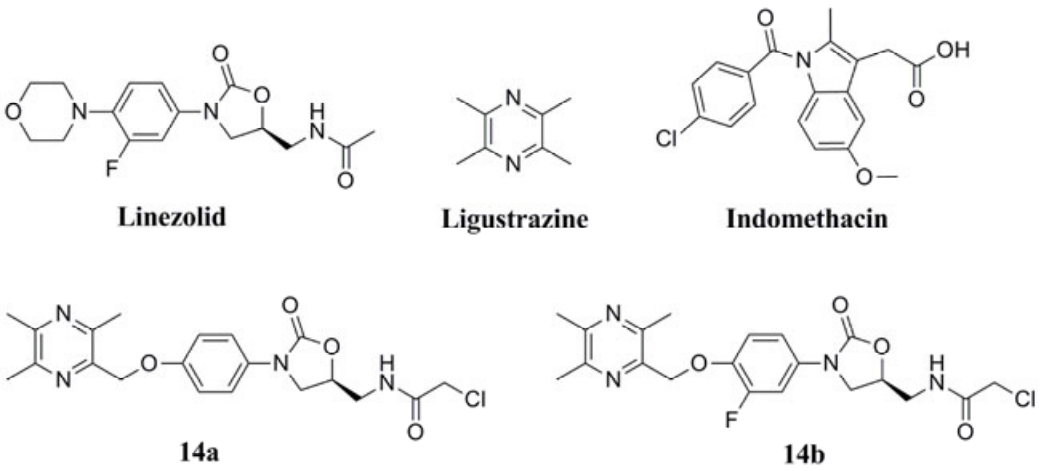


Figure 1. Chemical structures of linezolid, ligustrazine, indomethacin, **14a** and **14b**.

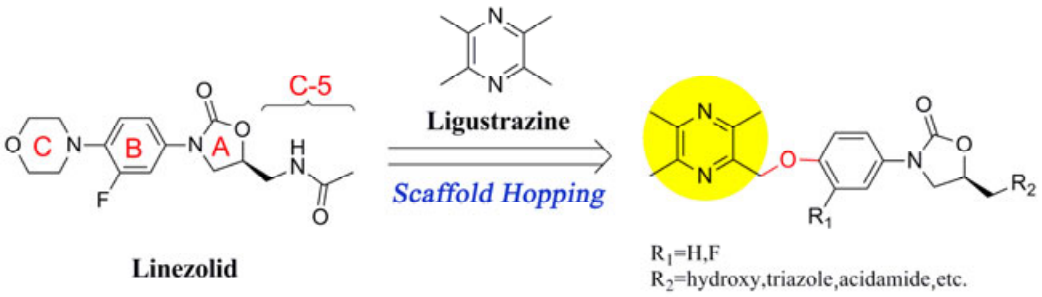


Figure 2. Design strategy for oxazolidinone derivatives

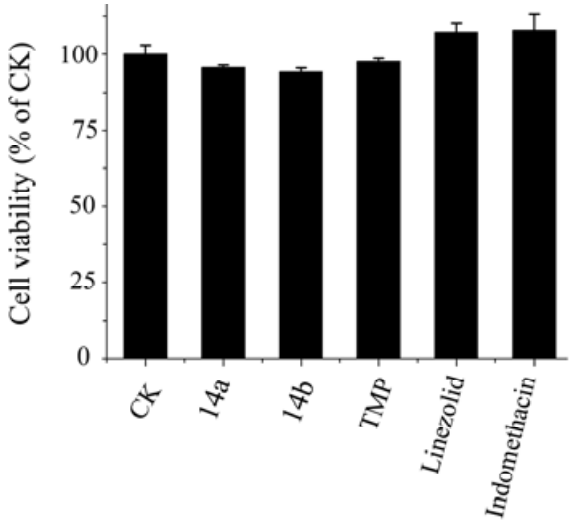


Figure 3. Effects of compounds **14a**, **14b**, linezolid and indomethacin on the viability of RAW264.7 cells at the concentration of 10 μM, except TMP of 200 μM. Data were presented as means ± SD (n=3). CK was indicated that the cells were cultured with fresh medium only.

