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SYNTHESIS OF POTENTIAL γ-LACTAM ANTIBIOTICS

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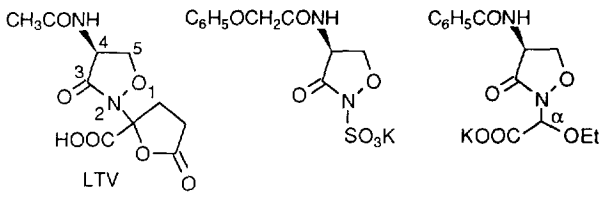
Abstract: Synthesis and biological testing of new 2-[4-(2-(2-amino-4-thiazolyl)-(Z)-2-(methoxyimino) acetylamino)-3-oxo-2-isoxazolidinyl]-2-aryloxy acetic acids analogs of lactivicin are described. The new products were obtained by addition of cycloserine derivatives on t-butyl 2-aryloxy-2-bromo acetate. Substitution on the aryloxy group modifies its leaving ability and allows different levels of activation of the γ-lactam ring.

Lactivicin (LTV, **1**) is a novel antibiotic isolated from *Empedobacter lactamgenus* YK-258 and *Lysobacter albus* YK-422, active against Gram-positive and negative bacteria.[1,2] Although possessing a γ-lactam ring in its structure, LTV presents β-lactam like biological activities : potent antibacterial activity, affinity to penicillin binding proteins and susceptibility to β-lactamases. The mode of action of LTV is believed to involve irreversible acylation of penicillin-binding proteins, which inhibits the growth of bacteria.[3,4]

Recent articles have reported the synthesis of lactivicin analogues. Takeda described the synthesis and antibacterial activities of a wide variety of 4-modified lactivicin derivatives.[5,6,7] Baldwin reported the synthesis of different compounds in which the acylating potential of the lactam ring is increased either by an electron withdrawing group on nitrogen (**2**) or by the presence of a good leaving group at the C-α position of the heterocycle nitrogen (**3**) (Scheme 1).[8,9]

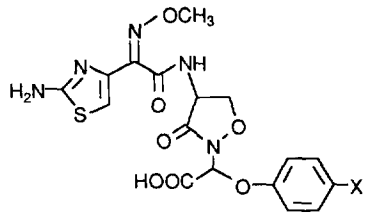
Scheme 1.



1 **2** **3**

In a further development of these observations, we have developed a synthesis of compounds **4** where the γ-lactam carbonyl group is activated by an aryloxy substituent at the C-α position. Furthermore, substitution on the aryl moiety allows modulation of the leaving ability of the aryloxide ion and consequently

the acylating ability of **4**. The 2-(2-amino-4-thiazolyl)-(Z)-2-methoxyiminoacetyl group was chosen as the C-4 side chain by analogy with results obtained on β-lactam antibiotics, i.e penicillins and cephalosporins [10], in order to increase the potential biological activity.



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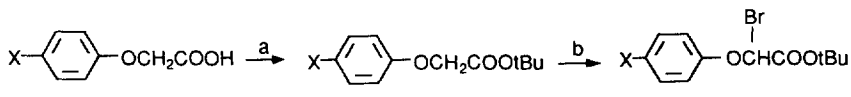
The synthetic route chosen was based on the N-alkylation of a cycloserine derivative **7** with t-butyl 2-aryloxy-2-bromo acetate **6**, prepared in two steps from the corresponding aryloxyacetic acid by esterification with tert-butyl alcohol followed by bromination with NBS (Scheme 2).[11]

Scheme 2.



