European Journal of Medicinal Chemistry 46 (2011) 65–70

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Discovery of a novel nitroimidazolyl–oxazolidinone hybrid with potent anti Gram-positive activity: Synthesis and antibacterial evaluation

Ali Khalaj [a], Maryam Nakhjiri [b], Amir Soheil Negahbani [b], Marjaneh Samadizadeh [c], Loghman Firoozpour [b], Saeed Rajabalian [d], Nasrin Samadi [e], Mohammad Ali Faramarzi [f], Neda Adibpour [g], Abbas Shafiee [a], Alireza Foroumadi [a,d,*]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy & Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b] Drug Design & Development Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Department of Chemistry, Science and Research Campus, Islamic Azad University, Tehran, Iran
[d] Kerman Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran
[e] Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[f] Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14174, Iran
[g] Department of Medicinal Chemistry, School of Pharmacy, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran

ARTICLE INFO

Article history:
Received 1 May 2010
Received in revised form
13 October 2010
Accepted 13 October 2010
Available online 10 November 2010

Keywords:
Oxazolidinone
Nitroimidazole
In vitro antibacterial activity
Cytotoxicity

ABSTRACT

A number of linezolid analogues containing a nitroaryl-1,3,4-thiadiazole moiety, were prepared and evaluated as antibacterial agents against a panel of Gram-positive and Gram-negative bacteria. Among synthesized compounds, nitrofuran analogue **1b** exhibited more potent inhibitory activity, with respect to other synthesized compounds and reference drug linezolid. The target compounds were also assessed for their cytotoxic activity against normal mouse fibroblast (NIH/3T3) cells using MTT assay. The results indicated that compound **1c** exhibit potent antibacterial activity against Gram-positive bacteria at non-cytotoxic concentrations.

1. Introduction

Antibiotic resistance is a major problem in hospitals as well as in community settings. Gram-positive pathogens such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium* and *Streptococcus pneumoniae* are becoming resistant to most of the existing antibiotics [1]. Oxazolidinones are a new class of totally synthetic antibiotics with activity against Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE) [2–5]. E. I. du Pont de Nemours & Company scientists were the first to discover, in the late 1970s, the antimicrobial properties of oxazolidinones during their research on plant antimicrobials [6]. The oxazolidinone, exemplified by DuP 721 (Fig. 1), is a relatively new class of orally active, totally synthetic antibacterial agent discovered by scientists at Du Pont [7]. However, DuP 721 was discontinued following Phase I clinical trials because it was shown that DuP 721 exhibited lethal toxicity in rats in drug safety studies conducted at the Upjohn company. Subsequent studies at Pharmacia and Upjohn Co. have resulted in two potent oxazolidinone antibacterial agents, eperezolid and linezolid (Fig. 1) [8]. These oxazolidinones inhibit protein synthesis by acting against the formation of the 70S initiation complex, and they are generally considered to be bacteriostatic [9,10]. The approval of linezolid (LZD), expanded therapeutic options to include other enterococcal species and permitted use of oral therapy [11]. Linezolid is currently approved to treat skin and respiratory infections caused by Gram-positive pathogens, including multidrug-resistant staphylococci, enterococci, and streptococci [2]. Although this is a rather expensive drug, treatments are considered more cost effective than using competing drugs [12]. Linezolid is considered a relatively safe drug and the main toxicity issue reported to date refers to the possible development of thrombocytopenia upon prolonged treatment [13–15]. Thus, the synthesis and antibacterial evaluation of this group of compounds in order to modify and eliminate their unwanted side effects, are in progress [16]. Ranbezolid (Fig. 1), an

* Corresponding author. Department of Medicinal Chemistry, Faculty of Pharmacy & Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran. Tel.: +98 21 66954708; fax: +98 21 66461178.
E-mail address: aforoumadi@yahoo.com (A. Foroumadi).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.10.015

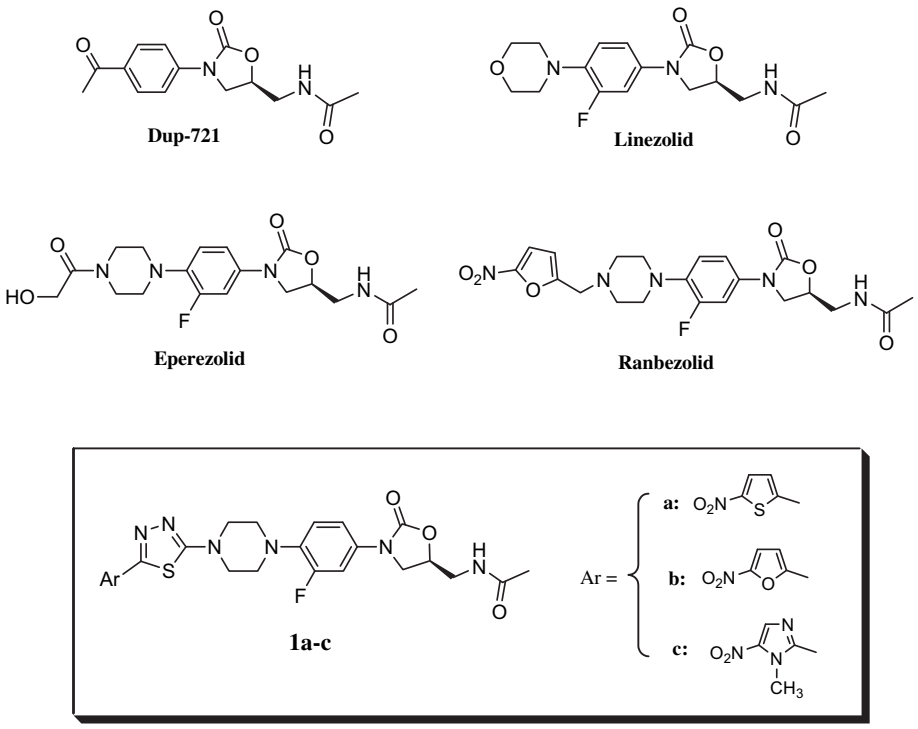


Fig. 1. Structures of four recognized oxazolidinone drugs and novel oxazolidinone molecules (**1a**–**c**).

investigational oxazolidinone [17], with an additional 5-nitrofuran moiety in oxazolidinone class of antibacterial drugs, showed excellent in vitro activity against Gram-positive pathogens [18]. Ranbezolid has some advantages over linezolid in the oxazolidinone series and its mode of action against Staphylococci and structural modeling studies of its interaction with ribosomes have been recently reported [19].

