THE EFFECT OF OXAMICETIN AND SOME AMICETIN ANALOGS ON RIBOSOMAL PEPTIDYL TRANSFERASE*

F. W. LICHTENTHALER, J. ČERNÁ and I. RYCHLIK

Institut für Organische Chemie, Technische Hochschule Darmstadt, 61 Darmstadt, West-Germany, and Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6, Czechoslovakia

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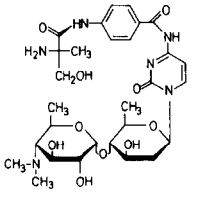
1. Introduction

Oxamicetin (**1**), a new antibiotic isolated recently from the fermentation broth of *Arthrobacter oxamicetus* Tomita and Kawaguchi [2,3], has been shown to be a close structural analog to amicetin [4]. It also resembles the latter in its antibacterial spectrum, being somewhat more active against gram-negative bacteria, but less active than amicetin against gram-positive and acid-fast bacteria [3]. These structural as well as biological similarities strongly suggested that oxamicetin–like amicetin (**2**), bamicetin (**3**), gougerotin, and blasticidin S – is to be allotted to the aminoacyl-4-aminohexosyl-cytosine group of antibiotics [5,6], that are all acting upon the acceptor site of both prokaryotic and eukaryotic ribosomes [7]. This paper provides confirmatory evidence for this suggestion by evaluation of oxamicetin in the fragment reaction and fragment binding assays, and, together with the behaviour of some amicetin analogs (**4**–**6**) in these systems, allows further assessment of steric and functional group characteristics as required for inhibitory activity.

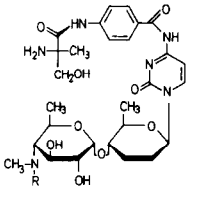
2. Materials and methods

Ribosomes were prepared from *Escherichia coli* B, as described elsewhere [8]. The transfer of the Ac-Leu-residue from the CACCA–Leu-Ac fragment to puromycin was measured according to Monro et al. [9]. For assaying the CACCA–Leu-Ac binding to the donor site, the procedure of Celma et al. [10] was used, whilst the CACCA–Phe binding to the acceptor site was determined according to the assay of Pestka [11]. Oxamicetin (**1**), in form of its monohydrochloride, and amicetin (**2**), were generous gifts from Dr H. Kawaguchi, Bristol-Banyu Research Institute, Tokyo; samples of plicacetin (**4**), cytimidine (**5**), and cytosamine triacetate (**6**) were kindly provided by Dr T. H. Haskell, Parke-Davis, Ann Arbor, Michigan [12].

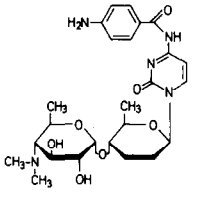
* Nucleosides, XXIV. For part XXIII see [1].



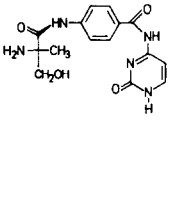
1 OXAMICETIN



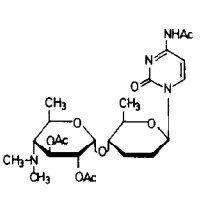
2 AMICETIN (R=CH_3)
3 BAMICETIN (R=H)



4 PLICACETIN



5 CYTIMIDINE



6 CYTOSAMINE TRIACETATE

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3. Results and discussion

3.1. *Effect of 1–6 on the puromycin reaction with CACCA–Leu–Ac as donor substrate*

The effects of the two nucleoside antibiotics (**1**,**2**) and their structural analogs **3**–**6** on the 70S-ribosome catalyzed AcLeu-transfer from CACCA–LeuAc to puromycin are illustrated in fig.1. In this assay, oxamicetin (**1**) proved to be a somewhat more potent inhibitor of the overall peptidyl transferase activity than amicetin (**2**), but the inhibition course is practically identical for both antibiotics. This behaviour is similarly exhibited by in vivo screenings with intact *E. coli* bacteria and a number of other test organisms [4], thus clearly indicating that the gross antibacterial activity of oxamicetin – like amicetin (**2**) and bamicetin (**3**) – is due to interaction with the peptidyl-transferase centre of the larger ribosomal subunit.

