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Synthesis and antibacterial activity of isothiazolyl oxazolidinones and analogous 3(2H)-isothiazolones

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ABSTRACT

The synthesis and antibacterial activity of several new 5-((3-oxoisothiazol-2(3H)-yl)methyl)-3-phenyl-oxazolidin-2-ones **8** and analogous 2-(4-substituted phenyl)-3(2H)-isothiazolones **3** and **4** substituted at 4 and/or 3-positions of the phenyl moiety with different groups of which some have shown to increase the antibacterial activity of both 3-aryl-2-oxazolidinones and 3(2H)-isothiazolones is described. The most active compounds were isothiazolyl oxazolidinones **8a,j** with unsubstituted and **8b** with 4-F substituted phenyl rings which showed activities higher than analogous 3(2H)-isothiazolones and comparable or superior to linezolid, vancomycin, and ciprofloxacin against some tested microorganisms. The change in position of F and/or the use of larger substituents gave compounds with reduced or no activity. Evaluation of cytotoxicity to mouse fibroblast (NIH/3T3) cells indicated that these compounds exhibit antibacterial activity at non-cytotoxic concentrations.

1. Introduction

In recent years 3-aryl-2-oxazolidinones have been subject of many investigations since they are a class of totally synthetic antimicrobial agents with a novel mechanism of action involving inhibition of bacterial protein synthesis at a very early stage and are active against Gram-positive bacteria resistant to methicillin (MRS) and vancomycin (VRS) [1]. Originally these compounds were developed from an extensive SAR studies on 5-(hydroxymethyl)-3-aryl-2-oxazolidinone, S-6123 [2] which had weak *in vitro* activity against certain human pathogens. Results showed that acetamidomethyl group is the best substituent for the 5-position of the 3-aryl-2-oxazolidinones, and the presence of strongly electron withdrawing groups at the 4-position of the 3-aryl moiety, enhance the antibacterial activity [3]. These findings resulted in introduction of Dup 721 and Dup 105 [3] into phase 1 of the clinical studies which were discontinued from further developments due to their toxicity profiles [4]. Subsequent investigations showed that a suitable electron donating amino substitution like piperazine or morpholine at the 4-position of the 3-aryl moiety can confer excellent antibacterial activity and a good safety profile and their effects are enhanced by one or two fluorine atoms flanking these substituents [4]. Linezolid and eperozolid [4] were emerged from the results of these investigations of which latter was not advanced to phase 3 clinical trial due to its shorter half life [5]. Linezolid as the first drug of this class of compounds has been approved for the treatment of skin soft tissue infections and bacterimia [1]. Further structural modifications mainly through replacement of 5-acetamido group with heterocyclic rings has led to the development of PH-027 [6], YX-10 [7], and AZD2563[8] which compared to linezolid have shown similar or more activity against Gram-positive microorganisms susceptible to this drug. Of these compounds PH-027 [6] has also shown activity against Gram-positive strains resistant to linezolid (Scheme 1).

On the basis of these reports and known antimicrobial activities of 3(2H)-isothiazolones [9] it seemed that replacement of 5-acetamidomethyl group of 3-aryl-2-oxazolidinones with 3(2H)-isothiazolones enhance the antibacterial activities of both classes of compounds. This paper describes the synthesis of 5-((3-oxoisothiazol-2(3H)-yl)methyl)-3-phenyloxazolidin-2-ones **8** substituted at the 4 and/or 3-positions of the phenyl moiety with groups of which some have shown to potentiate antimicrobial activity of 3(2H)-isothiazolones [10,11] and 3-aryl-2-oxazolidinones [3,4] and comparison of their antibacterial activities with analogous 3(2H)-isothiazolones **3a-k**, **4a**,**b**,**d-k** (Scheme 2), and vancomycin, linezolid and ciprofloxacin as reference drugs.

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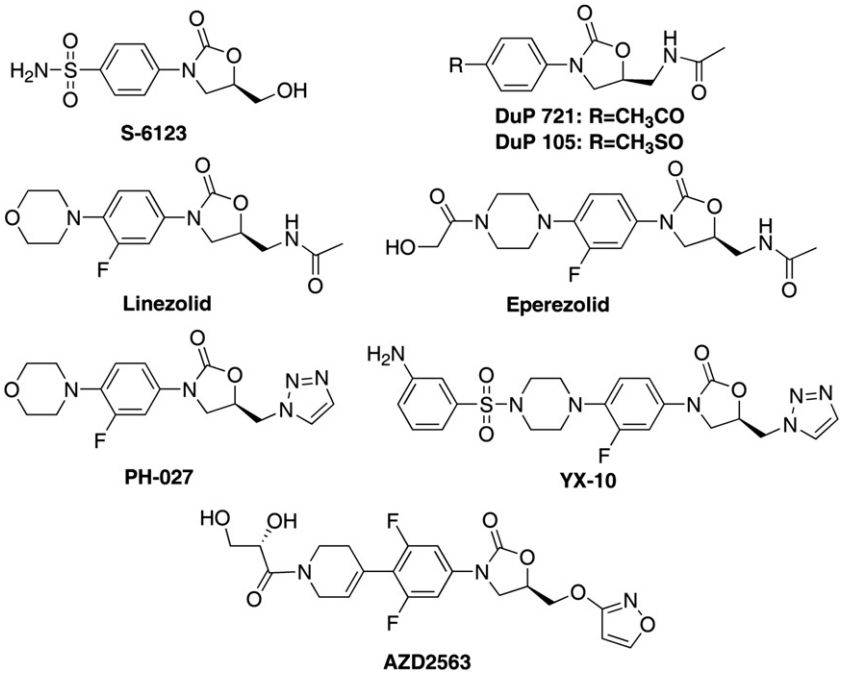


Scheme 1. Structures of S-6123, Dup-721, Dup-105, linezolid, eperezolid, PH-027, YX-10 and AZD2563.

