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Synthesis and Structure of Silver Complexes with Nicotinate-Type Ligands Having Antibacterial Activities against Clinically Isolated Antibiotic Resistant Pathogens

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The synthesis and low-temperature X-ray crystal structures of five new silver complexes, $[Ag_2$-μ-O,O′(2-aminonicotinium)$_2(NO_3)_2]_n$ (**7**), [Ag(isonicotinamide)$_2$-μ-O,O′$(NO_3)]_2$ (**8**), [Ag(ethyl nicotinate)$_2$](NO_3) (**9**), [Ag(ethyl isonicotinate)$_2(NO_3)$] (**10**), and [Ag(methyl isonicotinate)$_2(H_2O)](NO_3)$ (**11**), are presented and fully characterized by spectral and elemental analysis. The antimicrobial activities of these complexes were screened using 12 different clinical isolates belonging to four pathogenic bacteria, *S. aureus*, *S. pyogenes*, *P. mirabilis*, and *Ps. Aeruginosa*, all obtained from diabetic foot ulcers. These tested bacteria were resistant for at least 10 antibiotics commonly used for treatment of diabetic foot ulcers. Compounds **7** and **8** had considerable activity against *Ps. Aeruginosa* (MIC values 2–8 μg/mL), compound **9** against *S. aureus* (MIC 4–16 μg/mL) and *S. pyogenes* (MIC 2–4 μg/mL), and also **9** and **11** against *P. mirabilis* (MIC 1–16 μg/mL). All complexes were non-toxic for daphnia at concentrations above 512 μg/mL overnight.

Introduction

Currently we are seeing a revival of silver in the medical practice, principally in the addition of silver to medical instruments and in wound dressings (especially burns and chronic wounds) to avoid infections.[1,2] There is also much interest in investigating and applying new more sophisticated Ag(I) compounds for their antimicrobial activity.[1,3−7] One approach to such compounds is to combine known biologically benign molecules with suitable donor groups with Ag(I) and investigate their properties.[1b]

One attractive class of ligands comprises nicotinic acid, **1**, and nicotinamide (vitamin B3, **3**) and their derivatives;

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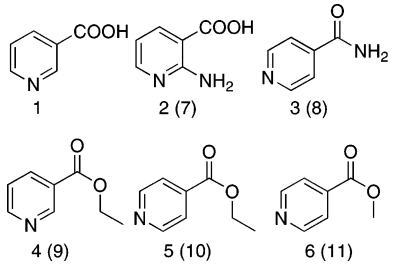
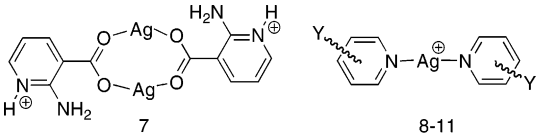
Chart 1. Nicotinic Acid, **1**, and the Related Compounds **2**−**6** Used as Ligands for Ag(I) in This Study[a]



[a] In parentheses we give the number of the resulting silver(I) compound.

Chart 2. Principal Interactions in Complexes **7**−**11** with Ligands **2**−**6**



see Chart 1. Nicotinic acid and nicotinamide are essential for the human body, although their sinister names might suggest otherwise. Nicotinic acid lowers cholesterol and triglycerides, protects the body against atherosclerosis, and has antibacterial properties.[8] The lack of nicotinic acid causes "pellagra", which affects epithelia and the nervous system. Moreover, nicotinamide and isonicotinamide as such were found to have antifungal and antimicrobial activity.[9] The other ligands in this study are not as well documented, but **4** and **5** have found various applications in the pharmaceutical industry and cosmetics (although the later is not necessarily a quality indicator).

Herein we report the synthesis, structure, and antimicrobial activities of five new Ag(I) complexes **7**−**11** with ligands **2**−**6** in Chart 1.

Results and Discussion

Synthesis. The preparations involve the straightforward mixing of a water solution of $AgNO_3$ with the ligand dissolved in ethanol in molar proportions 1:2 giving the desired $[AgL_2]NO_3$ complexes [Ag(isonicotinamido)$_2$-μ-O,O′$(NO_3)]_2$ (**8**), [Ag(ethyl nicotinate)$_2$](NO_3) (**9**), [Ag(ethyl isonicotinato)$_2(NO_3)$] (**10**), and [Ag(methyl isonicotinate)$_2$-$(H_2O)](NO_3)$ (**11**), in 90% yields. Compound **7** was obtained using equimolar proportions of $AgNO_3$ and 2-aminonicotinate giving [Ag_2-μ-O,O′(2-aminonicotinium)$_2$]$(NO_3)_2$, with similar yield; see Chart 2.