The analogs (**4**–**6**), however, show considerably decreased inhibitory activity. When comparing values at 50% inhibition (fig.1), plicacetin (**4**), lacking the α-methylseryl portion of amicetin and, due to its elaboration by the amicetin producing organism [13] being either a degradation product of (**2**), or the biological precursor thereof, is 10 times less active than amicetin. This clearly stresses the importance of the aminoacyl part, i.e. the presence of 'structural feature II' [6] for full activity. On the other hand, loss of the disaccharide portion in the amicetin molecule has an even more profound effect on the AcLeu transfer to puromycin, as evidenced by the activity of cytimidine (**5**), that is reduced by a factor of 50. A still poorer inhibitor of peptidyl transferase is cytosamine triacetate (**6**), the activity – as compared to amicetin – being reduced by two orders of magnitude.

3.2. *Effect of oxamicetin, amicetin and analogs 3–6 on substrate binding to the donor and acceptor site*

With respect to substrate-interaction at the acceptor site, oxamicetin markedly decreased the binding of the acceptor substrate (CACCA–Phe) to the acceptor site (table 1). Although this effect is slightly weaker than in the case of amicetin (cf. table 1) and bamicetin [5], it nonetheless suggests a practically identical mode of three antibiotics. The analogs (**4**–**6**), however, lacking either the aminoacid part (**4**), the disaccharide unit (**5**) or the aminoacyl–aminobenzoic acid portion of the amicetin molecule, are nearly devoid of inhibitory activity in acceptor substrate binding, the difference towards amicetin approaching two orders of magnitude (cf. table 1).

Whilst thus inhibition of the fragment reaction of compounds **1**–**6** is paralleled by the inhibitory result from acceptor substrate binding, there are some significant differences in their interference with the binding of the donor substrate. When evaluated for interaction with the ethanol-dependent binding of CACCA–Leu-Ac to 70 S ribosomes, amicetin like bamicetin [5] and

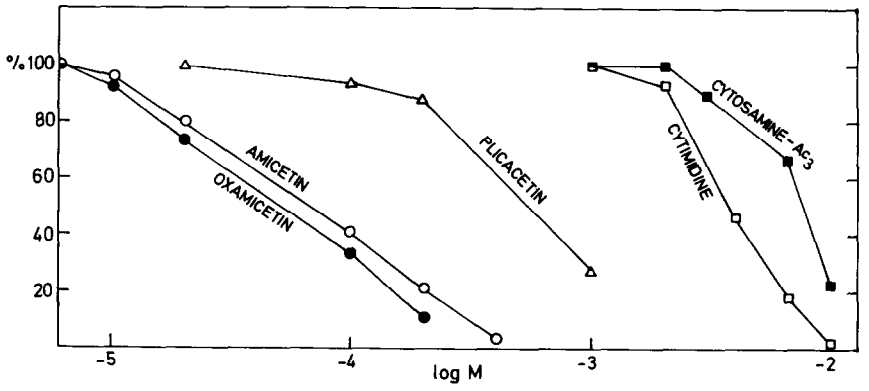

Fig.1. The effect of oxamicetin (**1**, ○–○–○), amicetin (**2**, ●–●–●), plicacetin (**4**, △–△–△), cytimidine (**5**, □–□–□) and cytosamine triacetate (**6**, ■–■–■) on the fragment reaction of AcLeu–pentanucleotide with puromycin. Reaction mixtures contained ribosomes (110 mg protein) and Ac[^{14}C]Leu pentanucleotide (1100 cpm). Log M, concentration of tested compounds (calculated on the basis of final volume after addition of methanol). %, AcLeu–puromycin formation as % control without inhibitors (about 800 cpm transferred).

Table 1
The effect of oxamicetin, amicetin, cytimidine and of cytosamine triacetate on the CACCA–[^{3}H]Phe and CACCA–[^{14}C]Leu-Ac binding to 70 S ribosomes

	Conc. (mM)	CACCA–[^{3}H]Phe cpm	%	CACCA–[^{14}C]Leu cpm	%
Control	–	983	100	656	100
Oxamicetin (1)	0.1	746	76	802	122
	1	532	54	964	147
	3	432	44	1122	171
Amicetin (2)	0.1	691	70	669	102
	1	314	32	754	115
	3	137	15	750	115
Cytimidine (5)	1	1017	103	793	121
	3	826	84	1214	185
	10	516	52		
Cytosamine	1	872	89	556	85
triacetate (6)	3	775	79	532	81
	10	694	70	394	60