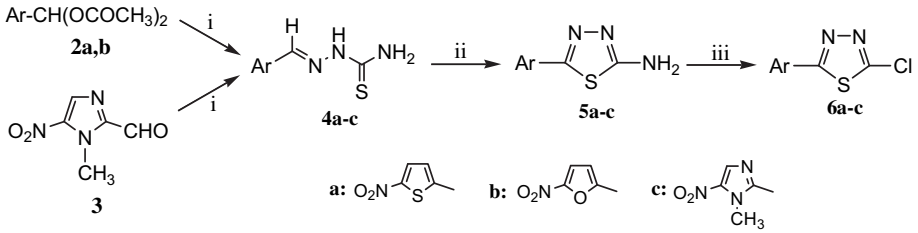
In recent years, we have reported the synthesis and antibacterial activity of several nitroaryl-1,3,4-thiadiazole-quinolone hybrids [20–23], which some of them showed potent antibacterial activity against Gram-positive bacteria. In continuation our research program to find new antibacterial agents for the treatment of infectious diseases, herein we would like to report the synthesis and in vitro antibacterial profile of a series of nitroaryl-1,3,4-thiadiazole-oxazolidinone hybrids **1a**–**c** (Fig. 1) which led to the discovery of *N*-((3-(3-fluoro-4-(4-(5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl)piperazin-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide **1c** as a potent antibacterial agent against Gram-positive bacteria.

2. Chemistry

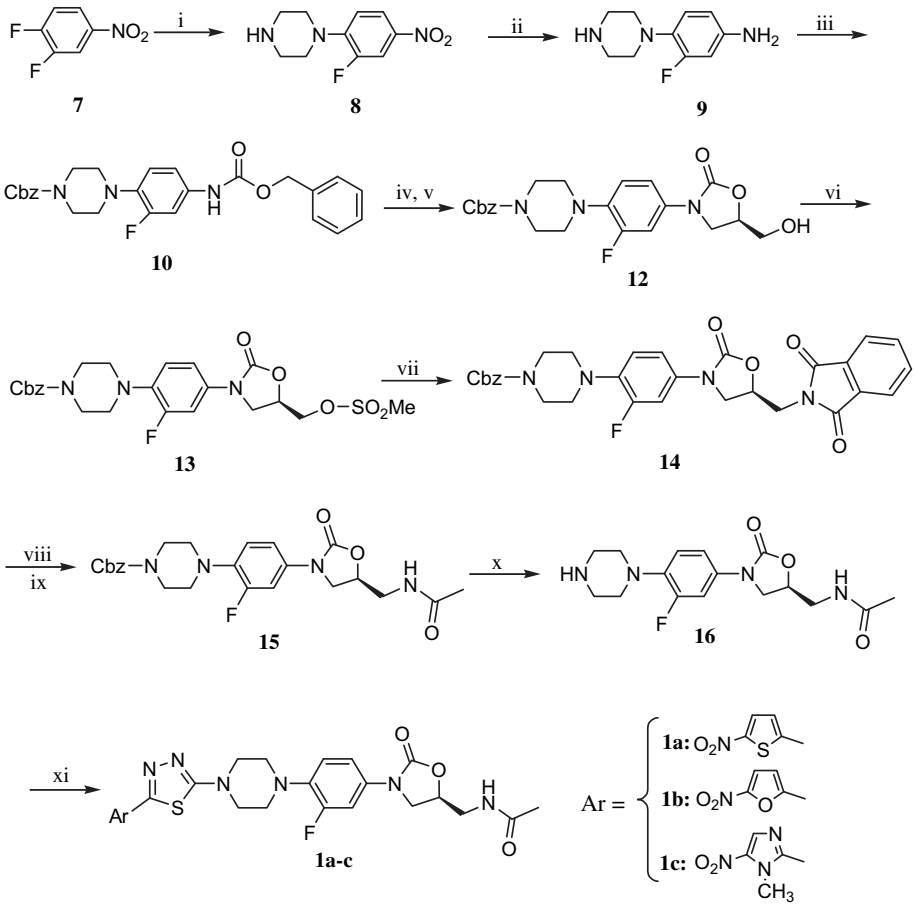
Our synthetic pathways to nitroaryl-1,3,4-thiadiazoles **6a**–**c** and target compounds **1a**–**c** are presented in Schemes 1 and 2. The required 1,3,4-thiadiazoles **6a**–**c** were obtained according to the method previously reported by us [20,24]. The key intermediate oxazolidinone **16** was prepared according to the method reported in the literature [25] with some modifications. Thus, the reaction of commercially available 3,4-difluoronitrobenzene **7** with excess piperazine, selectively gave the *p*-substituted nitrobenzene **8**. Catalytic reduction of **8** and subsequent reaction of the amine with benzylchloroformate afforded carbamate **10**. Reaction of **10** with *n*-BuLi (THF, −78 °C) followed by addition of (*R*)-glycidyl butyrate (**11**) provided (5*R*)-(hydroxymethyl)-2-oxazolidinone **12**. Activation of hydroxyl group as mesylate **13** and subsequent displacement of mesyl group with phthalimide gave compound **14**. Compound **14** was deprotected with methylamine to afford the intermediate 5-(aminomethyl)oxazolidinone which was reacted with Ac_2O to provide compound **15**. Catalytic deprotection of **15** provided the key intermediate piperazine **16** [25]. Finally, reaction of compounds **6a**–**c** with piperazine **16** afforded target compounds **1a**–**c**.

