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Antibiotics GE23077, novel inhibitors of bacterial RNA polymerase. Part 3: Chemical derivatization

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Abstract—GE23077 is a novel RNA polymerase inhibitor that is isolated from the fermentation broth of an *Actinomadura* sp. It is a cyclic heptapeptide complex made up of four factors, differing in the structure of acyl group connected to the side chain of an α,β-diaminopropanoic acid moiety and in the configuration of the stereocenter of an α-amino-malonic acid residue. Although GE23077 shows strong inhibitory activity on both Rifampicin-sensitive and -resistant polymerases, it exhibits poor antimicrobial activity. The most reasonable explanation for this property has been based on the lack of penetration of the molecule across the bacterial membrane, owing to its strong hydrophilic character. To improve penetration, several parts of the molecule were accordingly modified with the aim of altering the physico-chemical properties of GE23077. The current SAR study has identified moieties important for RNA polymerase activity.

1. Introduction

GE23077 (see Fig. 1) is a new metabolite that has been discovered in the course of a screening program for new inhibitors of RNA polymerase. Its structure has been elucidated[1] and is new. GE23077 is a cyclic heptapeptide containing four unusual amino acids: iso-serine (iso-Ser), α-amino-malonic acid (Ama), α,β-diaminopropanoic acid (Apa), and β,γ-dihydroxyglutamine (Dhg). It is a mixture of four major factors called A1, A2, B1, and B2, having molecular weights of 803 (A1, A2) and 805 Da (B1, B2), respectively. These factors differ in the nature of the side chain of the α,β-diaminopropanoic acid residue, where the amino group forms an amide with 2-methyl-2-butenoic acid (factor A) and 3-methyl butanoic acid (factor B).

Acidic character of an α-malonic proton is thought to be the origin of an isomerization process between A1, A2 and B1, B2.

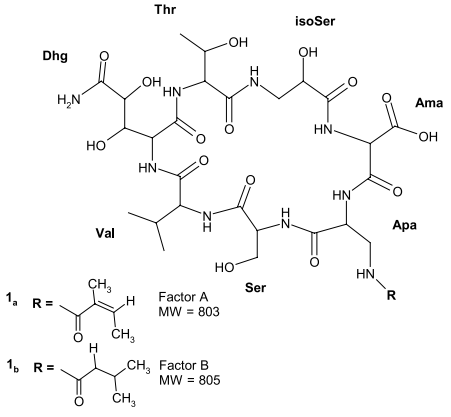

Figure 1. Structures of GE23077-factors A and B.

GE23077 is a potent inhibitor of *E. coli* Rifampicin-sensitive (rifa-s) and -resistant (rifa-r) RNA polymerase.[2] Although its inhibitory activity on the purified enzyme seems to be very high (IC_{50} on *E. coli* and *B. subtilis* RNA polymerase at 0.02 mg/l),[2] its antimicrobial activity is weak and restricted only to a few microorganisms.

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The most reasonable explanation seems to be one of poor penetration of the molecule across the bacterial membrane, owing to its strong hydrophilic character.

To improve penetration of GE23077 across membranes, the molecule has been accordingly modified with the aim of altering its physico-chemical properties. After preliminary screening, three amino acids in the core structure were found to be amenable to chemical modification: Ama, Apa, and Dhg.

2. Results and discussion

2.1. α-Amino-malonic acid derivatives (Ama)

To effect a change in the total charge of the molecule and to increase its lipophilicity, negative charge of the carboxylic group was eliminated by decarboxylation or condensation processes with different alcohols and amines. Decarboxylation of the GE23077 complex took place under basic conditions (TEA, DMF, 60 °C for 8 h, 60% yield) or heating the molecule without solvent for a few days at 90 °C, to give the corresponding glycine derivative (**2**) (see Table 1). Esters at the Ama position ($\mathbf{3_{a–c}}$) were prepared using three different strategies, depending on the alcohol to be condensed: alkyl halide and $KHCO_3$ in DMSO (45% yield), alkyl chloroformate and pyridine in DMF (60–80% yield), or a saturated solution of HCl in the desired alcohol (95% yield). Amides $\mathbf{4_{a–d}}$ (see Table 1) were prepared by condensation of 2 equiv of the selected amine with 1.5 equiv of PyBOP as a condensing agent and TEA as base (pH 8, 20–30% yield).

To prevent epimerization, the Ama Cα was derivatized, blocking the keto–enol isomerization. For this purpose, GE23077-methylester ($\mathbf{3_a}$) was dissolved in THF/LiBr[3] and treated with potassium *tert*-butoxide at 0 °C to generate an anion at the Ama Cα position. Different electrophiles were studied, but the anion reacted successfully only with *t*-butylacrylate and allylbromide to give the corresponding Ama-alkylated methylester derivatives. On increasing the temperature to about 25 °C, the Ama-ester group was eliminated, giving free carboxylic acid. Subsequent decarboxylation was carried out, as described for GE23077 itself, giving rise to alkylated-decarboxylated derivatives (**5**) (13% yield) and after deprotection was carried out with TFA at RT to a carboxylic group, derivative (**6**) (15% yield).