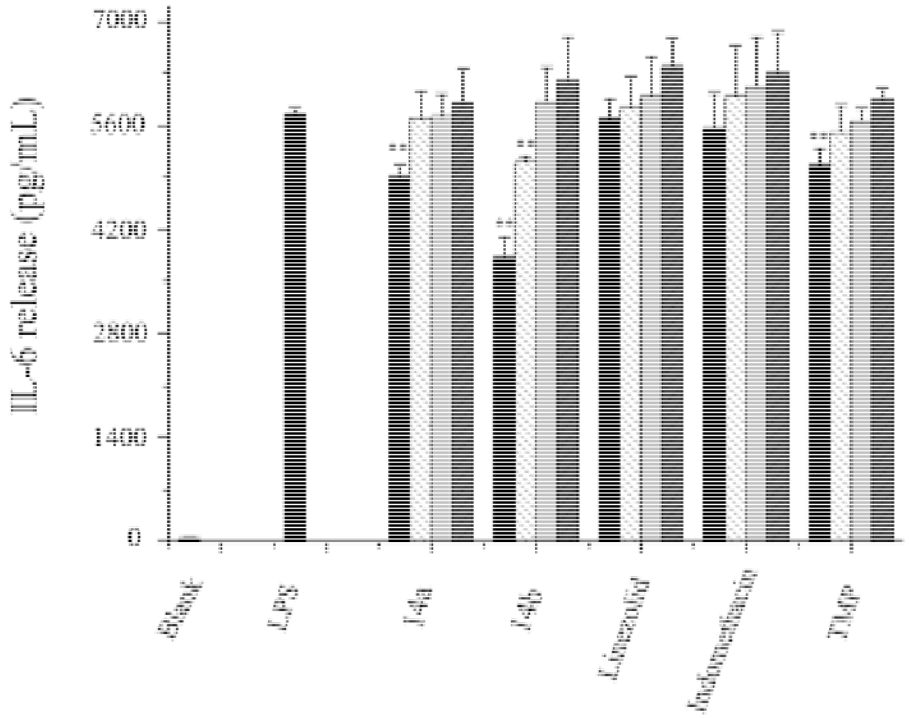
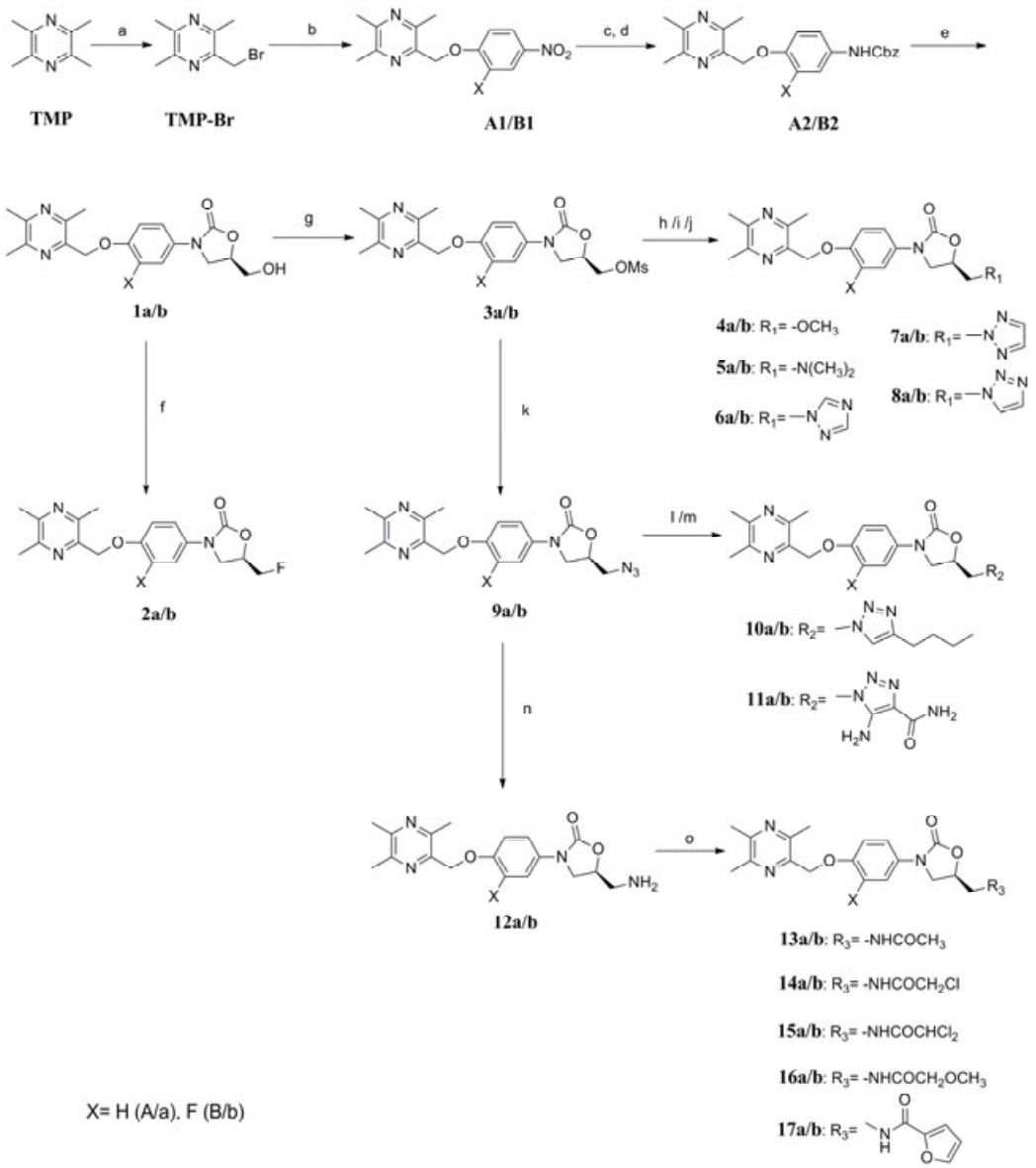