Structures. Ag(I) has preference for a linear coordination, likely because it has s- and p-orbitals available for bonding. However, also due to the symmetric d^{10} character, a substantial number of coordination numbers and geometries have been obtained.[10] Thus, it can also bind bidentate ligands to form one-dimensional polymeric chains,[11] as well as di- and polynuclear complexes.[12] Recently many Ag(I) complexes with pyridine and pyridine derivatives have been synthesized and characterized by X-ray crystallography.[11] The crystal structure of the silver(I) complex with 2-aminopyridine and chlorobenzoic acid, $[Ag_2(C_6H_4ClCO_2)_2$-$(C_5H_6N_2)_2]$, gave three-coordinated Ag(I) with bonds to two O atoms and one N atom from three different ligands.[13] On the contrary, the Ag(I) atom in [Ag(pyridine-2,6-dicarboxylic acid)(pyridine-2-carboxylic acid-6-carboxylate)]·$2H_2O$ was found to have a distorted tetrahedral coordination geometry.[14] Silver(I) nicotinic acid, H[Ag(nicotinate)$_2$] (nicotinic acid is 3-pyridinecarboxylic acid), was previously investigated and revealed two different structural types, in both of which Ag(I) is three-coordinated.[15] As for nitrate salts, we have lately analyzed a number of such compounds, both by X-ray diffraction and by using the Cambridge Structural Database.[7,16]

Crystallographic data for **7**−**11** are found in Table 1.

[Ag_2-μ-O,O′(2-aminonicotinium)$_2$]$(NO_3)_2$ (7). The coordination unit of this compound consists of a binuclear silver dicarboxylate complex, also containing a short Ag···Ag interaction at 2.9183(3) Å with the nitrate anions doubly hydrogen bonded to the peripheral protonated pyridine and amino group; see Chart 2 and Figure 1. Sheets are built up by additional NH_2···O hydrogen bonds (2.975(3) Å, 125-(3)°), weaker C−H···O hydrogen bonds (3.274(3) Å, 169°), and very weak Ag···ONO_2^- (2.945(3) Å) interactions. There is efficient "$\pi-\pi$ stacking" between the pyridine rings (offset angle 19.6°, ring−ring distance 3.262(3) Å, centroid−centroid distance 3.518(3) Å) and possibly some weak interactions in the columnar arrangements of the binuclear silver dicarboxylate units (the intersheet Ag···Ag distance is 3.518(3) Å); see Figure 2. Some relevant geometrical data are summarized in Tables 2 and 3.

[Ag(isonicotinamido)$_2$]NO_3 (8). This compound and all the following compounds show the classical linear N−Ag−N coordination geometry with negligible interactions with the nitrate group (Ag···O 2.714(1) Å). An ORTEP type plot is shown in Figure 3, and geometrical data are given in Table 4. One amide group (N2B, O1B) forms a $R_2^2(8)$ amide−amide hydrogen-bonded ring and further hydrogen bonds to the nitrate which together with the second amide

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Table 1. Crystallographic Data for **7**−**11**

param	**7**	**8**	**9**	**10**	**11**
ligand	2-aminonicotinic acid (**2**)	isonicotinamide (**3**)	ethyl nicotinate (**4**)	ethyl isonicotinate (**5**)	methyl isonicotinate (**6**)
formula	$C_6H_6AgN_3O_5$	$C_{12}H_{12}AgN_5O_5$	$C_{16}H_{18}AgN_3O_7$	$C_{16}H_{18}AgN_3O_7$	$C_{14}H_{16}AgN_3O_8$
fw	308.01	414.14	472.20	472.20	462.17
T (K)	173(2)	173(2)	173(2)	173(2)	173(2)
λ (Å)	0.710 73	0.710 73	0.710 73	0.710 73	0.710 73
cryst syst	triclinic	triclinic	triclinic	triclinic	triclinic
space group	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$
a (Å)	3.5183(1)	7.3026(1)	7.2836(2)	6.9450(1)	7.3257(1)
b (Å)	10.6365(1)	8.9139(2)	7.8177(2)	12.0900(3)	10.0792(2)
c (Å)	11.3204(1)	11.1363(2)	16.2800(4)	12.4690(2)	12.4069(1)
α (deg)	81.368(1)	95.966(1)	77.062(1)	64.437(1)	69.001(1)
β (deg)	86.303(1)	99.428(1)	86.046(1)	76.351(1)	76.142(1)
γ (deg)	83.541(1)	100.698(1)	81.727(1)	74.229(1)	81.098(1)
V (Å^3)	415.691(13)	695.91(2)	893.42(4)	900.39(3)	827.95(2)
Z	2	2	2	2	2
ρ_{calc} (g cm^{-1})	2.461	1.976	1.755	1.742	1.854
μ (mm^{-1})	2.432	1.485	1.173	1.164	1.268
F(000)	300	412	476	476	464
cryst size (mm^3)	0.40 × 0.08 × 0.04	0.38 × 0.14 × 0.03	0.30 × 0.10 × 0.06	0.36 × 0.18 × 0.03	0.14 × 0.06 × 0.04
θ (deg)	2.47−32.81	2.35−32.94	2.57−32.90	3.08−29.27	2.31−29.15
measd reflcns	7382	12 298	15 873	13 821	12 233
unique reflcns	2899	4864	6232	4915	4443
R(int)	0.0334	0.0292	0.0316	0.0515	0.0495
compl ($\theta = 30°$) (%)	99.3	99.4	99.5	99.7	99.6
data/restr/param	2899/0/145	4864/0/220	6232/0/264	4915/0/249	4443/2/257
GOF on F^2	0.986	1.008	0.980	1.001	1.039
R1 ($I > 2\sigma$)	0.0289	0.0271	0.0304	0.0447	0.0425
wR2 ($I > 2\sigma$)	0.0584	0.0624	0.0711	0.0917	0.0726
R1 (all data)	0.0406	0.0352	0.0439	0.0773	0.0798
wR2 (all data)	0.0632	0.0664	0.0777	0.1042	0.0839
largest diff peak and hole (e·Å^{-3})	0.636	0.446	0.398	0.953	0.495
	−0.895	−0.867	−0.599	−0.971	−0.711