2. Chemistry

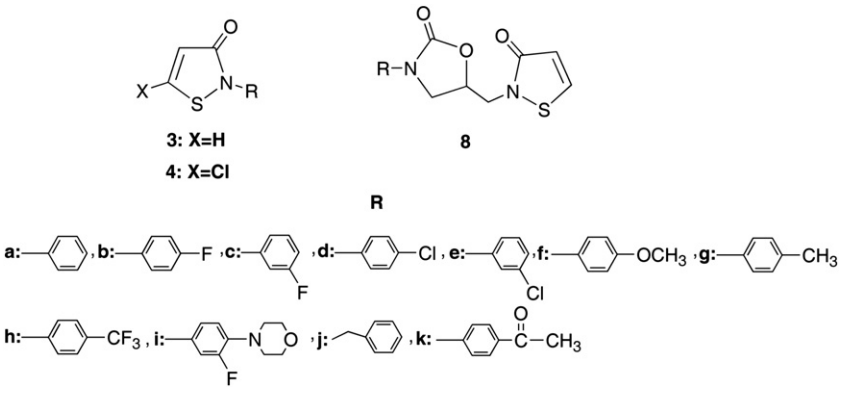
Isothiazolones **3** and **4** as outlined in Scheme 3 and described previously [10,12] were prepared through the reaction of sulfuryl chloride with dithiodipropionamides **2** which in turn were obtained by the reaction of dithiodipropionyl chloride with amines **1** (Scheme 3).

5-((3-oxoisothiazol-2(3H)-yl)methyl)-3-phenyloxazolidin-2-ones **8** (Scheme 4) were obtained by nucleophilic displacement of the hydroxyl sulfonate esters of 1,3-oxazolidin-2-one methane-sulfonates **7** with 3(2H)-isothiazolone **3l**. All 1,3-oxazolidin-2-one methanesulfonates **7** were prepared by the reaction of methansulfonyl chloride with 5-hydroxymethyl-2-oxazolidinones **6** [4] which were prepared by alkylation–cyclization of carbamates **5** with glycidyl butyrate in the presence of n-butyl lithium in THF at −78 °C [4]. Carbamates **5a–k** were synthesized through the reaction of arylamines **1** with ethyl chloroformate [13]. Except **1i**, which was prepared by the nucleophilic displacement of 4-fluoro atom of 3,4-difluoronitrobenzene with excess of morpholine followed by the reduction of the nitro group to amine [4], other amines **1** which were used in this study, are commercial (Merck Co).

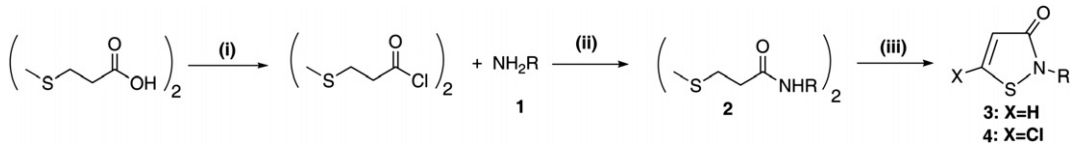
3. Results and discussion

Reviewing of the antibacterial activities of 3(2H)-isothiazolones **3a–k** and **4a,b,d–k** (Table 1) indicate that with exception of the activities of **4f,g,h** against *Pseudomonas aeruginosa*, and **4a** against *Staphylococcus aureus*, the activity of other compounds against Gram-positive bacteria were comparable or higher than those against Gram-negative bacteria. Furthermore with exception of activities of **4a,b** against *S. aureus*, **4h** against *Staphylococcus epidermidis*, **4j** against *P. aeruginosa* and **4k** against *Salmonella typhimurium*, other 3(2H)-isothiazoleones substituted at the 5-position of the isothiazolone ring with chloro in comparison with unsubstituted analogous compounds, showed higher or equal activity.

As it was anticipated introduction of 3(2H)-isothiazolone at the 5-position of oxazolidin-2-ones resulted in compounds which in general were more active than linezolid and vancomycin against tested gram negative bacteria. The most active compounds of this series were unsubstituted phenyl derivatives **8a,j** and 4-fluorophenyl derivative **8b** which with exception of the lower activity of **8j** against *Escherichia coli*, showed higher activities than analogous 3(2H)-isothiazolones against all tested microorganisms. These compounds also in comparison to the reference drugs showed higher or comparable



Scheme 2. Structures of 2-oxazolidinones **8** and 3(2H)-isothiazolones **3a–k**, **4a,b,d–k**.



Scheme 3. (i): $SOCl_2$, reflux, 1 h; (ii): NaOH 25%, 0–5 °C, r. t. 18 h; (iii): SO_2Cl_2, 0–10 °C, r. t. 18 h.

activities against some tested microorganisms. Particularly, the activity of **8a** the most potent compound of this study was higher than linezolid and comparable to vancomycin against *S. aureus*, higher than ciprofloxacin against *S. epidermidis* and equal to ciprofloxacin against all tested Gram-negative bacteria. The activity of **8j**, another compound with unsubstituted phenyl ring was higher than linezolid and vancomycin and comparable to ciprofloxacin against *S. aureus* and higher than vancomycin and ciprofloxacin against *S. epidermidis*. Moreover the activity of the 4-fluorophenyl derivative **8b** was higher than linezolid and comparable to vancomycin against *S. aureus*.

While substitution of the 4-position of the phenyl ring of 3(2H)-isothiazolones with chloro, methoxy, and methyl group increased their antibacterial activities against all (**3d** and **4d**) or some tested bacteria like *S. aureus* (**4f**,**g**), *P. aeruginosa* (**4f**,**g**) and *S. epidermidis* (**3f**,**g** and **4f**) in comparison to their unsubstituted analogous **3a** and **4a**, replacement of 4-fluroro atom of the phenyl ring of isothiazolyl oxazolidinone **8** with these groups (**8d**,**f** and **g**) diminished their activities. Similarly substitution of the phenyl ring with trifluoromethyl (**8h**) [14], acetyl (**8k**) [3], and 3-fluoro-4-(4-morpholinyl) (**8i**) [4], which have been reported to increase the potency of 3-aryl-2-oxazolidinones resulted in less active compounds. Also changing the position of fluoro and chloro from 4 to 3 (**8c**,**e**), reduced their antibacterial activities.

Antibacterial activity of compounds **8a**,**b**,**j** which showed lower MICs against tested gram-positive bacteria listed in Table 1 were further evaluated against two clinical isolates of *S. aureus* resistant to methicillin and results are presented in Table 2. All these compounds were found inferior to linezolid with MIC > 4 which is greater than susceptibility break point for this antibiotic.