3. Pharmacology

Compounds **1a**–**c** were evaluated for their antibacterial activity against Gram-positive (*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus warneri*, *Staphylococcus lentus*, *Staphylococcus xylosus*, *Staphylococcus saprophyticus*,



Scheme 1. Synthetic procedure for the synthesis of the intermediate compounds **6a**–**c**. Reagents and conditions: (i) thiosemicarbazide, EtOH, HCl, reflux; (ii) $(NH_4)\,Fe(SO_4)_2$, H_2O, reflux; (iii) $NaNO_2$, HCl, Cu.



Scheme 2. Synthetic procedure for the synthesis of the target compounds **1a**–**c**. Reagent and condition: (i) Piperazine, CH_3CN, reflux; (ii) H_2, 5% Pd/C, THF; (iii) Benzyloxycarbonyl–Cl (Cbz–Cl), $NaHCO_3$ (or Na_2CO_3), acetone–H_2O; (iv) n-BuLi, THF −78 °C; (v) (*R*)-glycidyl butyrate (**11**); (vi) MsCl, Et_3N, CH_2Cl_2; (vii) potassium phthalimide, CH_3CN, H_2O, reflux; (viii) aqueous $MeNH_2$, EtOH, reflux; (ix) Ac_2O, pyr; (x) Pd/C, H_2, MeOH–CH_2Cl_2; (xi) (**6a**–**c**), Et_3N, EtOH, reflux.

Micrococcus luteus, *Corynebacterium glutamicum*, *Bacillus subtilis* PTCC 1023, MRSA 3, MRSA 5 and MRSA 17) and Gram-negative (*Escherichia coli* ATCC 8739, *Klebsiella pneumonia* ATCC 10031, and *Pseudomonas aeruginosa* ATCC 9027) bacteria using conventional agar-dilution method [26]. The MIC (minimum inhibitory concentration) values were determined by comparison to linezolid and ciprofloxacin as reference drugs.

4. Results

The individual minimum inhibitory concentrations (MICs, μg/mL) obtained for compounds **1a**–**c** against Gram-positive and Gram-negative are presented in Table 1. The IC_{50} values obtained for in vitro cytotoxic activity of the test compounds **1a**–**c** and **16** against normal mouse fibroblast cell line (NIH/3T3) are shown in Table 2.

5. Discussion

MIC values of the tested derivatives indicate that compounds **1a**–**c** and **16** showed a higher antibacterial activity against Gram-positive rather than Gram-negative bacteria. Table 1 reveals that compounds **1b** and **1c** (MICs 0.006 μg/mL) followed by **1a** were superior in inhibiting the growth of *Staphylococcus warneri* in comparison to linezolid and ciprofloxacin as reference drugs. Compound **1b** was the most active compound against *S. lentus*, its activity was found to be 65–130 times better than reference drugs. Antibacterial evaluation of compounds **1a**–**c** and **16** against *Micrococcus luteus* reveals that compounds **1c** (MICs 0.195 μg/mL) followed by **1b** (MICs 0.391 μg/mL) possessed a significant activity. In addition compounds **1b** and **1c** were the most potent against *C. glutamicum*, *S. sarophyticus* and MRSA 5 with MIC values of 0.024–0.195 μg/mL. Moreover the latter compounds were more potent than reference drugs. Furthermore, compound **1b** was the most active compound against *S. aureus*, *S. epidermidis* and MRSA 3. Generally, most of the compounds have shown poor or no activity (MIC $\geq$ 6.25 μg/mL) against Gram-negative bacteria with the exception of compound **1c** against *K. pneumonia* which showed potency (MIC = 0.781 μg/mL), although not superior to those of ciprofloxacin and linezolid (MICs = 0.195 and 0.391 μg/mL, respectively). Eventually it can be inferred from Table 1 that compound **16** exhibited weak activity against all of the Gram-positive and Gram-negative bacteria; moreover, among compounds tested, the 5-nitrofuran counterpart **1b**, showed the highest activity against nearly all of the tested Gram-positive bacteria, superior to the reference drugs linezolid and ciprofloxacin.

For further pharmacological study, in vitro cytotoxic activity of the test compounds **1a**–**c** and **16** against normal mouse fibroblast cell line (NIH/3T3) was investigated using MTT colorimetric assay [27]. The IC_{50} values obtained for these compounds are shown in Table 2. While nitrothiophene and nitrofuran derivatives (**1a** and **1b**) showed moderate toxicity against mouse fibroblast cell line, nitroimidazole analogue (**1c**) showed no toxicity at concentration

Table 1
In vitro antibacterial activities of compounds **1a**–**c**, **16** and reference drugs against selected strains (MICs in μg/mL).