Two different complexes corresponding to the two Cα epimeric forms were obtained for each electrophile (their ratio depending on the nature of the reagent).

2.2. α,β-Diaminopropanoic acid derivatives (Apa)

Deacylation of GE23077 was considered to replace the lateral chain with more lipophilic ones. Ozonolysis of the complex (**1**) was carried out and, as expected, factor A ($\mathbf{1_a}$) was converted into an α-ketone product (**7**) (see Chart 1), while factor B ($\mathbf{1_b}$) remained unchanged. The α-ketone product (**7**) was subjected to reductive amination with benzylamine hydrochloride and sodium cyanoborohydride to give the desired benzylamine derivative ($\mathbf{8_a}$). Ama-decarboxylated benzylamine derivative ($\mathbf{8_b}$) and alcoholic derivative on the side chain (**12**) (see Table 1) generated by direct reduction of **7** by sodium cyanoborohydride were obtained as by-products of this reaction. Hydrogenolysis of $\mathbf{8_a}$ in water with Pd 10% on carbon as catalyst gave the alanine derivative (**9**) in good yield. Using PtO_2 as a catalyst in water/methanol (1:3), two products were obtained in a 1:1 ratio. The first one was the expected derivative **9**, the other its hexahydro derivative ($\mathbf{8_c}$) that was produced by an unexpected reduction of its aromatic ring to a cyclohexane, instead of hydrogenolysis of its benzylamine moiety. To our knowledge, reduction of the aromatic rings by heterogeneous catalytic hydrogenation occurs only under harsh conditions (high temperatures and pressures, for example, Rh, Pt under 5–10 atm pressure, 50–100 °C or Ni, Pd under 100–200 atm pressure, 100–200 °C), except for some natural product substrates.[4]

The alanine derivative (**9**) reacted with the pentafluorophenol-activated esters of organic lipophilic acids, such as R^5CO_2H (see Table 1, with R = COCHMeNHCOR[5]) at 25 °C in the presence of 2 equiv of TEA (pH 8–10) to yield different lipophilic amides at the Apa position (entries $\mathbf{10_{a–f}}$, Table 1). It took nearly 2–5 h for the majority of the acids to be used up in the reactions and about two days for the bulky ones to be consumed.

To verify if lipophilic-chain derivatives on the Apa moiety were more active if they retained the amine functionality as derivatives $\mathbf{8_a}$ and **9**, we made the α-ketone product (**7**) react with different lipophilic amines, such as R^4NH_2 (see Table 1, with R = COCHMeNHR[4]), through a reductive amination step to obtain products $\mathbf{11_{a–d}}$.

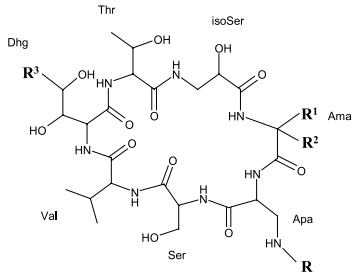
2.3. β,γ-Dihydroxyglutamine derivatives (Dhg)

To transform the primary amide of Dhg into a cyano group, GE23077 was made to react with cyanuric chloride[5] in aqueous (0.2%) DMF albeit unsuccessfully since extreme degradation of the substrate took place. The same reaction performed on GE23077 methylester ($\mathbf{3_a}$) gave a large number of different adducts, but when HCl was added and the reaction was left to stir overnight, the desired Dhg-cyano derivative (**13**) was obtained as the main product (see Chart 2).

Reduction of the cyano group was carried out with the use of $CoCl_2$ and sodium borohydride at 0 °C in methanol,[6] giving the desired primary amine derivative (**14**). This last compound reacted with pentafluorophenol-activated esters of organic lipophilic acids at 25 °C in the presence of TEA (pH 8–9) to yield different lipophilic amides ($\mathbf{15_{a–d}}$). The yields reported earlier were calculated based on preparative-HPLC-purified products.