The cytotoxic properties of compounds **8a**,**b**,**d**,**j** were also investigated on normal mouse fibroblast (NIH/3T3) cell line using MTT colorimetric assay [15] and the results are shown in Table 3.These compounds were not cytotoxic at concentration below 162 μg/mL after 24 h incubation and compound **8j** showed lower cytotoxicity at 162–273 μg/mL range. A comparison between MIC and IC_{50} of the tested compounds suggests that compounds **8a**,**b**,**d**,**j** exhibit in vitro antibacterial activity at non-cytotoxic concentrations.

The results of this study suggests that replacement of the 5-acetamidomethyl of the 3-aryl-2-oxazolidinones with 3(2H)-isothiazolones enhance the potency and antibacterial spectrum of both classes of compounds only when the 4-position of the 3-aryl group is unsubstituted or substituted with small size fluoro atom which might be due to dual modes of action. The change in position of F and/or the use of larger groups whether with electron-donating (CH_3, OCH_3) or electron-withdrawing properties (Cl, CF_3 and $COCH_3$) and irrespective of differences in lipophilicity of substituents resulted in compounds with reduced or no antibacterial activity.

4. Experimental

4.1. Chemistry

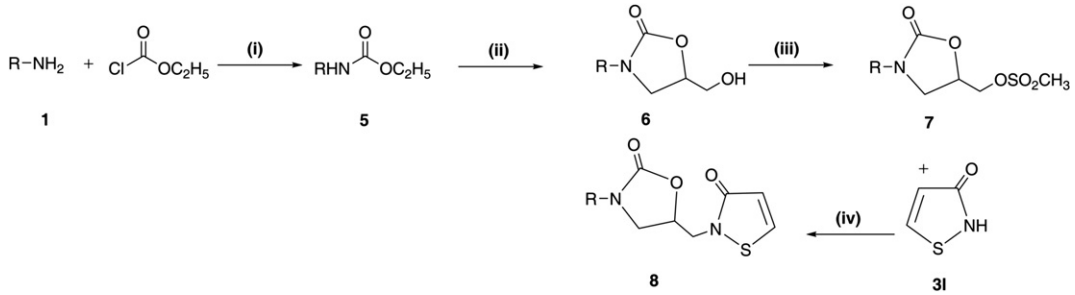
All common reagents and solvents were obtained from Merck Co. Column chromatography was carried out using silica gel (Kieselgel 60, 230–400 mesh, Merck). Melting points were determined on a MEL-270 Sibata melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 500 MHz, a Varian Unity Plus 400 (300.866 MHz) and a Bruker 80 MHz spectrometers using DMSO-d_6 and $CDCl_3$ as solvent. Chemical shifts (δ) are reported in ppm relative to TMS as internal standard. Mass spectra were obtained on a Finningan TSQ-70 instrument. Infrared spectra were recorded on a Nicolet Magna IR 550 spectrometer. Elemental analyses for C, H and N were performed using a Heracus CHN–O-rapid elemental analyzer and the results are within ±0.4% of the theoretical values. All compounds were characterized by the above techniques. Analytical and spectroscopic data for the known compounds were consistent with reported literature values and data only for new compounds are presented. Ciprofloxacin, linezolid, and vancomycin were commercial.

*4.1.1. Synthesis of 3(2H)-isothiazolones **3**,**4** and 5-((3-oxoisothiazol-2(3H)-yl)methyl)-3-phenyloxazolidin-2-one **8***

*4.1.1.1. Preparation of 3(2H)-isothiazolones (**3**,**4**).* Preparation of compounds **3a**,**d**,**f**–**h**,**k**,**4a**,**d**,**f**–**h**,**k** [10], **3e**,**j**,**l** and **4j** [12] by the reaction of corresponding dithiodipropionamides **2** with sulfuryl chloride have been described previously and physicochemical data for those of **3b**,**c**,**i** and **4b**,**c**,**e**,**i** which were prepared by the same method, are as follows.

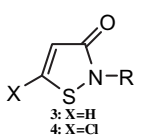
*4.1.1.1.1. 2-(4-fluorophenyl)isothiazol-3(2H)-one (**3b**).* Yield 20.4%, mp 97–100 °C (toluene). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.32(d, 1H, J = 8.5 Hz), 7.12–7.15(m, 2H), 7.51–7.54(m, 2H), 8.16(d, 1H, J = 8.5 Hz). MS (EI) m/z: 194.0(M^+). Anal. Calcd. For C_9H_6FNOS: C, 55.37; H, 3.10; N, 7.18. Found: C, 55.35; H, 3.11; N, 7.20.

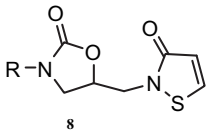
*4.1.1.1.2. 5-chloro-2-(4-fluorophenyl)isothiazol-3(2H)-one (**4b**).* Yield 70%, mp 119 °C (ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.36(s, 1H), 7.12–7.15(m, 2H), 7.46–7.49(m, 2H). MS (EI) $m/$



Scheme 4. (i): Pyr., 0–5 °C, r. t. 12 h; (ii): n-Buthyl lithium, Glycidyl butyrate, −78 °C, r. t. 18 h; (iii): CH_3SO_2Cl, 0 °C, r. t.; (iv): NaH, DMF, r. t. overnight.

Table 1
MIC (μg/mL) values of compounds **3a–k**,**4a**,**b**,**d–k**,**8a–k** and vancomycin and ciprofloxacin as reference drugs.