Assay of CACCA-Phe binding was determined according to Pestka [11]. Reaction mixtures containing CACCA–[^{3}H]Phe (4700 cpm) 70 S ribosomes (8 A_{260} units) and 20% ethanol were incubated at 24°C for 20 min. Assay of CACCA–[^{14}C]Leu-Ac binding was determined according to Celma et al. [10]. The incubation mixtures containing CACCA–[^{14}C]Leu-Ac (2800 cpm) and 70 S ribosomes (11 A_{260} units) were incubated at 0°C for 60 min.

plicacetin [5] exhibit only a very poor increase in binding to the donor site (14–17% over control at 1–3 mM)*. Oxamicetin, however, and, surprisingly, cytimidine as well, had a very pronounced stimulatory effect on the donor substrate binding (71 and 85% over control at 3 mM), in its size comparable to that observed [5] for gougerotin.

When trying to rationalize this behaviour in terms of structure-activity relationships, one is tempted to attribute the high stimulatory effect of cytimidine (**5**) to the lack of the bulky disaccharide residue as in amicetin, i.e. the presence of a molecule that with respect to its major structural features [6] is more resembling the strongly stimulating blasticidin S and gougerotin than amicetin. Whilst this indeed may be the case, it is on the other hand somewhat startling, that introduction of an additional hydroxyl group into the disaccharide portion of amicetin (i.e. oxamicetin) causes practically the same increase of donor substrate binding as removal of the disaccharide portion altogether. Clearly more analogs need to be evaluated to unravel these peculiarities.

Cytosamine triacetate (**6**) similarly displays some exceptional behaviour. Unlike all other antibiotics and analogs of this group tested so far, it decreases the binding of donor substrate to the donor site of peptidyl transferase, e.g. at 10 mM to an extent of 40%. These characteristics of (**6**), i.e. inhibition of substrate binding to the acceptor as well as to the donor site, are reminiscent of the action of macrolide antibiotics spiramycin and carbomycin or of lincomycin [7,10,14], and might point towards an analogy of the respective disaccharide units.

4. Conclusions

Oxamicetin (**1**), differing from amicetin (**2**) by an additional hydroxyl group at C-3′ of the disaccharide

* However, when evaluating amicetin in the ethanol-dependent binding of CACCA–Leu-Ac to isolated 50 S subunits, the stimulation is quite substantial (93% over control at 1 mM) [10]. This discrepancy may be due to the different bacterial strains used (*E. coli* MRE 600 [10] versa *E. coli* B in this work), yet a more likely explanation is an apparent differentiation between 50 S and 70 S particles by amicetin and according to 70 S data [5] by bamicetin as well, an effect that is only parially exhibited by gougerotin [5,10].

unit, and, in fact, being a conceivable biosynthetic precursor thereof, is somewhat superior to amicetin in its inhibitory effect on the fragment reaction and in its stimulation of donor substrate binding, whilst substrate binding to the acceptor site is slightly lower. These effects of (1) and (2), nonetheless, and of bamicetin (3) [5] as well, are so closely resembling each other as to safely conclude the same mode of action for the three antibiotics, i.e. identical sites of interaction with the peptidyl transferase centre. Hence, allotment of oxamicetin to the aminoacyl-4-aminohexosyl-cytosine group of ribosomal inhibitors appears to be justified structurally as well as biologically.

When comparing the presently available biological data on antibiotics (1–3) with those on the other representatives of this group, i.e. blasticidin S and gougerotin there is ample evidence that all are acting upon the peptidyl transferase centre of prokaryotic and – in less generalizable form – of eukaryotic ribosomes. Some minor biological differences among these five antibiotics exist nevertheless, most notably between amicetin–bamicetin and gougerotin–blasticidin S [5,15–17], indicating that the exact sites of interaction within the peptidyl transferase centre are very close yet not identical [18]. Thus, for biological as well as for structural reasons, the aminoacyl-4-aminohexosyl-cytosine group of antibiotics is reasonably subdivided into the amicetin group (1–3), featuring the disaccharide residue as a unique structural element, and another, i.e. blasticidin S-gougerotin, which has the same major structural features [6] except the disaccharide unit.

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