Figure 4. The effects of compounds **14a**, **14b**, linezolid, indomethacin and TMP on the LPS-induced production of IL-6 in RAW264.7 cells. RAW264.7 cells were treated with **14a**, **14b**, linezolid, indomethacin (at the concentrations of 10 μM, 5 μM, 2.5 μM and 1.25 μM), TMP (at the concentrations of 200 μM, 100μM, 50 μM and 20μM) and LPS (100ng/mL) for 24 h. Data were presented as means ± SD (n=3). $^{*}P < 0.05$, $^{**}P < 0.01$ versus the LPS (treated with LPS only) group.



Scheme 1. Reagent and condition: (a) NBS, $(PhCO)_2O_2$, 24 h, reflux; (b) *p*-nitrophenol or 2-fluoro-4-nitrophenol, K_2CO_3, DMF, 85 °C; (c) H_2, 10% Pd/C, THF; (d) Cbz-Cl, Na_2CO_3, acetone-H_2O (10:1); (e) $LiN(Si(CH_3)_3)_2$, (*R*)-glycidyl butyrate, THF, -78 °C to r.t.; (f) DAST, Et_3N, CH_2Cl_2, r.t.; (g) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C to r.t.; (h) NaOMe, MeOH, r.t., 24 h; (i) K_2CO_3, dimethylamine hydrochloride, DMF, 60 °C; (j) NaH, triazole, DMF, r.t.; (k) NaN_3, DMF, 90 °C, 3 h; (l) 1-hexyne, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, β-CD, H_2O; (m) 2-cyanoacetamide, K_2CO_3, DMSO, r.t.; (n) H_2, 10% Pd/C, THF, 24 h; (o) R_3COCl, Et_3N, CH_2Cl_2, 0 °C to r.t.