Compound	X	R	*S. a*[a]	*S. e*[b]	*E. coli*[c]	*S. typh*[d]	*Ps. a*[e]
3a	H	Phenyl	3.25	3.25	12.5	25	12.5
4a	Cl	Phenyl	50	0.3	0.6	0.3	1.25
8a	H	Phenyl	0.75	0.75	0.01	0.01	0.01
3b	H	4-Fluorophenyl	1.5	3	6	12.5	12.5
4b	Cl	4-Fluorophenyl	3	3	6	6	12.5
8b	H	4-Fluorophenyl	0.75	1.5	0.75	0.75	0.75
3c	H	3-Fluorophenyl	6	3.25	12.5	12.5	12.5
8c	Cl	3-Fluorophenyl	50	100	100	100	100
3d	H	4-Chlorophenyl	3	1.5	10	10	10
4d	Cl	4-Chlorophenyl	0.1	0.1	0.3	0.3	0.6
8d	H	4-Chlorophenyl	1.5	1.5	12.5	12.5	25
3e	H	3-Chlorophenyl	10	12.5	25	25	25
4e	Cl	3-Chlorophenyl	6	10	12.5	12.5	12.5
8e	H	3-Chlorophenyl	12.5	12.5	12.5	12.5	12.5
3f	H	4-Methoxyphenyl	10	3	25	12.5	25
4f	Cl	4-Methoxyphenyl	1.5	0.25	1.5	1.5	0.01
8f	H	4-Methoxyphenyl	50	50	100	100	100
3g	H	4-Methylphenyl	25	2	25	25	25
4g	Cl	4-Methylphenyl	5	0.5	5	5	0.01
8g	H	4-Methylphenyl	25	25	25	25	25
3h	H	4-Trifluoromethylphenyl	25	0.25	25	25	25
4h	Cl	4-Trifluoromethylphenyl	5	5	25	12.5	0.1
8h	H	4-Trifluoromethylphenyl	50	100	100	100	100
3i	H	3-Fluoro-4-morpholinophenyl	12.5	10	25	25	25
4i	Cl	3-Fluoro-4-morpholinophenyl	10	10	25	25	12.5
8i	H	3-Fluoro-4-morpholinophenyl	6	12.5	25	12.5	12.5
3j	H	Benzyl	12.5	6	12.5	12.5	12.5
4j	Cl	Benzyl	0.5	0.5	0.5	12.5	25
8j	H	Benzyl	0.5	0.5	12.5	12.5	12.5
3k	H	4-Acetylphenyl	12.5	12.5	12.5	12.5	12.5
4k	Cl	4-Acetylphenyl	12.5	12.5	12.5	25	12.5
8k	H	4-Acetylphenyl	12.5	25	50	50	100
Vancomycin	–	–	0.75	0.75	>100	>100	>100
Ciprofloxacin	–	–	0.5	1	0.01	0.01	0.01
Linezolid	–	–	2	0.03	>100	>100	>100

[a] *S. a*: *Staphylococcus aureus* ATCC 29737.
[b] *S. e*: *Staphylococcus epidermidis* ATCC 12229.
[c] *E. coli*: *Escherichia coli* ATCC 8739.
[d] *S.typh*: *Salmonella typhimurium* ATCC 1639.
[e] *Ps. a*: *Pseudomonas aeruginosa* ATCC 9027.

z: 229.1(M^+), 194.1, 166.1. Anal. Calcd. For $C_9H_5ClFNOS$: C, 47.07; H, 2.19; N, 6.10. Found: C, 47.20; H, 2.12; N, 6.30.

*4.1.1.1.3. 2-(3-fluorophenyl)isothiazol-3(2H)-one (**3c**).* Yield 20%, mp 95–97 °C (triturated with n-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 6.35(d, 1H, *J* = 8.0 Hz), 7.0–7.05(m, 1H), 7.29–7.33(m, 1H), 7.39–7.5(m, 2H), 8.25(d, 1H, *J* = 8.0 Hz). MS (EI) *m*/*z*: 195.4(M^+). Anal. Calcd. For C_9H_6FNOS: C, 55.37; H, 3.10; N, 7.18. Found: C, 55.29; H, 3.05; N, 7.10.

*4.1.1.1.4. 5-chloro-2-(3-fluorophenyl)isothiazol-3(2H)-one (**4c**).* Yield 49%, mp 115 °C (n-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 6.38(s, 1H), 7.02–7.07(m, 1H), 7.28–7.31(m, 1H), 7.39–7.44(m, 2H). MS (EI) *m*/*z*: 228.4(M^+). Anal. Calcd. For $C_9H_5ClFNOS$: C, 47.07; H, 2.19; N, 6.10. Found: C, 47.19; H, 2.10; N, 6.02.

*4.1.1.1.5. 5-chloro-2-(3-chlorophenyl)isothiazol-3(2H)-one (**4e**).* Yield 57.3%, mp 118–120 °C (triturated with petroleum ether). ^{1}H

Table 2
MIC (μg/mL) values of compounds **8a**,**b**,**d**,**j** and linezolid as reference drug against two clinical isolates of *S. aureus* resistant to methicillin (MRSA).

Compound	$MRSA_1$	$MRSA_2$
8a	6	10
8b	10	12.5
8d	12.5	12.5
8j	8	12.5
Linezolid	2	2.5

Table 3
Cytotoxic activity of compounds **8a**,**b**,**d**,**j** in comparison with linezolid against mouse fibroblast (NIH/3T3) cell line.

Compound	IC_{50} (μg/mL)[a]
8a	162 ± 15.5
8b	174 ± 16
8d	194 ± 8.4
8j	273 ± 17.6
Linezolid	465

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

NMR (500 MHz, $CDCl_3$) δ: 6.35(s, 1H), 7.29–7.31(m, 1H), 7.37(t, 1H, $J = 8.0$ Hz), 7.41–7.44(m, 1H), 7.60(t, 1H, $J = 2.0$ Hz). MS (EI) m/z: 245.3(M^+). Anal. Calcd. For $C_9H_5Cl_2NOS$: C, 43.92; H, 2.05; N, 5.69. Found: C, 43.99; H, 2.19; N, 5.72.

*4.1.1.1.6. 2-(3-fluoro-4-morpholinophenyl)isothiazol-3(2H)-one (**3i**).* Yield 15%, mp 128–130 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.05–3.12(m, 4H), 3.79–3.88(m, 4H), 6.33(d, 1H, $J = 8.0$ Hz), 6.95 (d, 1H, $J = 9.0$ Hz), 7.17–7.20(m, 1H), 7.26–7.28(m, 1H), 8.14(d, 1H, $J = 8.0$ Hz). MS (EI) m/z: 278.8(M^+). Anal. Calcd. For $C_{13}H_{13}FN_2O_2S$: C, 55.70; H, 4.67; N, 9.99. Found: C, 55.78; H, 4.61; N, 9.74.

