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Synthesis, analysis and in vitro antibacterial activity of new metal complexes of sulbactam

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Abstract

Complexes of copper(II), nickel(II) and iron(III) with β-lactamase inhibitor sulbactam have been synthesized, characterized and identified by elemental analysis, IR and ^{1}H NMR spectroscopy. These complexes have been then tested for their in vitro antibacterial activity in combination with ampicillin against various bacterial species.

Keywords: β-Lactamase inhibitor; Sulbactam; Ampicillin; Antibacterial activity; Metal complexes of sulbactam

1. Introduction

Nowadays, β-lactams are the most widely used antibiotics [1]. The most widespread mechanism of bacterial resistance to β-lactam antimicrobials is the expression of β-lactamases that degrade these antibiotics [2]. One way of counteracting resistance to β-lactam antibiotics is to use them in combination with a β-lactamase inhibitor [3–6].

Sulbactam, a penicillanic acid sulfone (Fig. 1), has been characterized as a competitive or non-competitive β-lactamase inhibitor which has relatively little intrinsic antibacterial activity but which has been shown to extend the in vitro spectrum of β-lactam antibiotics substantially against resistant bacteria [7]. Fixed combinations of sulbactam with ampicillin have been successfully introduced into clinical use. When ampicillin and sulbactam are combined, sulbactam acts as inhibitor of the action of β-lactamases, allowing ampicillin to act by itself in the usual fashion without interference.

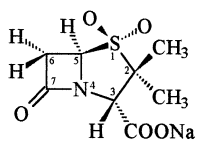

Fig. 1. Structure of sulbactam.

It would then be interesting to examine whether sulbactam will retain its action when combined with ampicillin in the form of metal complexes. Some articles appeared recently, dealing with metal complexes of β-lactam antibiotics [8–11]. We studied the formation of metal complexes of sulbactam with a number of chelating agents which are known to have a biological function.

2. Chemistry

In this paper synthesis and coordinating properties of copper(II), nickel(II) and iron(III) complexes of sulbactam are reported, and the relative changes that occur in its action upon complexation are explored.

Sulbactam contains electron-donor atoms such as nitrogen and oxygen, which easily coordinate with

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metal ions to form complexes. Its molecule is expected to act as a bidentate ligand occupying four coordination sites, while the remaining two are filled by water molecules thus giving octahedral complexes. The evidence concerning the structure and coordinating properties of the prepared complexes was based on spectroscopic and elemental analysis data.

3. Chemical experimental

The metal salts used for preparing the complexes were Merck products, p.a. grade: $Cu(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$ and $Fe(NO_3)_3 \cdot 9H_2O$. Sulbactam sodium salt was a gift from Antibiotic Co., Razgrad, Bulgaria.

The complexes were synthesized by mixing water solutions of the corresponding copper(II), nickel(II) and iron(III) salts with the water solutions of sulbactam sodium salts in amounts equal to metal:ligand molar ratio of 1:2. The reaction mixture was stirred at 25°C for 1 h. Then ethanol was added and the obtained precipitates were filtered and dried in a desiccator to constant weight.

The C, H and N contents were determined by elemental analysis. The copper, nickel and iron ions were determined complexometrically after mineralization and the sodium ions by means of flame photometry.

The water content of the synthesized compounds was determined using a Metrohn Herizall E55 Karl Fisher titrator.

IR spectra (Nujol) were recorded on a Shimadzu FTIR-8101M spectrometer.

^{1}H NMR spectra of sulbactam and its complexes were recorded at room temperature on a Brucker WP 100 (100 MHz) spectrometer in D_2O. Chemical shifts are given in ppm.

4. Antibacterial activity

The in vitro antimicrobial activity of the newly synthesized metal complexes of sulbactam in combination with ampicillin was compared with that of ampicillin combined with uncomplexed sulbactam against a number of clinically important and control strains.