group (N2A, O1B) gives a $R_3^4(10)$ ring. Thus, hydrogen bond interactions are formed giving a double chain of silver complexes; see Figure 4 and Table 5. These chains are further supported by weak Ag···Ag interactions of 3.1115(3) Å, whereas "$\pi-\pi$ stacking" seems less efficient in this case as the aromatic rings are clearly nonparallel. A packing diagram is provided in Figure 5. Recently, a room-temperature structure of the same compound was published.[17]

[Ag(ethyl nicotinate)$_2$](NO$_3$) (9). An ORTEP type picture of the molecular unit of this compound is shown in Figure 6. It comprises the classic linear coordination (N−Ag−N 175.37(5)°) of two ethyl nicotinate ligands with the nitrate clearly at a nonbonding distance (Ag···O 2.8246(16) Å); see also Table 6. The absence of classical hydrogen bonds makes the "$\pi-\pi$ stacking" between the pyridine rings important for the overall structure (offset angle 22.0(3)°, ring−ring distance 3.367(2) Å, centroid−centroid distance 3.697(2) Å), and in addition, there are some weak C−H···O hydrogen bonds, mostly to the nitrates. There are no Ag···Ag interactions in this compound. A packing diagram is shown in Figure 7.

[Ag(ethyl isonicotinato)$_2$(NO$_3$)] (10). This is the only one among the five complexes where the nitrate group can possibly be assigned as coordinated; see Figure 8. Although the shortest Ag−O distance is still rather long, 2.573(3) Å, the significant bending of the N−Ag−N axes to 148.6(1)° indicates an interaction, and in view of our recent analysis of the Cambridge Structural Database, we consider this as a borderline case.[7] Another indication of the difference compared to the isomer **9** is that no significant weak C−H···O

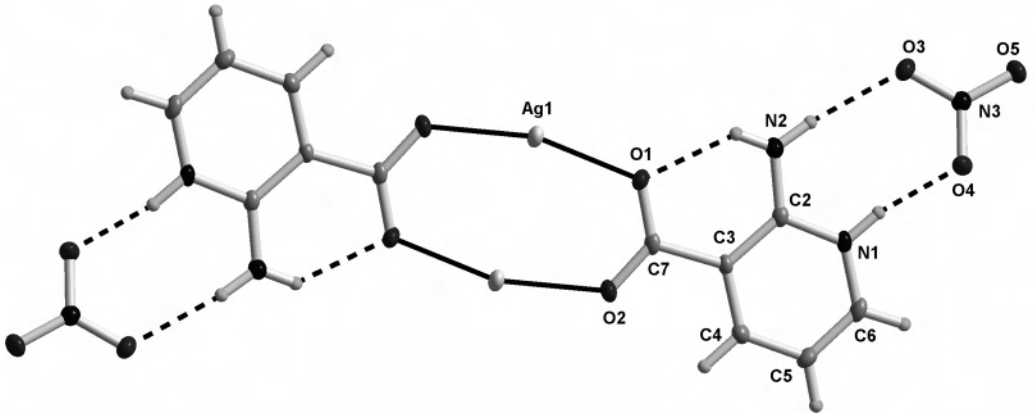

Figure 1. Thermal ellipsoid (50% probability level) drawing of **7**, [Ag$_2$-μ-O,O′(2-aminonicotinium)$_2$](NO$_3$)$_2$, indicating the most important intra- and intermolecular interactions.

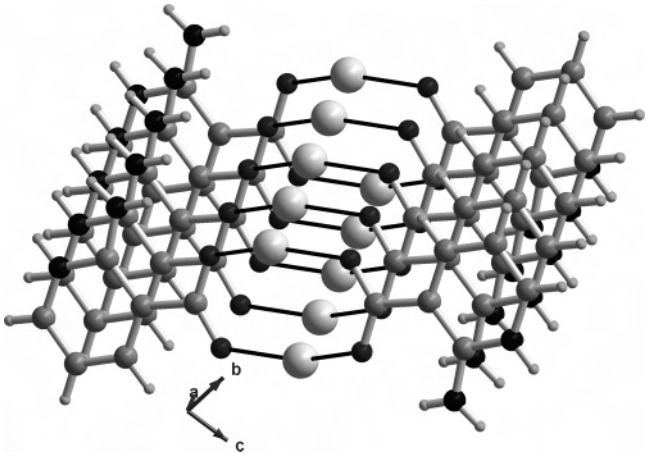

Figure 2. The stacking in **7**, $[Ag_2\text{-}\mu\text{-O,O}'(2\text{- aminonicotinium})_2](NO_3)_2$.