*4.1.1.1.7. 5-chloro-2-(3-fluoro-4-morpholinophenyl)isothiazol-3(2H)-one (**4i**).* Yield 45%, mp 135–137 °C (ethyl acetate-n-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.08–3.10(m, 4H), 3.86–3.88(m, 4H), 6.34(s, 1H), 6.97(t, 1H, $J = 9.1$ Hz), 7.15–7.19(m, 1H), 7.24–7.28(m, 1H). MS (EI) m/z: 314.8(M^+). Anal. Calcd. For $C_{13}H_{12}ClFN_2O_2S$: C, 49.61; H, 3.84; N, 8.90. Found: C, 49.75; H, 3.95; N, 8.82.

*4.1.1.2. Preparation of dithiodipropionamides (**2**).* Physicochemical data for the novel dithiodipropionamides **2b**,**c**,**i** which were prepared by the reported method for the preparation of known dithiodipropionamides **2a**,**d**,**f**–**h**,**j**,**k** [10] and **2e**,**l** [12] through the reaction of the dithiodipropionyl chloride with amines **1b**,**c**,**i** are as follows:

*4.1.1.2.1. 3,3′-disulfanediylbis(N-(4-fluorophenyl)propanamide) (**2b**).* Yield 94.8%, mp 169–174 °C. MS (EI) m/z: 198.1, 165.1, 136.1. IR (KBr, cm^{-1}): 3282, 3068, 2914, 1651, 1610. Anal. Calcd. For $C_{18}H_{18}F_2N_2O_2S_2$: C, 54.53; H, 4.58; N, 7.07. Found: C, 54.67; H, 4.66; N, 6.98.

*4.1.1.2.2. 3,3′-disulfanediylbis(N-(3-fluorophenyl)propanamide) (**2c**).* Yield 96%, mp 122–125 °C. MS (EI) m/z: 197.1, 166.2, 137.2. IR (KBr, cm^{-1}): 3372, 3106, 2940, 1663, 1596. Anal. Calcd. For $C_{18}H_{18}F_2N_2O_2S_2$: C, 54.53; H, 4.58; N, 7.07. Found: C, 55.45; H, 4.63; N, 7.12.

*4.1.1.2.3. 3,3′-disulfanediylbis(N-(3-fluoro-4-morpholinophenyl)-propanamide) (**2i**).* Yield 56.3%, mp 158–162 °C. MS (EI) m/z: 283.1, 249.9, 222.0. IR (KBr, cm^{-1}): 3294, 2912, 1664. Calcd. For $C_{26}H_{32}F_2N_4O_4S_2$: C, 55.11; H, 5.69; N, 9.89. Found: C, 54.91; H, 5.48; N, 9.95.

*4.1.1.3. General procedure for preparation of 5-((3-oxoisothiazol-2(3H)-yl)methyl)-3-phenyloxazolidin-2-one (**8**).* A mixture of 3(2H)-isothiazolone **3l** (0.03 g, 0.0003 mol), NaH (0.01 g) and mesylates **7** (0.0003 mol) in DMF (2 mL) under N_2 was stirred at room temperature overnight. The solution was then poured to a mixture of crushed ice-water and the solid was filtered. The solid was purified by chromatography using chloroform–ethyl acetate (2:1) to afford compounds **8a**–**k**.

*4.1.1.3.1. 5-((3-oxoisothiazol-2(3H)-yl)methyl)-3-phenyl-oxazolidin-2-one (**8a**).* Yield 15%, mp 115–118 °C (ethyl acetate-n-hexane). ^{1}H NMR (80 MHz, $CDCl_3$) δ: 3.9–4.3(m, 2H,), 4.68(d, 2H, $J = 4.5$ Hz), 5.03(m, 1H), 6.56(d, 1H, $J = 4.6$ Hz), 7.1–7.6(m, 5H), 8.5(d, 1H, $J = 4.6$ Hz). MS (EI) m/z: 276.0 (M^+), 174.9. Anal. Calcd. For $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14. Found: C, 56.62; H, 4.40; N, 10.05.

*4.1.1.3.2. 3-(4-fluorophenyl)-5-((3-oxoisothiazol-2(3H)-yl)methyl)oxazolidin-2-one (**8b**).* Yield 35%, mp 83–86 °C (ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.99(dd, 1H, $J = 8.5$, 6.0 Hz), 4.16(t, 1H, $J = 8.5$ Hz), 4.66(dddd, 2H, $J = 11.5$, 4.5 Hz), 5.02–5.05(m, 1H), 6.63(d, 1H, $J = 4.5$ Hz), 7.10(dd, 2H, $J = 9.0$, 8.5 Hz), 7.53 (dd, 2H, $J = 9.0$, 4.5 Hz), 8.47(d, 1H, $J = 4.5$ Hz). MS (EI) m/z: 293.8(M^+), 194.4, 150.4. Anal. Calcd. For $C_{13}H_{11}FN_2O_3S$: C, 53.05; H, 3.77; N, 9.52. Found: C, 53.09; H, 3.75; N, 9.56.

*4.1.1.3.3. 3-(3-fluorophenyl)-5-((3-oxoisothiazol-2(3H)-yl)methyl)oxazolidin-2-one (**8c**).* Yield 10%, oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.99(dd, 1H, $J = 8.8$, 6.4 Hz), 4.10–4.27(m,1H), 4.62–4.71(m, 2H), 5.02–5.07(m,1H), 6.63(d,1H, $J = 4.4$ Hz), 6.86–6.87(m, 1H), 7.25(dd,1H, $J = 7.0$, 1.5 Hz), 7.32–7.38(m,1H), 7.48–7.71(m, 1H), 8.47(d, 1H, $J = 4.4$ Hz). MS (EI) m/z: 293.9(M^+), 194.1. Anal. Calcd. For $C_{13}H_{11}FN_2O_3S$: C, 53.05; H, 3.77; N, 9.52. Found: C, 53.10; H, 3.79; N, 9.43.

