Hypothesis

STRUCTURAL BASIS FOR INHIBITION OF PROTEIN SYNTHESIS BY THE AMINOACYL-AMINOHEXOSYL-CYTOSINE GROUP OF ANTIBIOTICS*

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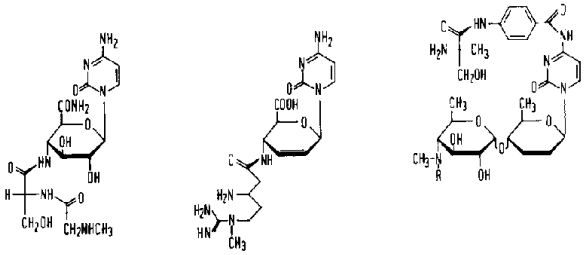
1. Introduction

Among the numerous antibiotics known to act on the larger ribosomal subunit [2, 3] a group consisting of gougerotin (**1**), blasticidin S (**2**), amicetin (**3**) and bamicetin (**4**), can clearly be delineated on the basis of their mode of action and their structural subunits, suggesting [4] the term **aminoacyl-4-aminohexosyl-cytosine** group of antibiotic inhibitors. The effects of the first three (**1**–**3**) on protein synthesis have been studied in great detail using various model systems with washed bacterial ribosomes [2, 3], native polyribosomes from *E. coli* [5] as well as with yeast [6, 7] and mammalian [7–9] ribosomal systems, whilst the inhibitory behaviour of bamicetin (**4**) in several bacterial model reactions has been evaluated only recently [4]. With respect to the transpeptidation step the inhibitory patterns of this group of antibiotics (**1**–**4**) are similar enough as to suggest competition for similar or even identical sites within the ribosomal peptidyl transferase, conceivably those associated with the aminoacyl as well as the cytosine recognition regions for the CCA-amino acid terminus of native tRNAs. Hence, it is clearly worthwhile looking for conformities between the molecular architecture of these aminoacyl aminohexosyl cytosine antibiotics and their mode of action.



First assessments of the structural features of pyrimidine and purine nucleoside antibiotics were undertaken by Fox et al. [10], who proposed – as requirements for affecting protein synthesis – an 'accessible' amino acid moiety attached to a 'carrier' nucleoside together with two basic centers at opposite sites of the molecule, one being the amino group of the amino acid, the other a nitrogen of the nucleobase (i.e. cytosine amino group) or, in the case of amicetin, the dimethylamino moiety in the disaccharide portion. These characterizations, however, are not without contradiction, since the somewhat exotic aminoacyl components of antibiotics **1**–**4**, to which the growing peptide chain cannot be transferred, are incommensurable with antibiotics of the puromycin type. Furthermore, the pK_a values of the respective groups in compounds **1**–**4** differ within too wide a range as to effect – in the same functional way – the activity of ribosomal peptidyl transferase which in fact shows a pH dependance suggesting the involvement of a functional group with a pK_a value in the range of 7.5–8 [11].

In connection with related studies aimed at tracing

* See [1].