Table 2. Selected Inter- and Intramolecular Silver Interactions in $[Ag_2\text{-}\mu\text{-O,O}'(2\text{-aminonicotinium})_2](NO_3)_2$ (**7**)[a]

interactn	*d* (Å)	interactn	angle (deg)
Ag1−O1	2.2133(16)	O1−Ag1−O2	157.76(7)
Ag1−O2	2.2170(16)	O1−Ag1−O5	124.57(6)
Ag1−O5	2.5627(18)	O2−Ag1−O5	77.49(6)
Ag1−Ag1^{i}	2.9183(3)	O1−Ag1−Ag1^{i}	82.31(4)
		O2−Ag1−Ag1^{i}	78.93(5)
		O5−Ag1−Ag1^{i}	144.82(4)

[a] Symmetry transformations used to generate equivalent atoms: (i) $-x$, $-y+1$, $-z+1$.

Table 3. Hydrogen Bond Data for $H_2[Ag_2\text{-}\mu\text{-O,O}'(2\text{-aminonicotiniate})_2](NO_3)_2$ (**7**)[a]

D−H···A	*d*(D−H) (Å)	*d*(H···A) (Å)	*d*(D···A) (Å)	−(DHA) (deg)
N1−H1···O4^{ii}	0.88	1.90	2.746(3)	161
N1−H1···N3^{ii}	0.88	2.68	3.544(3)	166
N2−H21···O3^{ii}	0.87(3)	2.12(3)	2.982(3)	171(3)
N2−H22···O1	0.81(3)	2.07(4)	2.700(3)	134(3)
N2−H22···O3^{iii}	0.81(3)	2.44(3)	2.975(3)	125(3)
C6−H6···O4^{iv}	0.95	2.34	3.274(3)	169
C4−H4···O2^{i}	0.95	2.39	2.727(3)	100

[a] Symmetry transformations used to generate equivalent atoms: (i) $-x$, $-y+1$, $-z+1$; (ii) $-x+1$, $-y+1$, $-z$; (iii) $x+1$, y, z; (iv) $x+2$, $y-1$, z.

hydrogen bonds can be found and the "$\pi-\pi$ stacking" is also weaker (offset angle 37.0(3)°, ring−ring distance 3.3−3.6 Å, centroid−centroid distance 3.813(2) Å). The Ag···Ag distance is rather too long to be considered an interaction,

Table 4. Selected Inter- and Intramolecular Silver Interactions in $[Ag(isonicotinamido)_2]NO_3$ (**8**)[a]

interactn	*d* (Å)	interactn	angle (deg)
Ag1−N1B	2.1677(14)	N1B−Ag1−N1A	171.03(5)
Ag1−N1A	2.1755(14)	N1B−Ag1−Ag1^{i}	88.42(4)
Ag1−Ag1^{i}	3.1115(3)	N1A−Ag1−Ag1^{i}	100.55(4)
Ag1−O3	2.7136(14)		

[a] Symmetry transformations used to generate equivalent atoms: (i) $-x$, $-y+1$, $-z+1$.

Table 5. Hydrogen Bond Data for $[Ag(isonicotinamido)_2]NO_3$ (**8**)[a]

D−H···A	*d*(D−H) (Å)	*d*(H···A) (Å)	*d*(D···A) (Å)	−(DHA) (deg)
N2A−H2A1···O2^{ii}	0.88	2.07	2.937(2)	168
N2A−H2A2···O1B^{i}	0.88	2.08	2.8866(19)	152
N2B−H2B1···O1B^{iii}	0.88	2.06	2.9341(19)	172
N2B−H2B2···O3^{iv}	0.88	2.05	2.882(2)	158
C2A−H2A···O3	0.95	2.44	3.187(2)	135
C2B−H2B···O3	0.95	2.46	3.222(2)	137
C3A−H3A···O1B^{v}	0.95	2.57	3.402(2)	147
C5B−H5B···O1A^{iv}	0.95	2.52	3.264(2)	135

[a] Symmetry transformations used to generate equivalent atoms: (i): $-x$, $-y+1$, $-z+1$; (ii) $-x-1$, $-y+1$, $-z$; (iii) $-x+2$, $-y+2$, $-z+2$; (iv) $-x+1$, $-y+2$, $-z+1$; (v) $x-1$, y, $z-1$; (vi) $x+1$, y, $z+1$.

3.2428(4) Å. The packing is shown in Figure 9, and geometric data are found in Tables 7 and 8.

[Ag(methyl isonicotinate)$_2$(H$_2$O)](NO$_3$) (11). All compounds are formed in an water−ethanol solution, but this is the only one to contain H_2O molecules, although the water can hardly be considered as coordinated (Ag···O 2.848(3) Å). The geometry is given in terms of an ORTEP type plot in Figure 10 and in metrics in Tables 7 and 8.

Although it is clear from Table 1 that **10** and **11** are not exactly isostructural, they nevertheless have a close resemblance in their molecular packing as can be seen comparing Figure 9 with Figure 11. Especially, the sheet formation is similar; however, the packing of the sheets is somewhat different in the two cases (Figure 12).