*4.1.1.3.4. 3-(4-chlorophenyl)-5-((3-oxoisothiazol-2(3H)-yl)methyl)oxazolidin-2-one (**8d**).* Yield 45%, mp 97–100 °C (triturated with petroleum ether). ^{1}H NMR (80 MHz, $CDCl_3$) δ: 3.9, 4.15(ddt, 2H, $J = 12.0$, 6.4, 8.8 Hz), 4.66(d, 2H, $J = 4.4$ Hz), 4.9–5.2(m, 1H), 6.6(d, 1H, $J = 4.7$ Hz), 7.35(d, 2H, $J = 10.4$ Hz), 7.45(d, 2H, $J = 10.4$ Hz), 8.45(d,1H, $J = 4.7$ Hz). MS (EI) m/z: 312.1($M + 2$), 310.7(M^+), 212.5, 167.4. Anal. Calcd. For $C_{13}H_{11}ClN_2O_3S$: C, 50.24; H, 3.57; N, 9.01. Found: C, 50.20; H, 3.53; N, 9.14.

*4.1.1.3.5. 3-(3-chlorophenyl)-5-((3-oxoisothiazol-2(3H)-yl)methyl)oxazolidin-2-one (**8e**).* Yield 45%, oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.99(dd, 1H, $J = 9.0$, 6.5 Hz), 4.16 (t, 1H, $J = 8.5$ Hz), 4.66(dddd, 2H, $J = 12.0$, 4.5 Hz), 5.02–5.06(m, 1H), 6.63(d, 1H, $J = 4.5$ Hz), 7.13(dddd, 1H, $J = 2.0$, 1.0 Hz), 7.31(t, 1H, $J = 8.0$ Hz), 7.48(dddd, 1H, $J = 2.5$, 1.0 Hz), 7.6(t, 1H, $J = 2.0$ Hz), 8.47(d, 1H, $J = 4.5$ Hz). Anal. Calcd. For $C_{13}H_{11}ClN_2O_3S$: C, 50.24; H, 3.57; N, 9.01. Found: C, 50.20; H, 3.51; N, 8.95.

*4.1.1.3.6. 3-(4-methoxyphenyl)-5-((3-oxoisothiazol-2(3H)-yl)methyl)oxazolidin-2-one (**8f**).* Yield 33%, mp 100–105 °C (triturated with n-hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.80(s, 3H), 3.95(dd, 1H, $J = 6.8$, 4.8 Hz), 4.14(t, 1H, $J = 6.8$ Hz), 4.64(dddd, 2H, $J = 9.2$, 3.6 Hz), 4.98–5.01(m, 1H), 6.61(d, 1H, $J = 4.0$ Hz), 6.92(d, 2H, $J = 7.2$ Hz), 7.45(d, 2H, $J = 7.2$ Hz), 8.45(d, 1H, $J = 4.0$ Hz). MS (EI) m/z: 306.9(M^+), 290.5, 275.5. Anal. Calcd. For $C_{14}H_{14}N_2O_4S$: C, 54.89; H, 4.61; N, 9.14. Found: C, 54.88; H, 4.64; N, 9.17.

*4.1.1.3.7. 5-((3-oxoisothiazol-2(3H)-yl)methyl)-3-p-tolyloxazolidin-2-one (**8g**).* Yield 39.3%, 76–80 °C(ethyl acetate-n-hexane).^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.33(s, 3H), 3.96(dd, 1H, $J = 7.2$, 5.2 Hz), 4.15(t, 1H, $J = 7.2$ Hz), 4.61, 4.67(dddd, 2H, $J = 9.6$, 4.0 Hz), 4.98–5.02(m, 1H), 6.61(d, 1H, $J = 4.0$ Hz), 7.19(d, 2H, $J = 6.8$ Hz), 7.43(d, 2H, $J = 6.8$ Hz), 8.45(d, 1H, $J = 4.0$ Hz). MS (EI) m/z: 290.5(M^+). Anal. Calcd. For $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65. Found: C, 57.96; H, 4.91; N, 9.63.

*4.1.1.3.8. 5-((3-oxoisothiazol-2(3H)-yl)methyl)-3-(4-(trifluoromethyl)phenyl)oxazolidin-2-one (**8h**).* Yield 30%, mp 90–93 °C (triturated with petroleum ether).^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.05(dd, 1H, $J = 9.2$, 5.5 Hz), 4.23(t,1H, $J = 7.4$ Hz), 4.69(dddd, 2H, $J = 11.5$, 4.5 Hz), 5.06–5.09(m,1H), 6.62(d,1H, $J = 5.0$ Hz), 7.65(d, 2H, $J = 9.0$ Hz), 7.71(d, 2H, $J = 9.0$ Hz), 8.48(d, 1H, $J = 5.0$ Hz). MS (EI) m/z: 343.9(M^+). Anal. Calcd. For $C_{14}H_{11}F_3N_2O_3S$: C, 48.84; H, 3.22; N, 8.14. Found: C, 48.92; H, 3.21; N, 8.16.

*4.1.1.3.9. 3-(3-fluoro-4-morpholinophenyl)-5-((3-oxoisothiazol-2(3H)-yl)methyl)oxazolidin-2-one (**8i**).* Yield 34%, mp 88–91 °C (ethyl acetate-n-hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.1–3.25(m, 4H), 3.74–4.03(m, 5H), 4.14(t, 1H, $J = 8.0$ Hz), 4.61–4.69(m, 2H), 5.0–5.07(m, 1H), 6.62(d, 1H, $J = 4.0$ Hz), 7.0–7.1(m, 1H), 7.23–7.30(m, 1H), 7.53(d, 1H, $J = 14.0$ Hz), 8.48(d, 1H, $J = 4.0$ Hz). MS (EI) m/z: 378.9(M^+), 377.5. Anal. Calcd. For $C_{17}H_{18}FN_3O_4S$: C, 53.82; H, 4.78; N, 11.08. Found: C, 53.76; H, 4.72; N, 11.07.

*4.1.1.3.10. 3-benzyl-5-((3-oxoisothiazol-2(3H)-yl)methyl)oxazolidin-2-one (**8j**).* Yield 31%, oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.58(t, 2H, $J = 6.8$ Hz), 4.3–4.5(m, 4H), 4.84–4.88(m, 1H), 6.75(d, 1H, $J = 4.4$ Hz), 7.28–7.40(m, 5H), 8.90(d, 1H, $J = 4.4$ Hz). MS (EI) m/z: 289.7(M^+). Anal. Calcd. For $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65. Found: C, 57.94; H, 4.88; N, 9.53.