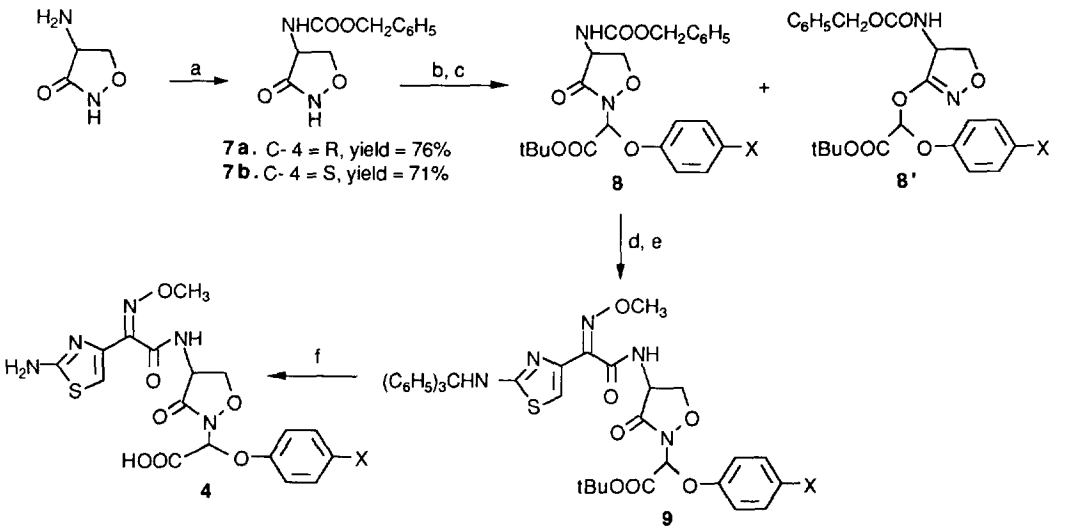
5a. X = H, yield =86%
5b. X = F, yield =83%
5c. X = Cl, yield =94%

6a. X = H, yield =92%
6b. X = F, yield =88%
6c. X = Cl, yield =70%

Reagents and conditions : a) t-BuOH, DCC, 4-pyrrolidinopyridine (0.1 eq), Et_2O, 25°C, 16h; b) NBS, UV, CCl_4, 60°C, 2h.

Reaction of (R) or (S)-cycloserine with benzylchloroformate gave N-benzyloxycarbonyl cycloserine **7**. Treatment of **7** with 1.1 eq of NaH in THF followed by addition of t-butyl 2-aryloxy-2-bromo acetate **6** gave essentially N-alkylation products **8** as a mixture of diastereomers. Competitive O-alkylation, very low in THF (less than 5% of **8'**), was more prevalent in DMF (ca 40% of **8'**). In the case of the (S)-cycloserine derivatives, separation of the two diastereomers was performed by chromatography on silica gel with ethyl acetate / hexane (25 / 75) as eluent. Hydrogenolysis of the Cbz protecting group with Pd / C gave an unstable amine, which was reacted immediately with 2-(2-tritylamino-4-thiazolyl)-(Z)-2-methoxyiminoacetic acid.[10] Removal of the acid labile protecting groups was achieved by treatment with a trifluoroacetic acid/ethanethiol mixture[12] affording new lactivicin analogues **4** in good yields.[13] Our results are summarized in Scheme 3 and Table 1.

Scheme 3.



Reagents and conditions : a) RCOCl (1.05 eq), $NaHCO_3$ (1.2 eq), $H_2O/(CH_3)_2CO$, 0°C, 4h; b) NaH (1.1 eq), THF, 0°C, 15 min; c) **6** (1.2 eq), THF, 0°C to 25°C, 3h; d) H_2, Pd (10%)/C, AcOEt/ phosphate buffer, 4h, 20°C; e) 2-(2-tritylamino-4-thiazolyl)-(Z)-2-(methoxyimino)acetic acid (1 eq), BOP (1 eq), Et_3N (1 eq), CH_2Cl_2, 20°C, 3h; f) CF_3COOH/CH_3CH_2SH (1:1) 0°C, 3h.

Table 1. Preparation of Lactivicin Analogs **4** from cycloserine (Scheme 3).