Solution NMR Studies. These compounds are not generally soluble in water but are all soluble or sparingly soluble in dimethyl sulfoxide (DMSO). Thus, as the solutions for the biological studies were prepared in this solvent (see below), all compounds were also characterized by ^{1}H NMR in DMSO-d_6. For all compounds, complex-induced shifts of about +0.02 ppm of the *meta*-protons were observed compared to the free ligand in the same solvent (complete

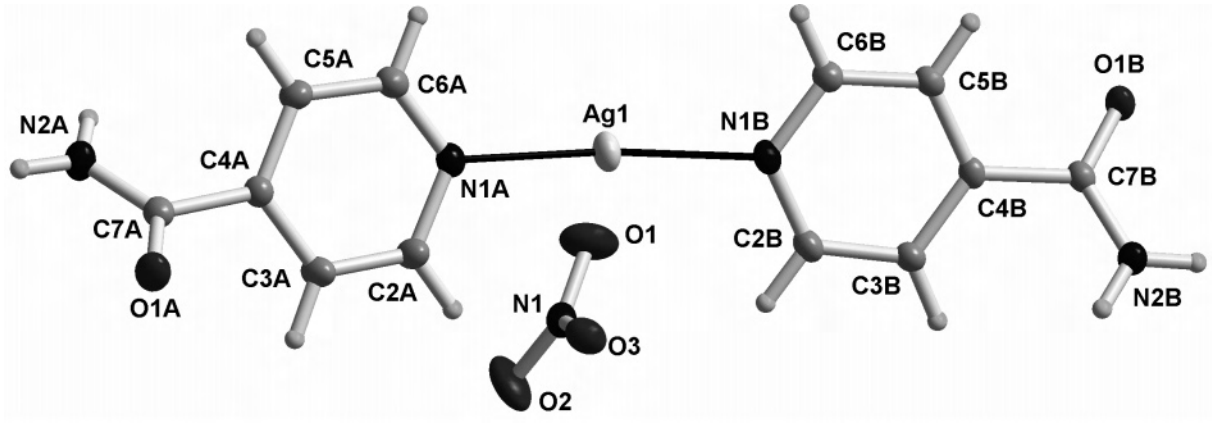

Figure 3. Thermal ellipsoid (50% probability level) drawing of **8**, $[Ag(isonicotinamido)_2]NO_3$. The N1A−Ag−N1B angle is 171.03(5)°, and the Ag···O3 distance is 2.7136(14) Å.