*4.1.1.3.11. 3-(4-acetylphenyl)-5-((3-oxoisothiazol-2(3H)-yl)methyl)oxazolidin-2-one (**8k**).* Yield 47%, mp 98–103 °C (triturated with petroleum ether). ^{1}H NMR (80 MHz, $CDCl_3$) δ: 2.59(s, 3H), 3.9–4.3(m, 2H), 4.7(d, 2H, $J = 8.0$ Hz), 4.9–5.2(m, 1H), 6.60(d, 1H, $J = 8.0$ Hz), 7.68(d, 2H, $J = 16.0$ Hz), 7.94(d, 2H, $J = 16.0$ Hz), 8.45(d, 1H, $J = 8.0$ Hz). MS (EI) m/z: 317.6(M^+), 218.1. Anal. Calcd. For $C_{15}H_{14}N_2O_4S$: C, 56.59; H, 4.43; N, 8.80. Found: C, 56.48; H, 4.36; N, 8.81.

*4.1.1.4. Preparation of mesylates (**7**).* The synthesis of mesylates **7a** [16], **7b**,**d**,**f** [17],**7c** [18],**7g**,**h** [19],**7i** [4] and **7k** [3] by the reaction of methanesulfonyl chloride with 5-hydroxymethyl oxazolidinones **6** and with exception that **7a** their physical and analytical data have been reported. Physicochemical data of **7a** and the novel mesylates **7e**,**j** which were prepared by a similar method, are as follows:

*4.1.1.4.1. (2-oxo-3-phenyl-1,3-oxazolidin-5-yl)methyl methanesulfonate (**7a**).* Yield 56%, mp 116–119 °C (n-hexane). ^{1}H NMR (80 MHz, $CDCl_3$) δ: 3.11(s, 3H), 3.8–4.1(m, 4H), 4.6–4.8 (m, 2H), 7.3–7.6(m, 5H). MS (EI) *m*/*z*: 271.1(M^+), 162.3. Anal. Calcd. For $C_{11}H_{13}NO_5S$: C, 48.70; H, 4.83; N, 5.16. Found: C, 48.52; H, 4.66; N, 5.20.

*4.1.1.4.2. [3-(3-chlorophenyl)-2-oxo-1,3-oxazolidin-5-yl]methyl methanesulfonate (**7e**).* Yield 50%, mp 78–80 °C (triturated with petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.03(s, 3H), 3.9(dd, 1H, $J = 9.0$, 6.4 Hz), 4.14 (t, 1H, $J = 8.2$ Hz), 4.55(dddd, 2H, $J = 12.0$, 4.2 Hz), 4.64–4.66(m,1H), 7.12(dddd,1H, $J = 2.0$, 0.6 Hz), 7.29(t,1H, $J = 6.2$ Hz), 7.45(ddt,1H, $J = 2.0$, 0.5, 1.2 Hz), 7.57(t, 1H, $J = 2.0$ Hz).MS (EI) *m*/*z*: 304.8(M^+),195. Anal. Calcd. For $C_{11}H_{12}ClNO_5S$: C, 43.21; H, 3.96; N, 4.58. Found: C, 43.01; H, 3.89; N, 4.61.

*4.1.1.4.3. (3-benzyl-2-oxo-1,3-oxazolidin-5-yl)methyl methanesulfonate (**7j**).* Yield 68%, oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.03(s, 3H), 3.31(dd, 1H, $J = 9.2$, 6.4 Hz), 3.51(t, 1H, $J = 5.6$ Hz), 3.78(dd, 1H, $J = 11.6$, 4.8 Hz), 4.20(dd, 1H, $J = 11.6$, 4.8 Hz), 4.41(s, 2H), 4.72–4.78(m,1H), 7.32–7.39(m, 5H). MS (EI) *m*/*z*: 285.1(M^+), 190.7. Anal. Calcd. For $C_{12}H_{15}NO_5S$: C, 50.52; H, 5.30; N, 4.91. Found: C, 50.54; H, 5.28; N, 4.83.

*4.1.1.5. Preparation of 5-hydroxymethyl oxazolidin-2-ones (**6**).* All 5-hydroxymethyl oxazolidin-2-one **6** of this study were prepared by the reaction of carbamates **5** with glycidyl butyrate in THF at −78 °C [4] in the presence of n-buthyl lithium. Physical and analytical data of **6a** [20], **6c** [18], **6g**,**h** [19], **6i** [4], prepared by this method and those of **6b**,**d**,**f** [17], **6j** [21] and **6k** [3] prepared by the reaction of arylamines with glycidol followed by dialkyl carbonate hetero-cyclization, have been described previously and for the novel compound **6e** are as follows:

*4.1.1.5.1. 3-(3-chlorophenyl)-5-(hydroxymethyl)oxazolidin-2-one (**6e**).* Yield 46%, mp 57–60 °C (ethyl acetate-n-hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.76(d, 1H, $J = 10.0$ Hz), 3.97–4.04(m, 4H), 4.74–4.76(m, 1H), 7.11(dddd, 1H, $J = 1.6$, 0.8 Hz), 7.29(t,1H, $J = 6.4$ Hz), 7.44(ddt,1H, $J = 1.6$, 0.4, 1.2 Hz), 7.59(t, 1H, $J = 2.0$ Hz). MS (EI) *m*/*z*: 226.8(M^+), 152.8. Anal. Calcd. For $C_{10}H_{10}ClNO_3$: C, 52.76; H, 4.43; N, 6.15. Found: C, 52.83; H, 4.49; N, 6.04.