4.1. Microorganisms

During 1997, 42 clinically important strains, belonging to eight different bacterial species, have been isolated from respiratory tract specimens, urogenital tract specimens, blood culture and feces (strains are listed in Tables 2 and 3). Most of them have been selected

Table 1
Elemental analysis data for the synthesized metal complexes of sulbactam

Complex	Found (Calc.) (%)					
	C	H	N	H_2O	Na	Metal
$Cu(C_8H_{10}NSO_5Na)_2 \cdot 2H_2O$	31.25 (31.53)	4.05 (3.94)	4.72 (4.59)	6.17 (5.91)	7.32 (7.55)	10.11 (10.34)
$Ni(C_8H_{10}NSO_5Na)_2 \cdot 2H_2O$	32.57 (32.81)	4.27 (4.10)	4.95 (4.78)	6.28 (6.15)	7.43 (7.86)	7.03 (6.68)
$Fe(C_8H_{10}NSO_5Na)_2 \cdot 2H_2O$	31.58 (31.89)	4.16 (3.98)	5.01 (4.65)	6.12 (5.98)	7.38 (7.64)	9.64 (9.27)

Table 2
Minimal inhibitory concentrations (MICs) in mg/l of ampicillin, sulbactam and their combinations against 42 clinical and three control strains

Microorganisms		Ampicillin		Sulbactam		Ampicillin + sulbactam	
Species	n[a]	MIC ranges	MIC_{90}[b]	MIC ranges	MIC_{90}	MIC ranges	MIC_{90}
Enterococcus faecalis	7	1–4	2.7	64–>128	>128	1–2	1.5
Moraxella catarrhalis	13	<0.06–2	1.7	<0.06–64	24	<0.03–2	0.5
Staphylococcus aureus	2	0.5	0.5	>128	>128	0.5–1	0.7
Escherichia coli	3	>1024	>1024	64–>64	>64	16–64	48
Shigella isangii	1	4	4	128	128	2	2
Klebsiella pneumoniae	8	1024–>1024	>1024	8–>64	>64	4–>64	>64
Acinetobacter baumann	7	1024–>1024	>1024	2–32	21	1–32	11
Pseudomonas aeruginosa	1	256	256	>128	>128	>64	>64
E. coli ATCC 25922		4	4	64	64	2	2
S. aureus ATCC 25923		<0.06	<0.06	>64	>64	0.06	0.06
P. aeruginosa ATCC 27853		512	512	>64	>64	32	32

[a] n, number of strains.
[b] MIC_{90}, minimal inhibitory concentration that inhibits 90% of the strains.

Table 3
Minimal inhibitory concentrations (MIC) in mg/l of the metal complexes of sulbactam and their combinations with ampicillin against 42 clinical and three control strains[a]

Microorganisms		Cu–Sulb		Amp+Cu–Sulb		Fe–Sulb		Amp+Fe–Sulb		Ni–Sulb		Amp+Ni–Sulb	
Species	*n*	MIC range	MIC_{90}	MIC range	MIC_{90}	MIC range	MIC_{90}	MIC range	MIC_{90}	MIC range	MIC_{90}	MIC range	MIC_{90}
E. faecalis	7	>64	>64	2–8	5.6	>64	>64	1–2	1.7	16–64	>64	2–4	3.2
M. catarrhalis	13	<0.06–>64	>64	0.03–4	3	<0.06–>64	42	<0.03–64	1.5	<0.06–>64	64	<0.03–1	0.7
S. aureus	2	>64	>64	1	1	>64	>64	2–4	3.2	>64	>64	1–2	1.8
E. coli	3	>64	>64	>64	>64	>64	>64	32–64	57	>64	>64	32–>64	>64
S. isangii	1	>64	>64	>64	>64	64	64	64	64	128	128	8	8
K. pneumoniae	8	>64	>64	64–>64	>64	32–>64	>64	32–>64	>64	64–>64	>64	16–>64	>64
A. baumann	7	>64	>64	>64	>64	4–32	24	4–64	28	4–64	48	4–>64	52
P. aeruginosa	1	>64	>64	>64	>64	>64	>64	64	64	64	64	64	64
E. coli ATCC 25922		>64	>64	4	4	32	32	16	16	64	64	4	4
S. aureus ATCC 25923		>64	>64	1	1	>64	>64	0.12	0.12	>64	>64	2	2
P. aeruginosa ATCC 27853		>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	64	64

[a] Amp, ampicillin; Sulb, sulbactam; *n*, number of strains; MIC_{90}, minimal inhibitory concentration that inhibits 90% of the strains.

because of some challenge for antimicrobial treatment e.g. *Moraxella catarrhalis* producing β-lactamase (Bla) or *Escherichia coli* and *Klebsiella pneumoniae*, elaborating extended broad-spectrum β-lactamase (EsBla).