Microorganisms[a]	1a	1b	1c	16	Linezolid	Ciprofloxacin
B. s	0.781	0.391	0.098	12.5	0.391	0.195
C. g	6.25	0.195	0.195	1.563	0.781	0.391
E. c	>100	>100	>100	50	>100	0.012
K. p	100	50	0.781	25	6.25	0.003
M. l	6.25	0.391	0.195	1.563	0.781	3.125
MRSA 3	0.195	0.049	0.391	25	0.781	0.391
MRSA 5	6.25	0.195	0.195	12.5	0.781	0.391
MRSA 17	0.098	0.012	0.024	25	0.781	0.391
P. a	>100	>100	>100	>100	>100	0.0391
S. a	0.098	0.012	0.098	12.5	0.781	0.195
S. e	0.024	0.006	0.024	6.25	0.781	0.391
S. l	0.098	0.012	0.098	12.5	1.563	0.781
S. s	0.195	0.024	0.024	3.125	0.781	0.098
S. w	0.049	0.006	0.006	18	0.781	0.195
S. x	0.098	0.024	0.049	3.125	0.781	0.391

[a] B. s: *Bacillus subtilis* PTCC 1023; C. g: *Corynebacterium glutamicum* ATCC 13032; E. c: *Escherichia coli* ATCC 8739; K. p: *Klebsiella pneumonia* ATCC 10031; M. l: *Micrococcus luteus* ATCC 9341; MRSA 3; MRSA 5; MRSA 17; P. a: *Pseudomonas aeruginosa* ATCC 9027; S. a: *Staphylococcus aureus* ATCC 6538p; S. e: *Staphylococcus epidermidis* ATCC 12228; S. l: *Staphylococcus lentus* ATCC 29070; S. s: *Staphylococcus saprophyticus* ATCC 15305; S. w: *Staphylococcus warneri* ATCC 27836; S. x: *Staphylococcus xylosus* ATCC 29971.

of 200 μg/mL (IC_{50} > 200 μg/mL). The same result was obtained for compound **16** (Table 2).

Among target compounds, the linezolid analogue **1c**, showed the highest inhibition against nearly all of the tested Gram-positive bacteria, superior to the reference drugs, and displayed antibacterial activity at non-cytotoxic concentrations.

6. Conclusion

In light of this study, it can be inferred that the antibacterial and cytotoxic properties of target compounds are mainly determined by heteroaryl substituent and this scaffold can be served as a promising substituent for oxazolidinone class of antibacterial drugs.

In conclusion, we report the discovery of *N*-((3-(3-fluoro-4-(4-(5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl) piperazine-1-yl)phenyl)-2-oxooxazolidine-5-yl)methyl)acetamide **1c** as a potent antibacterial agent against Gram-positive bacteria.

7. Experimental protocols

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. The FT-IR spectra were obtained using a Nicolet 550 spectrometer (Potassium bromide disks). The ^1H NMR and ^{13}C NMR spectra were recorded on Bruker FT-500 and Bruker FT-80. Chemical shifts (δ) are in parts per million relative to internal tetramethylsilane. The mass spectra were run on a Finnigan mat TSQ-70 spectrometer at 70 eV. Thin layer chromatography (TLC) was performed on plates of silica gel 60 F254 plates. Silica gel 60, 0.040–0.063 mm (230–400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Most of the solvents and reagents were purchased from Merck, Aldrich and Fluka and used as such without purification.

Table 2
Cytotoxic activity of compounds **1a**–**c** and **16**, against mouse fibroblast (NIH/3T3) cell line.

Compound	IC_{50} (μg/mL)[a]
1a	184 ± 4.2
1b	21.8 ± 3
1c	>200
16	>200

[a] IC_{50} is the concentration required to inhibit 50% of cell growth. The values represent mean ± SD.

7.1. 1-(2-Fluoro-4-nitrophenyl)piperazine (*8*)

A solution of 12.0 g (75.42 mmol) of 3,4-difluoronitrobenzene in 150 mL of acetonitrile was treated with 16.24 g (188.6 mmol) of piperazine followed by warming at reflux for 3 h. The solution was cooled to room temperature and concentrated *in vacuo*. The resulting residue was diluted with 200 mL of water and extracted with ethyl acetate (3 × 250 mL). The combined organic layers were extracted with water (200 mL) and saturated sodium chloride solution (200 mL) followed by drying on sodium sulfate. The solution was concentrated *in vacuo* to afford an orange solid which was purified by gradient chromatography eluting initially with dichloromethane until the least polar fractions had eluted, and then elution was continued with 2% (v/v) ethanol–dichloromethane and then with 10% (v/v) ethanol–dichloromethane. These procedures afforded compound **8**, Yield 85%; mp 68.5–71 °C; IR: υ_{max} (KBr) 3022 (NH), 1518 and 1336 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): 7.94 (ddd, 1H, J = 9, 2.5 and 1 Hz, H_5 nitrobenzene), 7.85 (dd, 1H, J = 13 and 2.5 Hz, H_3 nitrobenzene), 6.88 (t, 1H, J = 9 Hz, H_6 nitrobenzene), 3.25–3.23 (m, 4H, piperazine), 3.04–3.01 (m, 4H, piperazine); MS: *m*/*z* 225 (37, M^+), 183 (100), 137 (37), 58 (43); Anal. Calcd for $C_{10}H_{12}FN_3O_2$: C, 53.33; H, 5.37; F, 8.44; N, 18.66. Found: C, 52.97; H, 5.21; F, 8.65; N, 18.52.

7.2. N-Carbobenzoxy-3-fluoro-4-(N-carbobenzoxypiperazinyl) aniline (*10*)

A mixture of 12.5 g (0.056 mol) of **8** and 1.4 g of 5% palladium on carbon in 115 mL of THF was stirred under 40 psi of H_2 for 1.5 h, while maintaining the reaction at room temperature. The reaction mixture was filtered through diatomaceous earth and the pad washed with 2 × 50 mL of THF. The filtrate was concentrated, and the solid **9** was azeotroped with 43 mL of acetone. The crude amine was immediately dissolved in 69 mL of acetone and added to a 500 mL three-neck flask equipped with a mechanical stirrer, containing 138 mL of 10% aqueous sodium carbonate. The mixture was cooled to 0 °C, and 18 mL (0.127 mol) of benzylchloroformate was added dropwise over 20 min while maintaining the temperature between 0 and 5 °C. The mixture was then stirred for 1 h at 5 °C and then allowed to stir overnight at room temperature. The mixture was decanted, and the product was extracted with