*4.1.1.6. Preparation of ethyl carbamates (**5**).* Carbamates **5a**–**k** were prepared by the reaction of ethyl chloroformate with arylamines. Preparation of **5b**,**c** [13], **5d**–**g** [22], and **5j** [23] under similar conditions, and of **5a** [24], **5h** [25], and **5k** [26] by the reaction of aryl isocyanates with alcohols have been reported previously. Physicochemical data for the novel carbamate **5i** are as follows:

*4.1.1.6.1. ethyl 3-fluoro-4-morpholinophenylcarbamate (**5i**).* Yield 94%, mp 150–152 °C(triturated with warm n-hexane).^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.32(t, 3H, $J = 6.7$ Hz), 4.25(q, 2H, $J = 6.7$ Hz), 3.11(bs, 4H), 3.93(bs, 4H), 6.65(bs, 1H), 6.98–7.04(m, 2H), 7.35(d, 1H, $J = 13.2$ Hz). MS (EI) *m*/*z*: 267.6 (M^+), 222.1. Anal. Calcd. For $C_{13}H_{17}FN_2O_3$: C, 58.20; H, 6.39; N, 10.44. Found: C, 58.05; H, 6.25; N, 10.36.

4.2. Antibacterial susceptibility testing

The in vitro antibacterial activities of the compounds **3a**–**k**,**4a**,**b**,**d**–**k**,**8a**–**k** and vancomycin, ciprofloxacin and linezolid as the reference drugs was determined by the conventional agar dilution method using Mueller Hinton agar medium [27].The tested Gram-positive organisms included two clinical isolates of *S. aureus* resistant to methicillin (MRSA), *S. aureus* ATCC 29737 and *S. epidermidis* ATCC 12229.Gram-negative bacteria used in the study were *E. coli* ATCC 8739, *S. typhimurium* ATCC 1639 and *P. aeruginosa* ATCC 9027. The tested compounds, vancomycin and ciprofloxacin were dissolved in DMSO while linezolid was dissolved in water. Suspensions of each of bacteria were prepared to contain approximately 10^6 colony forming units (CFU/mL) and applied to plates with two fold serially diluted compounds to be tested in distilled water in concentration ranging from 0.01 to 100 μg/mL and incubated at 37 °C for 18 h. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilution as used in the experiments. The minimum inhibitory concentration (MICs) values were the lowest concentration of compounds, which resulted in no visible growth on the plate and are listed in Table 1.

4.3. Cytotoxic activity

The in vitro cytotoxicity of compounds **8a**,**b**,**d**,**j** against normal mouse fibroblast (NIH/3T3) cell line was assessed using MTT colorimetric assay [15] according to the previously described method in literature and results are presented in Table 3.

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References

[1] B. Bozdogan, P.C. Appelbaum, Int. J. Antimicrob. Agents 23 (2004) 113–119.
[2] W.A. Gregory. US Patent 4461773(1984). Chem. Abst. 101 p211126n
[3] W.A. Gregory, D.R. Britelli, C.L.J. 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.
[4] 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.
[5] M.R. Barbachyn, C.W. Ford, Angew. Chem. Int. Ed. 42 (2003) 2010–2023.
[6] O.A. Philips, E.E. Udo, A.A.M. Ali, N. Al-Hassawi, Bioorg. Med. Chem. 11 (2003) 35–41.
[7] F. Houxing, X. Gang, C. Yilang, J. Zhiteng, Z. Shuhua, Y. Yushe, J. Ruyun. Eur. J. Med. Chem. 42 (2007) 1137–1143.
[8] M.B. Gravestock, D.G. Acton, M.J. Betts, M. Dennis, G. Hatter, A. McGregor, M.L. Swain, R.G. Wilson, L. Woods, A. Wookey, Bioorg. Med. Chem. Lett. 13 (2003) 4179–4186.
[9] S.N. Lewis, G.A. Miller, A.B. Law. US Patent 3761488 (1973).
[10] A. Khalaj, N. Adibpour, A.R. Shahverdi, M. Daneshtalab, Eur. J. Med. Chem. 39 (2004) 699–705.
[11] N. Adibpour, A. Khalaj, S. Rezaee, M. Daneshtalab, Folia. Microbiol. 52 (2007) 573–576.
[12] S.N. Lewis, G.A. Miller, M. Hausman, E.C. Szamborski. J. Het. Chem. (1971), 571–580.
[13] G.C. Finger, D.R. Dickerson, D.E. Orlopp, J.W. Ehrmantraut, J. Med. Chem. 7 (1964) 572–573.
[14] W.A. Gregory, D.R. Brittelli, C.L.J. Wang, H.S. Kezar III, R.K. Carlson, C.H. Park, P.F. Corless, S.J. Miller, P.R. Rajagopalan, M.A. Wuonola, R.J. McRipley, V.S. Eberly, A.M. Slee, M. Forbes, J. Med. Chem. 33 (1990) 2569–2578.
[15] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[16] H. Prucher, R. Gottschlich, A. Haase, M. Stohrer, C. Seyfried, Bioorg. Med. Chem. Lett. 2 (1992) 165–170.
[17] H. Pruecher, H. Boettcher. DE 3723797 (1989), Chem. Abstr. 111 p7390w.
[18] L. Jae-Gul, L. Wou-Bin. PCT Int. Appl. World 0194342 (2001), Chem. Abs. 136 37590v.
[19] Y. Toshihiko, T. Tatsumitsu. Japanian Kokai Tokkyo Koho 11322729 (1999), Chem. Abstr. 131 p351320r
[20] P.R. Manninen, S.J. Brickner, Org. Syn. 81 (2005) 112–117.
[21] Y. Osa, Y. Hikima, Y. Sato, K. Takino, Y. Ida, S. Hirono, H. Nagase, J. Org. Chem. 70 (2005) 5737–5740.
[22] M. Michman, S. Patai, Y. Wiesel, J. Chem. Soc. Perkin Trans. 1 (1977) 1705–1710.
[23] P.A. Argabright. US Patent 3526655(1970), Chem. Abstr. 73 p98584y.
[24] A.J. Bloodworth, A.G. Davies, J. Chem. Soc. B (1966) 125–127.
[25] H. Martin, A. Lukaszczyk dipl-ing, D. Duerr, G. Pissiotas, O. Rohr, A. Hubele, S. Janiak. DE 1802739(1969), Chem. Abstr. 71 p91056c.
[26] S. Witek, J. Bielawski, A. Bielawska, J. Prakt. Chemie 321 (1979) 804–812.
[27] A. Barry, Antibiotics in Laboratory Medicine. Willams and Wilkins, Baltimore, 1991, pp. 1–16.