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SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-ALKOXYSUBSTITUTED TRINEMS. PART II

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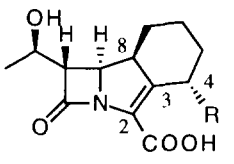
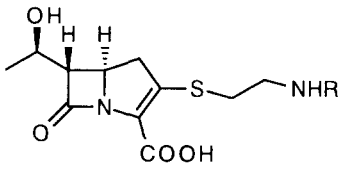
Abstract: The synthesis and the microbiological evaluation of a novel series of substituted tricyclic β-lactam derivatives (trinems, former named tribactams) is reported. Suitable basic groups were introduced within the key C-4 side chain with the aim of improving the antibacterial activity of this class of new antibiotics to *Pseudomonas* spp.. Among the different compounds prepared, basic derivatives **2** and **3** showed improved activity with respect to GV104326 **1** against these highly resistant strains.

The previous paper of this issue deals with the synthesis and the evaluation of the antibacterial activity of a novel series of non-basic 4-alkoxysubstituted trinems[1] (former named tribactams) structurally related to GV104326 **1** (Fig.1), the compound currently in phase II clinical studies. These tricyclic β-lactam derivatives, as most β-lactam antibiotics, shown reduced efficacy against the highly resistent strains *Pseudomonas aeruginosa*[2].

Figure 1





R = OCH_3 **1**
R = $O(CH_2)_nNH_2$ **2** (n= 2), **3** (n=3)
R = $OCH_2CH_2NHC(NH)R'$ **4** (R'=H), **5** (R'=CH_3)

R = H **6**
R = CH(NH) **7**

To extend the antibacterial action[3] of these compounds to *Pseudomonas* spp., in analogy with the naturally occurring thienamycin[4] **6** and its more chemically stable N-formimidoyl synthetic analogue imipenem[5] **7** (MK-0787), compounds **2, 3, 4** and **5** (Fig.1) bearing suitable basic groups within the C-4 side chain, were designed.

The synthetic route followed to obtain compounds **2** and **3** is shown in Scheme 1. In view of the comparable total yields of the synthesis of these derivatives, in this paper it will be discussed in detail only the synthesis of derivative **2**, the first compound prepared. In this event, the epoxy derivative **8**, key intermediate in the synthesis of GV104326 **1** prepared in large scale as previously reported[6], was reacted with ethylene glycol to give the dihydroxy derivative **9** as shown in Part I (previous paper of this issue). The following chemoselective protection of the primary hydroxyl group allowed to obtain intermediate **10** in 52% global yield from **8**, which was smoothly transformed into ketone **11** by Swern oxidation[7].