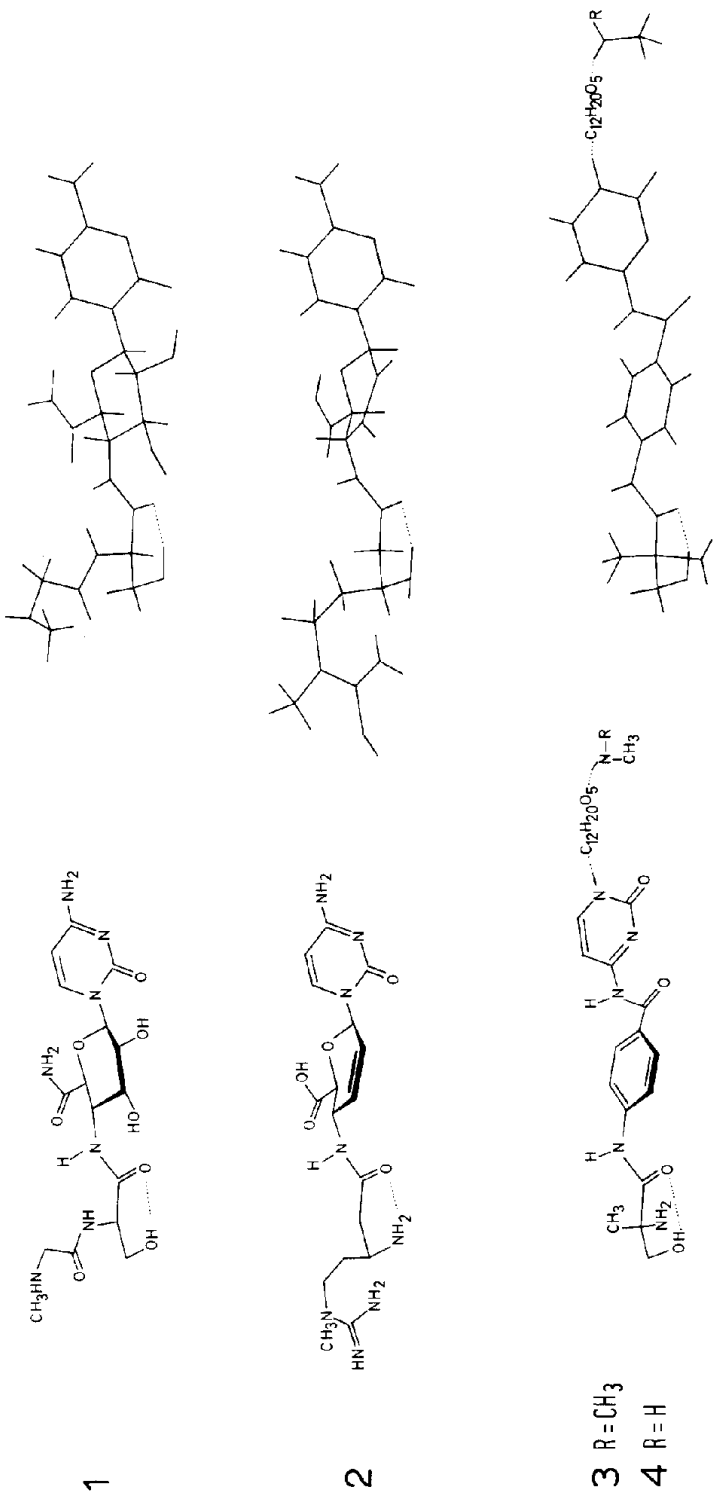


Fig. 1. Stereomodels of gougerotin (1) blasticidin S (2) amicetin (3) and bamicetin (4) (right) with atoms designated in the illustrations left. In the conformations shown, the cytosine portions of **1** and **2** are superimposable over the inverse cytosine portion of **3** and **4** (structural element **I**), as are the connecting links between nucleobase and peptide part (structural feature **II**). Similarly, the close resemblance in the peptide portions (structural characteristic **III**) clearly follow from the models.–The line drawings on the right were made from photographs of Dreiding stereomodels.

down structure–activity relationships of aminoacyl aminosugar nucleosides, we embarked on correlating inhibitory activity of the antibiotics **1**–**4**, as well as their presently available analogs with their structural and stereochemical characteristics. The results we render significant enough as to be presented.

2. Major structural features of antibiotics 1–4

With respect to the molecular architecture of the aminoacyl aminohexosyl cytosine antibiotics gougerotin, blasticidin S, amicetin and bamicetin (**1**–**4**), four major characteristic structural features, common to each, may be elicited from space-filling models or – more instructively – from Dreiding stereomodels (fig. 1):

I. The spatial arrangement of one oxygen and three nitrogen atoms is the same in the respective nucleobase portions. In gougerotin and blasticidin S, these are represented by N-1, N-3, N-4 and C-2-carbonyl oxygen atoms of the cytosine moiety (fig. 2A). In the case of amicetin and bamicetin, respectively, the same nitrogen atoms are involved, yet the order is reversed, whilst the oxygen atom is supplied by the amide oxygen of the *p*-aminobenzoyl moiety (fig. 2B). This congruity may best be demonstrated by superposing A on B as displayed in C.

II. A second characteristic grouping is being found in the same distance from the first structural feature and within an identical spatial arrangement; an amide bond representing the connecting link between the respective aminoacyl residues and the 4-aminohexuronic acid or 4-aminobenzoic acid moieties. The carbonyl portion of this 'peptide link' is derived from D-serine (gougerotin), ε-*N*-methyl-β-arginine (blasticidin S) and α-methyl-L-serine (amicetin and bamicetin); the amino part of this amide bond is represented by the 4-amino group found in each hexuronic acid (for **1** and **2**) and in *p*-aminobenzoic acid (for **3** and **4**).