To investigate the capability of GE23077 derivatives to inhibit bacterial RNA polymerase, a transcription cell-free assay using the purified *E. coli* enzyme and a specific DNA template was set up.[7] The results (see Table 1) show that Apa moiety seems to be not so critical for

Table 1. RNA polymerase inhibition (IC_{50})[a] and minimal inhibitory concentration (MIC) against *Moraxella catarrhalis* of GE23077 derivatives[b]



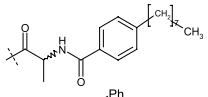
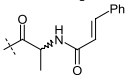
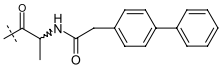
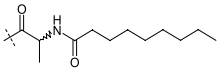
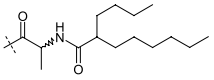
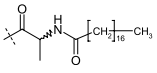
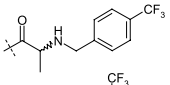
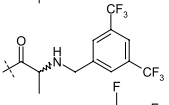
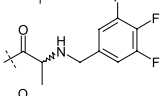
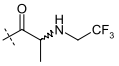
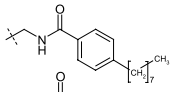
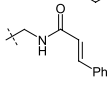
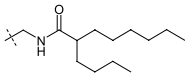
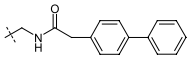
Derivative	R	R^1	R^2	R^3	IC_{50} (mg/l)	MIC (mg/l)
1	Natural chain	CO_2H	H	$CONH_2$	0.03	8
2	Natural chain	H	H	$CONH_2$	0.65	16
3_a	Natural chain	CO_2Me	H	$CONH_2$	0.15	8
3_b	Natural chain	CO_2Et	H	$CONH_2$	0.10	64
3_c	Natural chain	CO_2Allyl	H	$CONH_2$	0.40	nt
4_a	Natural chain	$CONHCH_2Ph$	H	$CONH_2$	0.30	128
4_b	Natural chain	CONMeOMe	H	$CONH_2$	0.40	nt
4_c	Natural chain	COTyr	H	$CONH_2$	nt	512
4_d	Natural chain	CO-L-Ala-L-Ala-OMe	H	$CONH_2$	>3.2	64
5	Natural chain	H	Allyl	$CONH_2$	2.24	32
6	Natural chain	H	$CH_2CH_2CO_2H$	$CONH_2$	0.20	8
7	COCOMe	CO_2H	H	$CONH_2$	0.07	8
8_a	$COCHMeNHCH_2Ph$	CO_2H	H	$CONH_2$	0.07	32
8_b	$COCHMeNHCH_2Ph$	H	H	$CONH_2$	1.05	128
8_c	$COCHMeNHCH_2$(cy-Hex)	CO_2H	H	$CONH_2$	0.42	16
9	$COCHMeNH_2$	CO_2H	H	$CONH_2$	0.28	64
10_a		CO_2H	H	$CONH_2$	0.07	>512
10_b		CO_2H	H	$CONH_2$	0.08	128
10_c		CO_2H	H	$CONH_2$	0.22	>512
10_d		CO_2H	H	$CONH_2$	0.09	256
10_e		CO_2H	H	$CONH_2$	0.13	512
10_f		CO_2H	H	$CONH_2$	0.57	>512
11_a		CO_2H	H	$CONH_2$	0.05	256
11_b		CO_2H	H	$CONH_2$	0.24	512
11_c		CO_2H	H	$CONH_2$	0.06	64
11_d		CO_2H	H	$CONH_2$	0.17	>512
12	COCHOHMe	CO_2H	H	$CONH_2$	0.11	64
13	Natural chain	CO_2Me	H	CN	1.37	128
14	Natural chain	CO_2Me	H	CH_2NH_2	3.14	32
15_a	Natural chain	CO_2Me	H		>3.2	>512
15_b	Natural chain	CO_2Me	H		>3.2	>512

Table 1 (*continued*)

Derivative	R	R^1	R^2	R^3	IC_{50} (mg/l)	MIC (mg/l)
15_c	Natural chain	CO_2Me	H		>3.2	>512
15_d	Natural chain	CO_2Me	H		>3.2	>512

nt, not tested.

[a] Each IC_{50} value reported in the table is obtained testing 10 different dilutions of compound ranging from 0.0031 to 3.2 mg/l.

[b] All compounds reported were characterized by ESI-MS (electrospray ionization) and NMR analysis.