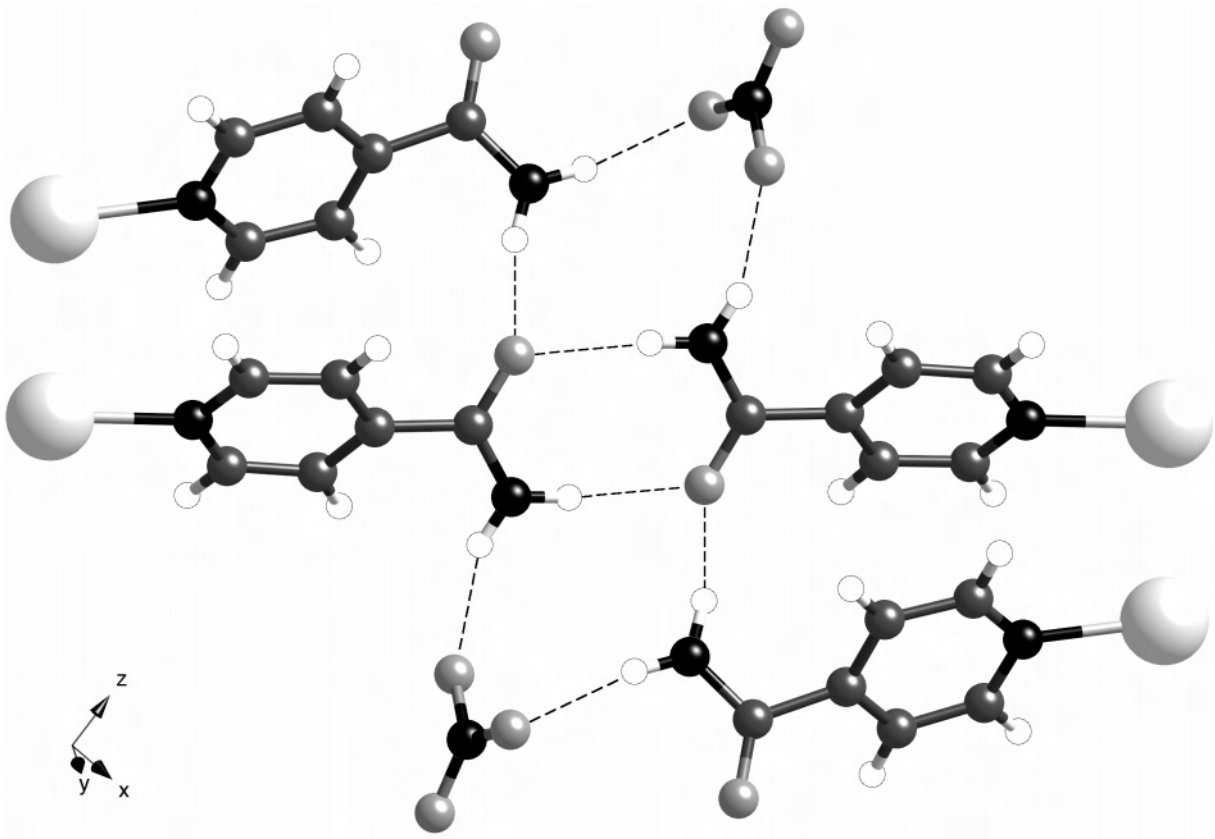

Figure 4. Hydrogen bond interactions in **8**, [Ag(isonicotinamido)$_2$]NO_3. See Table 5 for details. Ag···Ag distances are 3.1115(3) Å.

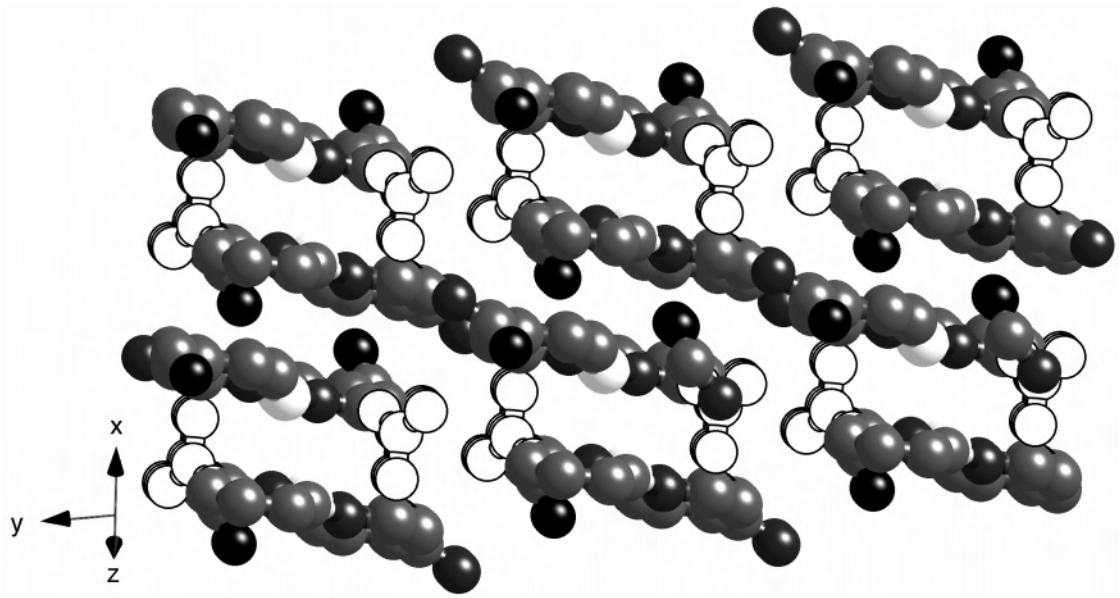

Figure 5. Packing diagram of **8**, [Ag(isonicotinamido)$_2$]NO_3. Nitrate groups are emphasized in white, and hydrogen atoms are omitted for clarity.

data are given in the Supporting Information). However, although these shifts are consistent, reproducible, and well outside the error margins of the method, they are small. Therefore, a ^{1}H NMR titration experiment was performed for [Ag(ethyl isonicotinate)$_2$(NO_3)] (**10**). This showed a typical titration curve for the *meta*-protons similar to those of other studies on silver−pyridyl systems, although the absolute values are smaller in magnitude;[18] see Figure S1. During the titration, all other chemical shifts of the system were constant within ±0.0005 ppm. That we get only small shift difference may be due to the interaction of the pyridyl group with the dipolar S^+-O^- group, as this in itself has been reported to give increases of the chemical shifts.[19]

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(18) Greco, N. J.; Hysell, M.; Goldenberg, J. R.; Rheingold, A. L.; Tor, Y. *Dalton Trans.* **2006**, 2288−2290.
(19) Rao, G. V.; Balakrishnan, M.; Venkatasubramanian, N.; Subramanian, P. V.; Subramanian, V. *Phosphorus Sulfur Relat. Elem.* **1976**, *1*, 83−5.