III. The carbonyl portion of this 'peptide link' is part of a further structural and steric conformity within antibiotics **1**–**4**. All four compounds are capable of developing an identical steric arrangement of a

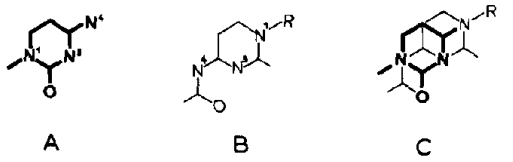


Fig. 2. Commensurability of the *N,O,N,N*-arrangements within the nucleoside portions of gougerotin/blasticidin S(A) with those of amicetin and bamicetin (B); a superposition of A on B is represented by C.

hydroxyl or an amino group in relation to the rest of the molecule, as illustrated in the stereomodels (fig. 2) by an admittedly arbitrary hydrogen bonding of –OH···OC-type in the serine portions of **1**, **3** and **4** and of the type $-NH_2\cdots O{=}C$ in the arginine moiety of **2**.

IV. Besides these sterically fixed structural characteristics a spatially less localized functional feature can be deduced from the structures of the four antibiotics: a 'terminal' *N*-methylamino group. In gougerotin and blasticidin S this grouping is contained in the sarcosyl- and *N*-methyl guanidine parts, respectively, whilst amicetin and bamicetin exhibit methylamino functions in their disaccharide portions.

Some conformational and functional group aspects contributing to the total activity may have been omitted, e.g., the carboxamide or carboxylic acid function in gougerotin and blasticidin S, that is unparalleled in amicetin and bamicetin, as well as others. Nevertheless, the four characteristic features outlined above appear to be the minimal structural requirements for inhibition of protein biosynthesis within this group of antibiotics–a conclusion, that is sustained by the inhibitory behaviour of the presently available analogs.

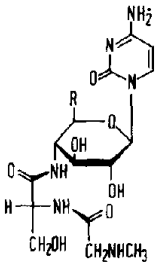
3. Evaluation of analogs

The evaluation of the analogs **5**–**28** with respect to their effects on protein synthesis, shows that none of these structural features may be altered or removed without substantial or complete loss of inhibitory activity.

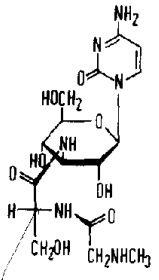
Replacement of the cytosine moiety in blasticidin S (**2**) by uracil ('desaminoblasticidin' **16**)–a modification within the first structural element–results in a diminution of inhibitory activity to one thousandth

or less [12]. As compared to this, alterations in the connecting link between the structural elements **I** and **II** appear to be less incisive. Replacement of the 5'-carboxamido function by a carbomethoxy or a *N*, *N*-dimethyl carbamido group (compounds **7** and **6**, resp.) results in reduced inhibitory activity (about 10%

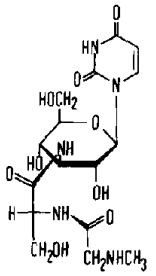
ANALOGS OF GOUGEROTIN (1):



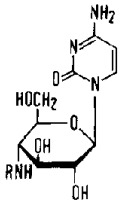
1 R = $CONH_2$
5 R = $CONHCH_3$
6 R = $CON(CH_3)_2$
7 R = $COOCH_3$
8 R = CH_2OH



9

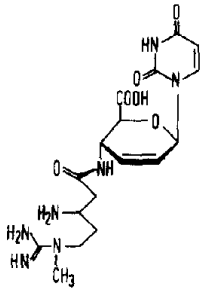


10

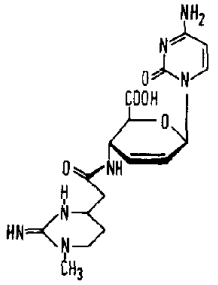


11 R = D-Ser
12 R = D-Ala
13 R = D-Phe
14 R = Sar-D-Phe
15 R = Sar-D-Ala

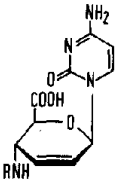
ANALOGS OF BLASTICIDIN S (2):



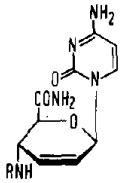
16
Deamino-blasticidin S



17
Cytomycin

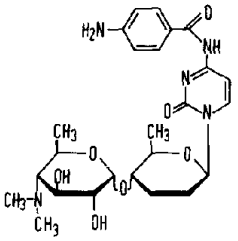


18 R = H
19 R = L-Ala
20 R = L-Phe

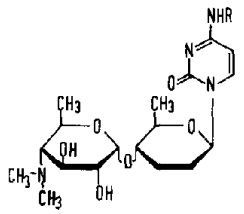


21 R = L-Ala
22 R = L-Phe

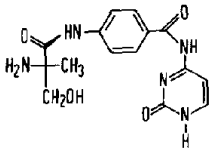
ANALOGS OF AMICETIN (3):



23
Plicacetin



24 R = benzoyl
25 R = m-nitrobenzoyl
26 R = m-dinitrobenzoyl
27 R = acetyl



28
Cytimidine

of that of gougerotin [13]). Similarly, the corresponding *N*- methyl carbamido (**5**[13]) and hydroxymethyl analogs (**8**[14,15]) exhibited a decrease in biological activity and are only about one third as active [13, 16].