chloroform (3 × 50 mL) and dried over sodium sulfate then dried *in vacuo*, and then was decolorized by activated charcoal in hot methanol and filtrated to give off-white solid compound **10**. Yield 52%; mp 159–161 °C; IR: υ_{max} (KBr) 3283 (NH), 1716 and 1690 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$): 7.39–7.32 (m, 10H, phenyl), 6.98 (d, 1H, J = 9 Hz, H_2 nitrobenzene), 6.85 (t, 1H, J = 9 Hz, H_6 nitrobenzene), 6.70 (s (brs), 1H, H_5 nitrobenzene), 5.19 (s, 2H, CH_2O), 5.17 (s, 2H, CH_2O), 3.67 (t, 4H, J = 5 Hz, piperazine), 2.99 (s (brs), 4H, piperazine); MS: *m/z* 463 (54, M^+), 328 (40), 91 (100), 88 (66); Anal. Calcd for $C_{26}H_{26}FN_3O_4$: C, 67.37; H, 5.65; F, 4.10; N, 9.07. Found: C, 67.58; H, 5.26; F, 4.32; N, 8.84.

7.3. (R)-[N-3-[3-fluoro-4-[N-1-(4-carbobenzoxy)piperazinyl]-phenyl]-2-oxo-5-oxazolidinyl] methanol (**12**)

To a mixture of 4.33 g (10.06 mmol) of **10** in 50 mL of dry THF at −78 °C under argon was added 6.8 mL (10.88 mmol) of 1.6 M *n*-butyllithium–hexane dropwise over 5 min. After 1.5 h, 1.55 mL of (*R*)-glycidyl butyrate **11** was added and the mixture allowed stirring at −78 °C for 1 h and then at room temperature for 3.5 h (the mixture became thick with solid precipitation). Saturated aqueous ammonium chloride (25 mL) was added followed by 25 mL of ethyl acetate and 5 mL of water. The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to provide **12** as a white solid. The solid was triturated with ethyl acetate–hexane (1: 1) in a warm water bath for 30 min and then refrigerated; the solids were collected by vacuum filtration to provide **12** as white solids. Yield 82%; mp 149–150 °C; IR: υ_{max} (KBr) 3426 (OH), 1741 and 1664 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$): 7.46 (dd, 1H, J = 14.5 and 2.5 Hz, H_2 fluorobenzene), 7.39–7.37 (m, 4H, H_2, H_3, H_5 and H_6 phenyl), 7.36–7.32 (m, 1H, H_4 phenyl), 7.13 (dd, 1H, J = 9 and 2.5 Hz, H_6 fluorobenzene), 6.92 (t, 1H, J = 9 Hz, H_5 fluorobenzene), 5.17 (s, 2H, benzylic CH_2), 4.77–4.72 (m, 1H, H_5 oxazolidinone), 4.03–3.98 (m, 2H, CH_2OH), 3.97–3.93 (m, 1H, H_4 oxazolidinone), 3.8–3.74 (m, 1H, H_4 oxazolidinone), 3.68 (t, 4H, J = 5 Hz, piperazine), 3.02 (t, 4H, J = 5 Hz, piperazine); MS: *m/z* 429 (66, M^+), 338 (25), 294 (33), 265 (40), 239 (25), 91 (100); Anal. Calcd for $C_{22}H_{24}FN_3O_5$: C, 61.53; H, 5.63; F, 4.42; N, 9.78. Found: C, 61.32; H, 5.54; F, 4.67; N, 10.02.

7.4. (R)-N-[[3-[3-Fluoro-4-[N-1-(4-carbobenzoxy)piperazinyl]-phenyl]-2-oxo-5-oxazolidinyl]methyl]phthalimide (**14**)

To a mixture of 3.5 g (8.15 mmol) of **12** and 2.5 mL (17.97 mmol) of triethylamine in 88 mL of methylene chloride at 0 °C was added 0.75 mL (9.69 mmol) of methanesulfonyl chloride dropwise over 2 min. The mixture was stirred at 0 °C for 1.5 h and at room temperature for 3 h, the mixture was washed with 74 mL water, and the aqueous layer was extracted with methylene chloride (13 mL). Ethyl acetate (63 mL) was added to the combined organic layers; these were dried (Na_2SO_4) and concentrated under reduced pressure to give **13** as a yellow foamy solid.

IR: υ_{max} (KBr) 1751 and 1695 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$): 7.56–7.32 (m, 6H, H Phenyl, H_2 fluorobenzene), 7.1–6.53 (m, 2H, H_5 and H_6 fluorobenzene), 5.16 (s, 2H, CH_2 benzylic), 5.05–4.53 (m, 1H, H_5 oxazolidinone), 4.54–4.40 (m, 2H, CH_2O), 4.30–3.53 (m, 2H, H_4 oxazolidinone), 3.52–3.56 (m, 4H, piperazine), 3.15–2.95 (m, 7H, CH_3 and piperazine); Anal. Calcd for $C_{23}H_{26}FN_3O_7S$: C, 54.43; H, 5.16; F, 3.74; N, 8.28; S, 6.32. Found: C, 54.79; H, 4.88; F, 3.51; N, 8.39; S, 6.04.