Entry	X	C-4 configuration	**8** Rf[a]	Isolated yield (%)		
				8[b]	**9**[b]	**4**[c]
a	H	R	-	52[d]	50	85
b	H	S	0.40	27[e]	72	89
c	H	S	0.32	28[e]	63	50
d	F	S	0.31	31[e]	72	47
e	F	S	0.24	23[e]	80	54
f	Cl	S	0.30	20[e]	54	54
g	Cl	S	0.25	22[e]	62	54

[a] With ethyl acetate/hexane (1:1) as eluent.

[b] Yield of purified products by silica gel chromatography.

[c] Yield after washing with Et_2O.

[d] Mixture of diastereomers after chromatography (d.r. = 50/50).

[e] Refers to pure diastereomer after chromatography. The absolute configuration was not determined.

Compounds **4b-g** were tested for in vitro antibacterial activities. MICs were determined by conventional micro-dilution method according to NCCLS recommendations for Gram-negative and positive bacteria sensitive to cephalosporins. None of the new compounds tested inhibited the growth of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 at concentration below 64 µg/ml. The low biological activity of our cycloserine analogues is similar to that observed by Baldwin with compounds **2** (MIC > 500 µg ml^{-1}) and **3** (MIC > 1 mg ml^{-1}). In our case, the lack of activity may be attributed to a partial hydrolysis of compounds **4** in aqueous solution during the biological trials.

References and notes

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13. All new compounds exhibit IR, ^1H NMR spectra and mass spectra in agreement with the structures indicated. As examples, we report here the ^1H NMR data of compounds **4** : δ_H(400 MHz, acetone D_6 / D_2O) : **4a.** Diastereomeric ratio : 50 / 50. 3.96 and 3.98 (3H, 2s); 4.22 and 4.32 [1H, 2dd, (J 7.5 and 10.0 Hz) and (J 7.5 and 10.0 Hz)]; 4.70 and 4.77 [1H, 2 dd, (J 7.5 and 7.5 Hz) and (J 7.5 and 7.5 Hz)]; 5.08 and 5.24 [1H, 2dd, (J 7.5 and 10.0 Hz) and (J 7.5 and 10.0 Hz)]; 6.38 and 6.44 (1H, 2s); 7.02-7.51 (6H, m). **4b**. 4.00 (3H, s); 4.33 (1H, dd, J 9.0 and 9.0 Hz); 4.79 (1H, dd, J 9.0 and 9.0 Hz); 5.45 (1H, m); 6.48 (1H, s); 7.00-7.48 (6H, m). **4c**. 3.97 (3H, s); 4.20 (1H, dd, J 9.0 and 9.0 Hz); 4.74 (1H, dd, J 9.0 and 9.0 Hz); 5.10 (1H, dd, J 9.0 and 9.0 Hz); 6.43 (1H, s); 7.12 (1H, s); 7.15-7.40 (5H, m). **4d**. 3.97 (3H, s); 4.36 (1H, dd, J 8.5 and 8.5 Hz); 4.80 (1H, dd, J 8.5 and 8.5 Hz); 5.25 (1H, dd, J 8.5 and 8.5 Hz); 6.45 (1H, s); 7.09 (1H, s); 7.15-7.40 (4H, m). **4e**. 3.91 (3H, s); 4.19 (1H, dd, J 8.5 and 8.5 Hz); 4.75 (1H, dd, J 8.5 and 8.5 Hz); 5.08 (1H, dd, J 8.5 and 8.5 Hz); 6.39 (1H, s); 6.91 (1H, s) ; 6.95-7.20 (4H, m). **4f**. 4.03 (3H, s); 4.30 (1H, dd, J 7.5 and 10.0 Hz); 4.87 (1H, dd, J 7.5 and 7.5 Hz); 5.32 (1H, dd, J 7.5 and 10.0 Hz); 6.39 (1H, s); 7.01 (1H, s); 7.03-7.55 (4H, m). **4g**. 4.04 (3H, s); 4.27 (1H, dd, J 7.5 and 10.0 Hz); 4.90 (1H, dd, J 7.5 and 7.5 Hz); 5.20 (1H, dd, J 7.5 and 10.0 Hz); 6.55 (1H, s); 7.13 (1H, s); 7.15-7.57 (4H, m).

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