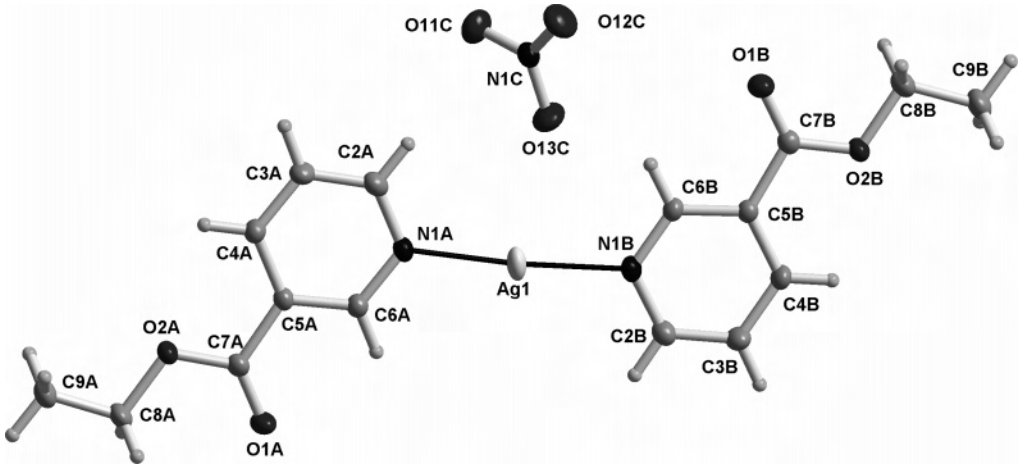

Figure 6. Thermal ellipsoid (50% probability level) drawing of [Ag(ethyl nicotinate)$_2$](NO$_3$) (**9**). The N1A−Ag−N1B angle is 174.37(5)°, and the Ag···O12C distance is 2.825(2) Å.

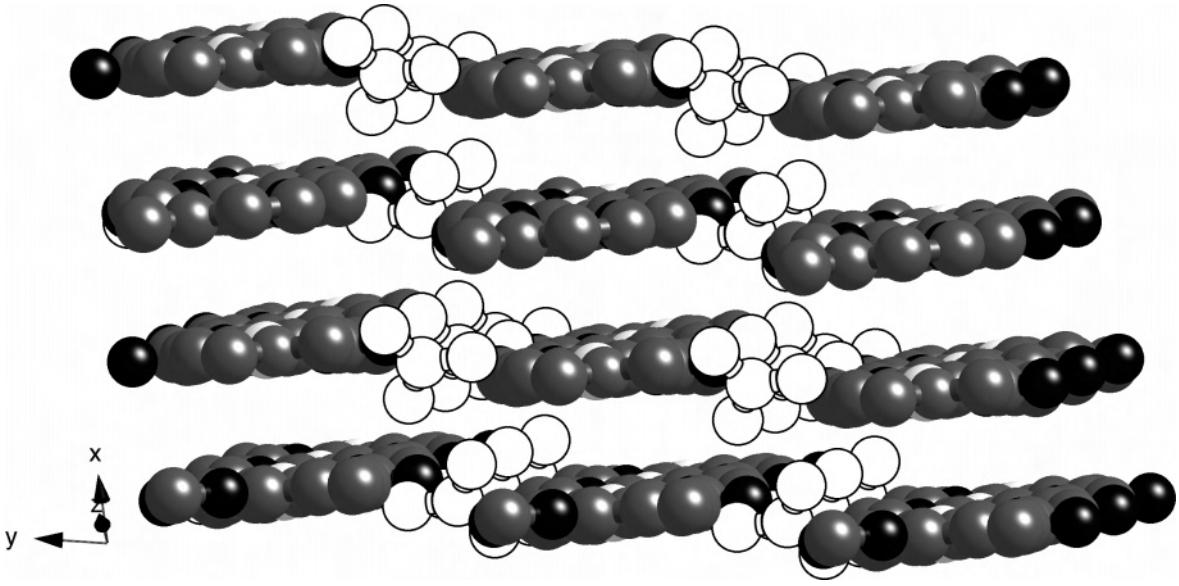

Figure 7. Packing diagram of [Ag(ethyl nicotinate)$_2$](NO$_3$) (**9**). Nitrate groups are emphasized in white, and hydrogen atoms are omitted for clarity.

Table 6. Selected Inter- and Intramolecular Silver Interactions in [Ag(ethylnicotinate)$_2$](NO$_3$) (**9**)[a]

interactn	*d* (Å)	interactn	angle (deg)
Ag1−N1A	2.1463(14)	N1A−Ag1−N1B	175.37(5)
Ag1−N1B	2.1523(14)		
Ag...O12C^{i}	2.8246(16)		

[a] Symmetry transformations used to generate equivalent atoms: (i) *x*, 1 + *y*, *z*.

On the other hand, potentiometrically determined stability constants for 1:1 and 1:2 complexes of Ag^+ and pyridine in 0.1 mol·dm^{-3} tetraethylammonium perchlorate DMSO solutions[20,21] are rather small, i.e., log K_1 = 1.41,[21] so that our solutions may in fact contain various amounts of uncomplexed silver ions,[17] especially since our ligands are somewhat less basic than pyridine itself.[22] Then again, it is not evident how to extrapolate the 0.1 M ionic strength data to 100% DMSO so further studies of the solution behavior are needed.