This was taken up into 126 mL of acetonitrile and 0.63 mL of water and 4.57 g (24.55 mmol) of potassium phthalimide was added. The mixture was heated to reflux for 48 h and then concentrated under reduced pressure to a yellow gum. The gum was triturated with 100 mL of ethyl acetate–hexane (1:1) followed by the addition of 50 mL of ethyl acetate. Upon concentration of the mixture under reduced pressure to 25 mL, a white precipitate formed; this was cooled to 4 °C, and the solids were collected to provide compound **14**. The filtrate was passed over a silica gel column, eluting with methylene chloride. The appropriate fractions were combined to provide **14** as a light yellow solid. Yield 66%; mp 127–129 °C; IR: υ_{max} (KBr) 1746 and 1710 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$): 8.00–7.52 (m, 4H, H phtalimide), 7.56–7.30 (m, 7H, H Phenyl, H_2 and H_6 flurobenzene), 5.14 (s, 2H, CH_2 benzylic), 5.00–4.58 (m, 1H, H_5 oxazolidinone), 4.40–4.20 (m, 2H, CH_2N), 4.15–3.90 (m, 2H, H_4 oxazolidinone), 3.69 (t, 4H, J = 5 Hz, piperazine), 3.00 (t, 4H, J = 5 Hz, piperazine); Anal. Calcd for $C_{30}H_{27}FN_4O_6$: C, 64.51; H, 4.87; F, 3.40; N, 10.03. Found: C, 64.32; H, 5.21; F, 3.31; N, 10.23.

7.5. (S)-N-[[3-[3-Fluoro-4-[N-1-(4-carbobenzoxy)piperazinyl]-phenyl]-2-oxo-5-oxazolidinyl] methyl]acetamide (**15**)

A mixture of 1.51 g (2.7 mmol) of **14** and 3 mL of 40% methylamine in water (34.85 mmol) and 30 mL of ethanol was heated to reflux for 5.5 h; then additional methylamine solution (0.5 mL) was added. The mixture was heated to reflux for 1 h and then concentrated under reduced pressure. The residue was dissolved in 15 mL of pyridine, the mixture cooled to 0 °C, and acetic anhydride (5 mL) added. The mixture was allowed to stir at room temperature overnight and then concentrated under reduced pressure to provide crude **15** as a white solid. The crude was placed upon a silica gel column and eluted with an ethanol–methylene chloride gradient (0–7% EtOH); the appropriate fractions were combined to provide compound **15** as a white solid. Yield 33%; mp 174–176 °C; IR: υ_{max} (KBr) 3313 (NH), 1751, 1685 and 1644 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$): 7.45 (dd, 1H, J = 14.5 and 2.5 Hz, H_2 fluorobenzene), 7.39–7.37 (m, 4H, H_2, H_3, H_5 and H_6 phenyl), 7.36–7.32 (m, 1H, H_4 phenyl), 7.09–7.06 (m, 1H, H_6 fluorobenzene), 6.92 (t, 1H, J = 9 Hz, H_5 fluorobenzene), 5.97 (t, 1H, J = 6 Hz, CONH), 4.80–4.74 (m, 1H, H_5 oxazolidinone), 4.50–4.00 (t, 1H, J = 9 Hz, CH_2NH), 3.76–3.71 (m, 2H, CH_2NH and H_4 oxazolidinone), 3.68 (t, 4H, J = 5 Hz, piperazine), 3.64–3.58 (m, 1H, H_4 oxazolidinone), 3.08–2.98 (m, 4H, J = 5 Hz, piperazine), 2.03 (s, 3H, CH_3CO); MS: *m/z* 470 (12, M^+), 426 (12), 335 (5), 177 (5), 105 (5), 91 (100), 56 (38); Anal. Calcd for $C_{25}H_{29}FN_4O_5$: C, 61.97; H, 6.03; F, 3.92; N, 11.56. Found: C, 61.74; H, 5.69; F, 3.99; N, 11.27.

7.6. (S)-N-[[3-[3-Fluoro-4-(N-1-piperazinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide, hydrochloride (**16**)

A mixture of 0.4 g (0.85 mmol) of **15** and 0.06 g of 10% palladium on carbon in 19 mL of methanol and 6 mL of methylene chloride was stirred under hydrogen for 48 h. The mixture was then filtered through diatomaceous earth. The filter cake was washed with 50 mL of methylene chloride followed by 25 mL of ethyl acetate, and the filtrate was concentrated to give off-white solid. The solid was triturated with 50 mL of 10% methanol–ethyl acetate in a warm water bath for 30 min, cooled to 0 °C and then was collected to provide **16** as a white solid. Yield 91%; mp 198–200 °C; IR: υ_{max} (KBr) 3436 and 3329 (NH), 1741 (C═O) and 1644 (CONH) cm^{-1}; ^1H NMR (DMSO): 9.03 (s (brs), 2H, NH_2^+), 8.26 (t, 1H, J = 6 Hz, CONH), 7.50 (dd, 1H, J = 15 and 2.5 Hz, H_2 fluorobenzene), 7.19 (dd, 1H, J = 9 and 2.5 Hz, H_6 fluorobenzene), 7.12 (t, 1H, J = 9 Hz, H_5 fluorobenzene), 4.71–4.68 (m, 1H, H_5 oxazolidinone), 4.07 (t, 1H, J = 9 Hz, CH_2NH), 3.69 (dd, 1H, J = 9 and 6 Hz, CH_2NH), 3.39 (t, 2H, J = 6 Hz, H_4 oxazolidinone), 3.22–3.21 (m, 4H, piperazine), 3.17–3.16 (m, 4H, piperazine), 1.81 (s, 3H, CH_3CO); MS: *m/z* 337 (45, M^+), 295 (100), 250 (53), 191 (32), 57 (32); Anal. Calcd for

$C_{17}H_{24}ClFN_4O_3$: C, 52.78; H, 6.25; Cl, 9.16; F, 4.91; N, 14.48. Found: C, 52.94; H, 6.10; Cl, 9.24; F, 4.73; N, 14.60.