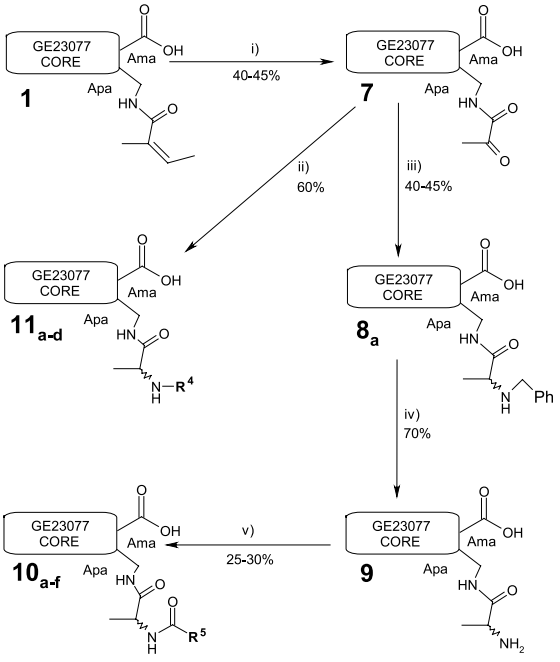


Chart 1. Reagents and conditions: (i) (1) O_3, DMF/MeOH (1:3), −78 °C; (2) Ph_3P, rt; (ii) R^4–NH_2·HCl, $NaBH_3CN$, DMF, rt; (iii) benzylamine·HCl, $NaBH_3CN$, DMF, rt; (iv) H_2, Pd/C 10%, rt, 1 atm, H_2O (on $\mathbf{8_a}$ as substrate); (v) 2 equiv TEA, 1.5 equiv $R^5COOPfp$, DMF, rt.

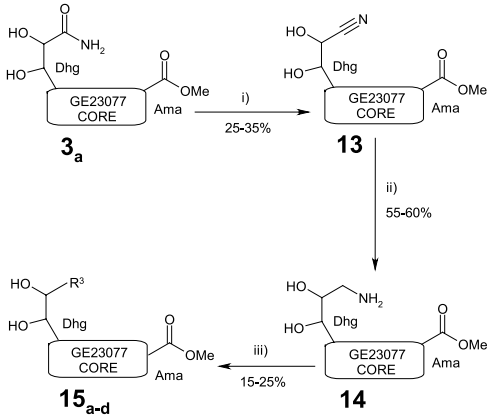


Chart 2. Reagents and conditions: (i) (1) 3 equiv $(ClCN)_3$, 0 °C, then rt, DMF (0.2% H_2O); (2) HCl, 0 °C then rt; (ii) (1) 34 equiv $NaBH_4$, 7 equiv $CoCl_2·6H_2O$, MeOH, 0 °C; (2) HCl 4%; (iii) 1.5 equiv $RCOOC_6F_5$, TEA pH 8–9, DMF, 1 h at rt.

binding, as derivatives at this position retain activities with IC_{50}'s in the same order of magnitude as GE23077 (entries **7**–**12**). On the contrary, the Ama and Dhg moieties seem to be relevant for binding to the enzyme. Indeed, the IC_{50} values of derivatives on the Ama position increase by one order of magnitude (entries **2**–**6**) and even more with a bulky substituent (entry $\mathbf{4_d}$). The interaction between the derivatives and the enzyme seems to be seriously balanced when the amide group on Dhg is modified (entries **13**–$\mathbf{15_d}$). In particular, binding seems to be lost when bulky, reversed amides are introduced at this position (entries $\mathbf{15_{a–d}}$). The fact that the Apa moiety can tolerate bulky substituents suggests that this residue either lies in a large cleft in the enzyme or points toward the solvent. The total loss of activity by Dhg derivatives, even for the non-bulky nitrile **13**, suggests a critical role for this amide moiety in enzyme binding.

Although many new derivatives are active against *Moraxella catharralis*, they did not show any significant antibacterial activity against other bacteria (data not shown). Against *M. catharralis*, compounds **2**, **3**, **6**, **7**, and $\mathbf{8_c}$ were equipotent to the parent compound.[8]

3. Conclusion

Although some derivatives possessed a significantly higher lipophilicity than GE23077, this improvement was not sufficient to confer increased antibacterial activity. Further efforts will thus be focused on the synthesis of new derivatives by modification of the hydroxylic moieties, present on the threonine, isoserine, and serine component amino acids of GE23077. Furthermore, we have planned to commence studies on the co-crystallization of GE23077 with its target enzyme, as these studies might provide a starting point for further rational design of new derivatives and analogs of GE23077.

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Monti, F.; Corti, E.; Miele, A.; Selva, E. *Eur. J. Biochem.* **2004**, *271*, 3146.
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7. The inhibition of RNA polymerase was measured in a cell-free transcription assay, as described by Ciciliato et al.,[2] with some modifications. Briefly, the reaction mixture (50 μl final volume) contained 20 mM Tris–acetate (pH 7.9), 50 mM KCl, 4 mM Mgacetate, 0.1 mM EDTA, 5 mM DTT, 10 μg/ml bovine serum albumin, 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 2 μM UTP, 0.01 pmoles pUC18 plasmid (Amersham Biosciences) as DNA template, 0.5 μCi [α-^{33}P]UTP, 0.2U *E.coli* RNA polymerase (Sigma), and 5 μl GE23077 or its derivatives. The activity of each sample is reported as its IC_{50}, that is a concentration giving 50% inhibition.
8. The Minimal inhibitory concentrations (MIC) of GE23077 and its derivatives were determined by the broth microdilution method according to standard methods (*NCCLS document* M7-A6 *Vol.23 No.2*, 2003). The strain of *Moraxella catharralis* tested belonged to the Vicuron Italia Srl collection.