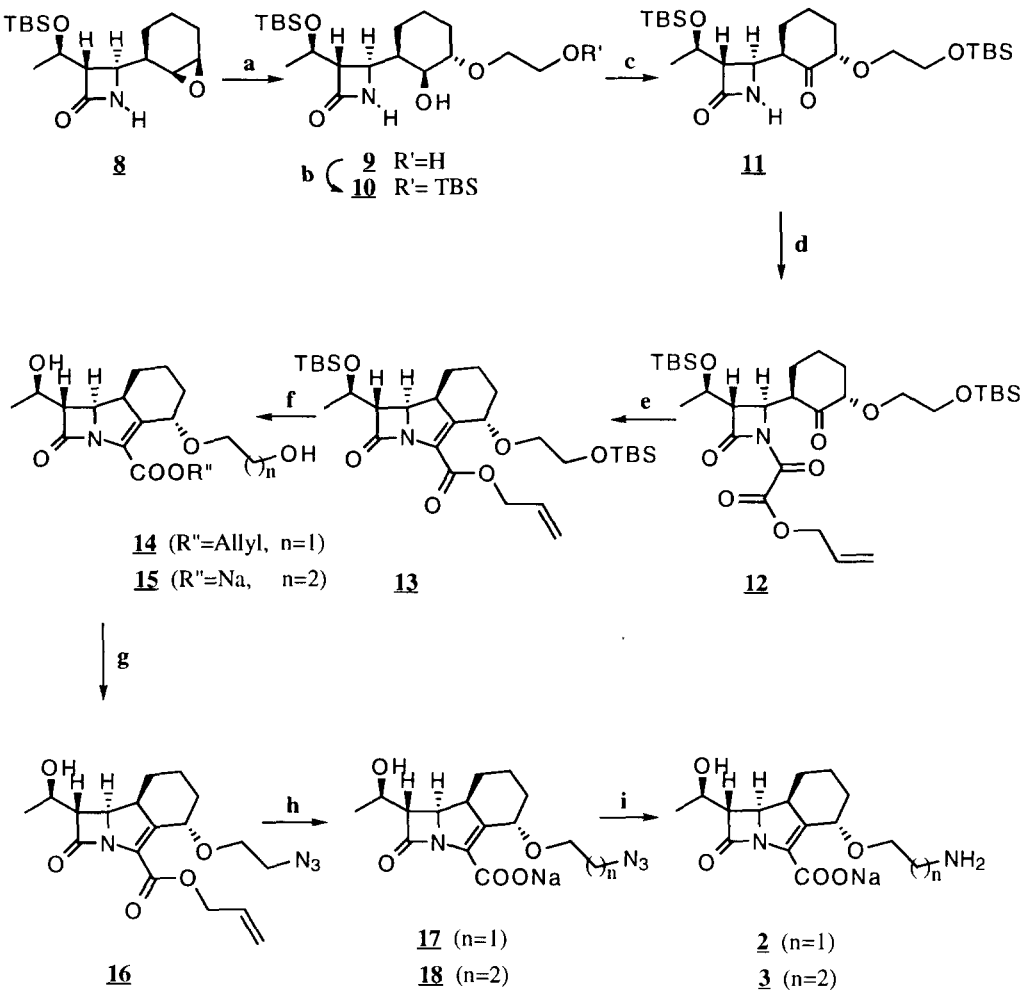
Tryciclic derivative **13** was obtained in 44% total yield from **11** by cyclization of the oxalamido intermediate **12**, quantitatively prepared treating **11** with stoichiometric amount of allyloxalylchloride and triethylamine in CH_2Cl_2 as solvent, and submitted without purification to the following thermal cyclization reaction[8] in the presence of an excess of $P(OEt)_3$. The following deprotection of the TBS (t-butyldimetylsilyl) protecting groups was carried out in 68% yields using TBAF in the presence of AcOH[9] to give the diol derivative **14**. This intermediate was transformed into the corresponding azido derivative **16** using NaN_3, CBr_4 and PPh_3 in DMF. This reaction was performed modifying the known procedure[10]: when a solution of CBr_4 (1 eq.) in dry DMF was added over 30 min into the reaction mixture, cooled at 0°C, containing the alchool derivative **7**, PPh_3 (1 eq.) and an excess of NaN_3 (5 eq.) in dry DMF the chemoselective substitution reaction occurred smoothly and compound **16** was isolated in 56% yield after purification by flash chromatography.

The following reduction of the azido group to the corresponding amine group was expected to be problematic due both to the high reactivity of the β-lactam ring towards nucleophiles and to the presence of a double bond within the five-membered ring. Therefore, this key step was carefully investigated. Considering that any attempt to reduce the azido group at this stage resulted in a complete decomposition of the starting material, it was decided to perform this reaction at buffered pH on the water soluble sodium salt derivative **17**, easily prepared by removal of the allyl ester protecting group with $Pd(PPh_3)_4$ in the presence of sodium 2-ethylhexanoate[11]. When this compound, dissolved in a 3:1 mixture of 0.05 M sodium phosphate buffer (pH=7) and THF, was hydrogenated in the presence of catalytic amount of $Pd/CaCO_3$ 5%[12] for 20 min, the target amino derivative **2** was isolated in reasonable yield after preparative HLPC purification, as a chemically stable compound.

The homologated analogue derivative **3** was successfully prepared in comparable total yield as derivative **2** following the same synthetic route using 1,3-propanediol instead of ethylene glycole in the opening reaction of the epoxide derivative **8**.

Amidine derivatives **4** and **5** (Fig.1, R = H and R = CH_3, respectively) were obtained from the available intermediate **2** as summarized in Sheme 2. In particular, to prepare formamidine derivative **4**, an aliquot of the starting compound **2** was dissolved at 5°C in 0.05 M sodium phosphate buffer (pH 7) and the pH of the solution was corrected at 8.5 with NaOH 0.1N. An excess of benzylformamidate x HCl[13], easily prepared according to the known procedure[14], was added portionwise over 10 min, mantainig the pH of the solution at 8.5 with NaOH 0.1N. The formation of the target compound and the desappearence of the starting material was monitored by HPLC. At the end the reaction the mixture was carefully neutralized, freeze dried and the solid residue was purified by preparative HLPC to give pure derivative **4** as a stable