7.7. General procedure for the synthesis of (S)-N-[[3-[3-fluoro-4-[4-(5-aryl-1,3,4-thiadiazol-2-yl)-1-piperazinyl] phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamides (**1a**–**c**)

To a mixture of 0.06 g (0.16 mmol) of **16** and 0.16 mmol of appropriate chloro-1,3,4-thiadiazole **6a**–**c** in 20 mL of ethanol, 0.2 mL triethylamine was added. The mixture was heated under reflux for 6 h (3 h for imidazole derivative) and then concentrated under reduced pressure. The crude product was placed on a silica gel column and eluted with ethanol–methylene chloride 5% to provide compounds **1a**–**c**.

7.7.1. (S)-N-[[3-[3-Fluoro-4-[4-[5-(5-nitrothiophene-2-yl)-1,3,4-thiadiazol-2-yl]-1-piperazinyl] phenyl]-2-oxo-5-oxazolidinyl] methyl]acetamide (**1a**)

Yield 41%; mp 271–273 °C (dec); IR: υ_{max} (KBr) 3339 (NH), 1746 (C=O), 1659 (CONH), 1521 and 1347 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): 7.87 (d, 1H, J = 4 Hz, H_4 thiophene), 7.56–7.46 (m, 1H, H_2 fluorobenzene), 7.40 (d, 1H, J = 4 Hz, H_3 thiophene), 7.10 (dd, 1H, J = 9 and 2.5 Hz, H_6 fluorobenzene), 6.95 (t, 1H, J = 9 Hz, H_5 fluorobenzene), 5.92 (t, 1H, J = 6 Hz, CONH), 4.78–4.75 (m, 1H, H_5 oxazolidinone), 4.03 (t, 1H, J = 9 Hz, CH_2NH), 3.80 (t, 4H, J = 5 Hz, piperazine), 3.78–3.74 (m, 1H, CH_2NH), 3.72–3.69 (m, 1H, H_4 oxazolidinone), 3.64–3.58 (m, 1H, H_4 oxazolidinone), 3.21 (t, 4H, J = 5 Hz, piperazine), 2.02 (s, 3H, CH_3CO); ^{13}C NMR (DMSO, 125 MHz): 172.7, 169.9, 155.6, 153.9, 153.6, 150.2, 149.6, 139.6, 133.8, 130.7, 119.9, 114.0, 106.7, 106.5, 71.5, 49.5, 47.3, 41.3, 39.4, 22.4; MS: m/z 547 (5, M^+), 528 (2), 504 (12), 320 (19), 306 (100), 263 (25), 234 (19), 15 (19), 57 (19); Anal. Calcd for $C_{23}H_{24}FN_7O_5S_2$: C, 49.19; H, 4.31; F, 3.38; N, 17.46. Found: C, 49.39; H, 4.08; F, 3.11; N, 17.69.

7.7.2. (S)-N-[[3-[3-Fluoro-4-[4-[5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl]-1-piperazinyl] phenyl]-2-oxo-5-oxazolidinyl] methyl]acetamide (**1b**)

Yield 47%; mp 277–278 °C; IR: υ_{max} (KBr) 3426 (NH), 1736 (C=O), 1629 (CONH), 1516 and 1352 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): 7.52–7.47 (m, 1H, H_2 fluorobenzene), 7.44 (d, 1H, J = 4 Hz, H_4 furan), 7.18 (d, 1H, J = 4 Hz, H_3 furan), 7.10 (dd, 1H, J = 9 and 2 Hz, H_6 fluorobenzene), 6.96 (t, 1H, J = 9 Hz, H_5 fluorobenzene), 5.92 (t, 1H, J = 6 Hz, CONH), 4.78–4.75 (m, 1H, H_5 oxazolidinone), 4.03 (t, 1H, J = 9 Hz, CH_2NH), 3.82 (t, 4H, J = 5 Hz, piperazine), 3.78–3.74 (m, 1H, CH_2NH), 3.72–3.69 (m, 1H, H_4 oxazolidinone), 3.64–3.58 (m, 1H, H_4 oxazolidinone), 3.22 (t, 4H, J = 5 Hz, piperazine), 2.02 (s, 3H, CH_3CO); ^{13}C NMR (DMSO, 125 MHz): 172.4, 169.9, 155.6, 153.9, 153.6, 151.5, 147.2, 145.5, 133.9, 131.6, 119.9, 114.0, 106.6, 106.5, 71.5, 49.5, 47.3, 41.3, 39.5, 22.3; MS: m/z 531 (31, M^+), 512 (38), 488 (52), 319 (52), 306 (100), 263 (100), 235 (72), 177 (58), 152 (64), 57 (50); Anal. Calcd For $C_{23}H_{24}FN_7O_6S$: C, 50.64; H, 4.43; F, 3.48; N, 17.97; S, 5.88. Found: C, 50.92; H, 4.68; F, 3.21; N, 18.09.

7.7.3. (S)-N-[[3-[3-Fluoro-4-[4-[5-(1-methyl-5-nitro-1H-imidazole-2-yl)-1,3,4-thiadiazol-2-yl]-1-piperazinyl] phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide (**1c**)