4.2. Media

Mueller–Hinton agar (MHA) (purchased from the National Center for Infections and Parasitic Diseases, Bulgaria) and MHA + 5% sheep blood were used.

4.3. Method

In vitro susceptibility determination has been performed by twofold serial dilution method in MHA [12]. Sulbactam (Sulb) and its metal complexes were combined with ampicillin (Amp) in ratio 1:1. The MIC_{90} value was calculated using a graphical method.

5. Results and discussion

Table 1 shows the data of the elemental analysis of the compounds obtained, serving as a basis for the determination of their empirical formulae and the results of the Karl Fisher analysis and of the flame photometry analysis.

The mode of bonding of the ligand to Cu(II), Ni(II), and Fe(III) ions was elucidated by recording the IR spectra of the complexes as compared with those of the free ligand.

The bands appearing in the IR spectrum of sulbactam sodium salt are at 1780, 1599, 1400, 1302 and 1124 cm^{-1}. The band at 1780 cm^{-1} can be attributed to the stretching vibrations of the carbonyl group (β-lactam), the two bands at 1599 and 1400 cm^{-1} can be related to the stretching vibrations of the carboxilate ion and the two bands at 1302 and 1124 cm^{-1} correspond to the sulfo-group. In all the complexes the $\nu_{C=O}$ band was shifted from 1780 to 1790 cm^{-1}.

A broad band, characteristic for ν_{OH} of coordinated water was observed in the region 3500–3200 cm^{-1} in the spectra of all the complexes.

The band at 1599 cm^{-1} assigned as ν_{COO^-} is observed at 1668–1614 cm^{-1} in the copper complex, at 1626 cm^{-1} in the nickel complex, and at 1634 cm^{-1} in the iron complex.

The ν_{SO_2} band at 1302 and 1124 cm^{-1} shifted to 1315 and 1118 cm^{-1} in copper and nickel complexes and to 1317 and 1120 cm^{-1} in the iron complex.

Metal ion coordination with sulbactam by means of oxygen atom of the COO^- group was confirmed by ^1H NMR spectroscopy.

The proton spectrum of the starting sulbactam sodium salt was as follows: 5.05 (dd, 1H, $J_1 = 5$ Hz,

$J_2 = 2$ Hz, 5-H); 4.25 (s, 1H, 3-H); 3.60 (d, 1H, $J = 5$ Hz, 6α-H); 3.50 (d, 1H, $J = 2$ Hz, 6β-H); 1.60 (s, 3H, CH_3); 1.40 (s, 3H, CH_3) (see Fig. 1 for the numbering of C-atoms).

The proton spectra of the metal complexes of sulbactam were as follows: 5.05 (m, 1H, 5-H); 3.80 (s, 1H, 3-H); 3.70 (m, 1H, 6α-H); 3.50 (m, 1H, 6β-H); 1.60 (s, 3H, CH_3); 1.40 (s, 3H, CH_3).

In all complexes of sulbactam a change in the signal of the H at C-3 which shifted from 4.25 to 3.80 ppm on complexation was observed. This can be taken as evidence for the participation of the COO^- group in coordination.

Table 2 shows the results of susceptibility determination for ampicillin, sulbactam and ampicillin + sulbactam combination. The higher minimal inhibitory concentrations (MICs) of ampicillin are results of Bla production. Sulbactam possesses poor antibacterial activity and only the strains of *Acinetobacter* are inhibited (natural susceptibility). The effect of Amp + Sulb combining is more pronounced in *M. catarrhalis* (plasmid-determined Bla).

The results of the susceptibility determination for metal complexes of sulbactam and their combinations with ampicillin are summarized in Table 3. It seems that the new complexes exhibit similar activity to that of sulbactam, usually slightly lower. The activity of Amp + Fe–Sulb is more near the activity of Amp + Sulb against *Staphylococcus aureus* ATCC 25923 and especially the activity against *Enterococcus faecalis* clinical strains.

The susceptibility to the combination Amp + Ni–Sulb is also similar to that of Amp + Sulb for the control strains *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, as well as for the most of clinical strains e.g. *M. catarrhalis*.

In conclusion several new metal complexes of sulbactam have been synthesized and their combinations with ampicillin were found to exhibit biological activity that is slightly lower than that of the parent combination.

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