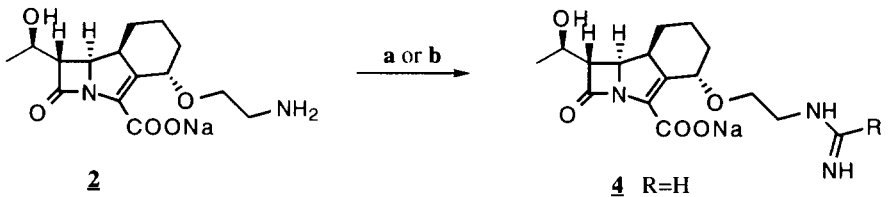
Scheme 1



a) Ethylene glycole, TsOH (c.a.), r.t., 24h; b) t-butyldimethylsilylchloride (1.2 eq), imidazole (1.2 eq), DMF, 0° C, 3h, 52% (two steps); c) DMSO (5 eq), oxalylchloride (2.5 eq), TEA (4.5 eq), CH_2Cl_2, -78° C, 30 min, addition of **10**, -20° C, 2h, 54%; d) allyloxalylchloride (1.3 eq), TEA (1.3 eq), K_2CO_3 (3.5 eq), CH_2Cl_2, 0° C, 1h; e) $P(OEt)_3$ (4 eq), o-xylene, reflux, 6h, 44% (two steps); f) TBAF (5 eq), AcOH (5 eq), THF, r.t., 48h, 68%; g) NaN_3 (5 eq), PPh_3 (1 eq), CBr_4 (1 eq), DMF, 2h, 0° C, 56%; h) $Pd(PPh_3)_4$ (c.a.), PPh_3 (0.1 eq), potassium ethylhexanoate (1 eq), r.t., 25 min, 96%; i) Pd/$CaCO_3$ 5%, THF/phosphate buffer 0.05 M (pH 7) 3:1, H_2 (1 atm), 5° C, 20 min.

compound. The acetamidine derivative **5** was similarly prepared in reasonable yield using commercially available ethylacetimidate x HCl.

Scheme 2



2

4 R=H

5 R=CH_3

a) **2**, THF/phosphate buffer 0.05 M (pH 7) 3:1, benzylformamidate x HCl (7 eq), NaOH 0.1 N, 5° C, 10 min; b) **2**, THF/phosphate buffer 0.05 M (pH 7) 3:1, ethylacetimidate x HCl (7 eq), NaOH 0.1 N, 5° C, 10 min.

To improve the yield of the process, the original preparation was slightly modified avoiding the purification of the amino derivative **2**: at the end of the hydrogenation reaction of compound **18**, the catalyst was filtered off and, after suitable dilution, it was repeated the same procedure described above. Following this semplified approach compounds **4** and **5** were obtained from the azido derivative **18** in 34% and 31% yield respectively[15].

The evaluation of the preliminary antibacterial activity of the different derivatives reported in Table 1, was carried out on a selected number of gram positive and gram negative bacteria including *Pseudomonas aeruginosa* and compared to Imipenem.

Table 1. *In vitro* antibacterial activity* (MIC mg/ml)[16] in comparison to imipenem.

Compound	S.a. 633	S.a. 853	E. faecalis 850	E. coli 1850	E. coli 1919	P.a. 1911	C. p. 615
Imipenem	0.12	0.12	2	0,5	0,5	4	0.03
2	0.5	0.5	8	0.5	0.5	8	0.12
3	0.2	0.2	4	0.5	0.5	16	0.06
4	0.25	0.5	2	1	1	32	0.12
5	1	2	16	1	0.5	32	0.12
15**	0.2	0.5	8	2	1	>32	0.03
17**	0.25	0.5	4	8	1	>32	0.03
18**	0.2	0.5	8	8	1	>32	0.06

* Minimum Inhibitory Concentrations (MIC) determined in Mueller Hinton broth: Anaerobes Schadler broth Inoculum = $5x10^5$ CFU/ml. **S. a. 663** = Staphylococcus aureus 663E ; **S. a. 853** = Staphylococcus aureus 853E β-Lactamases producing strains; **E. faecalis 850** = Enterobacter faecalis 850; **E. coli 1850** = Escherichia coli 1850E; **E. coli 1919** = Escherichia coli 1919E β-Lactamases producing strains; **P.a. 1911** = Pseudomonas aeruginosa 1911E; **C. p. 615** = Clostridium perfringens 615E.
** Compounds **15** and **18** were easily prepared from the corresponding allyl ester intermediates as reported in Scheme 1

These compounds shown good antibacterial profile against gram positive bacteria and moderate activity against gram negatives. In terms of activity against *Pseudomonas aeruginosa* the amino derivative **2** was the most active compound belonging to this series (MIC 8 *vs.* 4 for imipenem), further confirming the importance of the presence of basic groups to get activity against this high resistant strains. Finally all the compounds tested, as previous reported for this class of β-lactam derivatives[1], were found to be highly stable to human DHP-I.

Acknowledgments

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16. See note 10 of the previous paper of this issue.

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