Yield 80%; mp 261–262 °C; IR: υ_{max} (KBr) 3431 (NH), 1741 (C=O), 1654 (CONH), 1516 and 1362 (NO_2) cm^{-1}; ^1H NMR (DMSO): 8.05 (s, 1H, imidazole), 7.49 (dd, 1H, J = 14 and 2.5 Hz, H_2 fluorobenzene), 7.10 (dd, 1H, J = 9 and 2.5 Hz, H_6 fluorobenzene), 6.96 (t, 1H, J = 9 Hz, H_5 fluorobenzene), 5.94 (s (brs), 1H, CONH), 4.78–4.75 (m, 1H, H_5 oxazolidinone), 4.50 (s, 3H, NMe), 4.03 (t, 1H, J = 9 Hz, CH_2NH), 3.82 (t, 4H, J = 5 Hz, piperazine), 3.75 (dd, 1H, J = 9 and 7 Hz, CH_2NH), 3.71–3.70 (m, 1H, H_4 oxazolidinone), 3.69–3.59 (m, 1H, H_4 oxazolidinone), 3.22 (t, 4H, J = 5 Hz, piperazine), 2.02 (s, 3H, CH_3CO); ^{13}C NMR (DMSO, 125 MHz): 172.2, 169.9, 155.6, 153.9, 153.6, 149.3, 141.0, 140.4, 133.8, 133.1, 119.9, 114.1, 106.7, 106.5, 71.5, 49.5, 47.3, 41.3, 39.6, 35.0, 22.4; MS: m/z 545 (13, M^+), 502 (43), 307 (57), 263 (100), 235 (57), 57 (37); Anal. Calcd for $C_{23}H_{26}FN_9O_5S$: C, 49.37; H, 4.68; F, 3.40; N, 22.53; S, 5.73. Found: C, 49.62; H, 4.49; F, 3.72; N, 22.82.

Acknowledgments

This work was supported by grants from Tehran University of Medical Sciences and Iran National Science Foundation (INSF).

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2010.10.015.

References

[1] F.P. Tally, M.F. DeBruin, J. Antimicrob. Chemother. 46 (2000) 523–526.
[2] M.H. Wilcox, Expert Opin. Pharmacother. 6 (2005) 2315–2326.
[3] D.L. Stevens, B. Dotter, K. Madaras-Kelly, Expert Rev. Anti Infect. Ther. 2 (2004) 51–59.
[4] A.M. Nilius, Curr. Opin. Investig. Drugs 4 (2003) 149–155.
[5] R.C. Moellering, Ann. Intern. Med. 138 (2003) 135–142.
[6] A.R. Renslo, G.W. Luehr, M.F. Gordeev, Bioorg. Med. Chem. 14 (2006) 4227–4240.
[7] W.A. Gregory, D.R. Brittelli, C.L. Wang, M.A. Wuonola, R.J. McRipley, D.C. Eustice, V.S. Eberly, P.T. Bartholomew, A.M. Slee, M. Forbes, J. Med. Chem. 32 (1989) 1673–1681.
[8] J.S. Lee, Y.S. Cho, M.H. Chang, H.Y. Koh, B.Y. Chung, A.N. Pae, Bioorg. Med. Chem. Lett. 13 (2003) 4117–4120.
[9] R.N. Jones, D.M. Johnson, M.E. Erwin, Antimicrob. Agents Chemother. 40 (1996) 720–726.
[10] R. Norrby, Expert Opin. Pharmacother. 2 (2001) 293–302.
[11] D.I. Diekema, R.N. Jones, Drugs 59 (2000) 7–16.
[12] E. Fasani, F. Tilocca, A. Albini, Photochem. Photobiol. 85 (2009) 879–885.
[13] A.P. Johnson, IDrugs 6 (2003) 240–245.
[14] D.K. Hutchinson, Curr. Top. Med. Chem. 3 (2003) 1021–1042.
[15] L. Bernard, R. Stern, D. Lew, P. Hoffmeyer, Clin. Infect. Dis. 36 (2003) 1197.
[16] N. Adibpour, A. Khalaj, S. Rajabalian, Eur. J. Med. Chem. 45 (2010) 19–24.
[17] B. Das, S. Rudra, A. Yadav, A. Ray, A.V. Rao, A.S. Srinivas, A. Soni, S. Saini, S. Shukla, M. Pandya, P. Bhateja, S. Malhotra, T. Mathur, S.K. Arora, A. Rattan, A. Mehta, Bioorg. Med. Chem. Lett. 15 (2005) 4261–4267.
[18] D.B. Hoellman, G. Lin, L.M. Ednie, A. Rattan, M.R. Jacobs, P.C. Appelbaum, Antimicrob. Agents Chemother. 47 (2003) 1148–1150.
[19] V. Kalia, R. Miglani, K.P. Purnapatre, T. Mathur, S. Singhal, S. Khan, S.R. Voleti, D.J. Upadhyay, K.S. Saini, A. Rattan, V.S. Raj, Antimicrob. Agents Chemother. 53 (2009) 1427–1433.
[20] A. Foroumadi, S. Mansouri, Z. Kiani, A. Rahmani, Eur. J. Med. Chem. 38 (2003) 851–854.
[21] S. Jazayeri, M.H. Moshafi, L. Firoozpour, S. Emami, S. Rajabalian, M. Haddad, F. Pahlavanzadeh, M. Esnaashari, A. Shafiee, A. Foroumadi, Eur. J. Med. Chem. 44 (2009) 1205–1209.
[22] A. Foroumadi, S. Mansouri, S. Emami, J. Mirzaei, M. Sorkhi, N. Saeid-Adeli, A. Shafiee, Arch. Pharm. (Weinheim) 339 (2006) 621–624.
[23] A. Foroumadi, R. Ashraf-Askari, M.H. Moshafi, S. Emami, A. Zeynali, Pharmazie 58 (2003) 432–433.
[24] A. Foroumadi, M. Daneshtalab, A. Shafiee, Arzneimittelforschung Drug Res. 49 (1999) 1035–1038.
[25] S.J. Brickner, D.K. Hutchinson, M.R. Barbachyn, P.R. Manninen, D.A. Ulanowicz, S.A. Garmon, K.C. Grega, S.K. Hendges, D.S. Toops, C.W. Ford, G.E. Zurenko, J. Med. Chem. 39 (1996) 673–679.
[26] E.J. Baron, W.R. Bailey, S.M. Finegold, Bailey and Scott's Diagnostic Microbiology, eighth ed. Mosby, St. Louis, 1990.
[27] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.