A very pronounced effect on the inhibitory behaviour seems to be exerted by changes in distance between structural features **I** and **II** or their direction. As exemplified by fig. 3, gougerotin and its active 5′-hydroxymethyl analog **8** have identical molecular geometry with respect to features **I** and **II**, whereas in the entirely inactive analogue **9** [16,17], deviating from **8** only in the linkage of the peptide unit to the pyranoid amino function, the geometrical arrangements between cytosine nucleobase and the peptide link are distinctly different. Hence, it is not surprising that the gougerotin analog **10**[17] lacking structural as well as steric essentials, is devoid of any activity with respect to interference with protein synthesis at the peptide elongation stage [16]. For the same reasons, analogs of **10** with glycine, sarcosine, L-alanine, D-phenylalanine, L-lysine, and L-glutamine γ-methyl ester as aminoacyl components–which have been prepared [18] but apparently not evaluated biologically–are likely to be inactive.

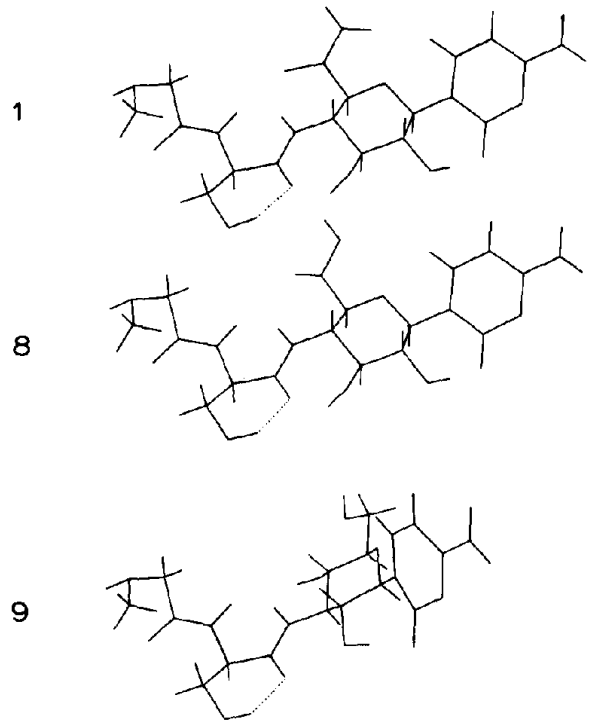


Fig. 3. Dreiding stereomodels of gougerotin analogs 8 and 9 as compared to gougerotin (**1**), clearly showing the congruity of 1 and 8 in gross stereochemical features in contrast to the disdistinctly different molecular geometry of 9.

Analogs of these antibiotics that lack the aminoacyl part altogether – and hence are missing structural elements **II** and **III** – show very little, if any, capabilities for interfering with the transpeptidation step. Thus, cytosinine (**18**), the nucleoside portion of blasticin S, is devoid of any activity [12], whilst plicacetin (**23**), missing the methylserine part of amicetin and thus being either its biological precursor or degradation product, is nearly inactive in this respect [4] – despite its reported antibacterial activity [19]. Correspondingly, a number of plicacetin analogues (**24**–**27**) that exhibit an antibacterial spectrum similar to amicetin [19], are likely to be inactive with respect to interference with peptide chain elongation. In this context, it would be interesting to learn about the bioactivity of cytimidine (**28**)[20], which exhibits structural features **II**–**III**, and, consequently, would be a closer functional analogue to amicetin than plicacetin.

Changes in the structural elements **III** are likewise accompanied with either some or complete loss of biological activity. Cytomycin (**17**), for example, is an extremely close analogue of blasticidin S, and is nearly inactive [12]. Thus, expectedly, compounds **19**–**22**, which have much less resemblance to blasticidin S in their aminoacyl parts than cytomycin, exhibit either no or only minimal inhibitory activity [21]. Variations of the sarcosyl-D-seryl unit in gougerotin similarly seems to lead to a decrease of biological activity as evidenced by the compounds **11**–**15**[15], all being less active [13] than the hydroxymethyl analog **8**.

Additional syntheses and testing of analogous compounds will certainly be required to render these structural rationalizations less tentative and to fully define the interactions of these inhibitors with their biological receptor, which in turn may help to elucidate the molecular architecture of the active centre of the peptidyl transferase. For the moment, these structure–activity rationalizations should serve to more effectively concentrate synthetic strategies in this field towards compounds of biological significance.

Acknowledgements

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