Antimicrobial Studies. Pressure ulcers and other wounds affect up to 10% of all hospitalized patients in the U.S. and cost more than 5 billion U.S. $/year.[23] Silver-containing wound dressing products find increasing use to prevent infections, and Silvazine (other trade names also exist), a 1% cream of the coordination polymer between silver(I) and sulfadiazine, patented in 1973, is used to treat burns.[24]

As for other Ag(I) compounds, silver(I) reacts with 2-mercaptonicotinic acid (mna) producing a variety of complexes with different biological activities. $Na_4[(HOCH_2)_3CNH_3]_2[Ag(mna)]_6\cdot 10H_2O$ was found to have effective antifungal and antibacterial properties,[6] $[(Et_3NH)^+]_2[Ag_6(\mu_3\text{-}Hmna)_4(\mu_3\text{-}mna)_2]_2\cdot 2DMSO\cdot H_2O$ has antiviral properties,[25] and $[Ag(Hmna)]_6\cdot 4H_2O$ has antimicrobial activity.[26]

(20) Grzejdziak, A.; Olejniczak, B.; Seliger, P. *J. Mol. Liq.* **2002**, *100*, 81−90.
(21) Cassol, A.; Dibernardo, P.; Zanonato, P.; Portanova, R.; Tolazzi, M. *J. Chem. Soc., Dalton Trans.* **1987**, 657−659.
(22) Berthelot, M.; Laurence, C.; Safar, M.; Besseau, F. *Perkin Trans. 2* **1998**, 283−290.
(23) Williamson, J. E. Dressing for success: New wound care products aid healing, efficiencies. *Healthcare Purchasing News* **2005**, *Jan*.
(24) Silver, S.; Phung, L. T.; Silver, G. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 627−634.

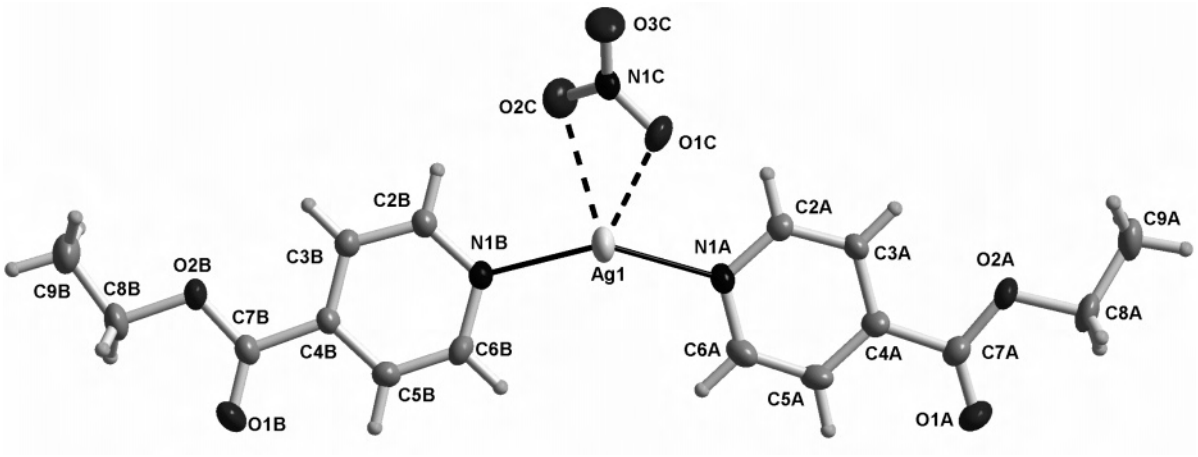

Figure 8. Thermal ellipsoid (50% probability level) drawing of [Ag(ethyl isonicotinato)$_2$(NO$_3$)] (**10**). The N1A−Ag−N1B angle is 148.6(1)°, and the Ag···O1C and Ag···O2C distances are 2.573(3) and 2.680(3) Å, respectively.

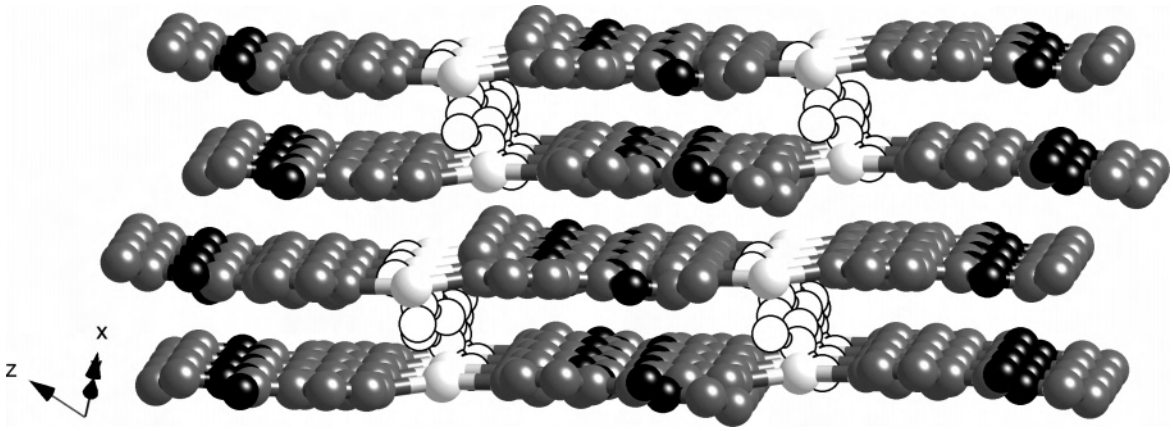

Figure 9. Packing in [Ag(ethyl isonicotinato)$_2$(NO$_3$)] (**10**). Hydrogen atoms have been omitted, and nitrate groups are in white for clarity.

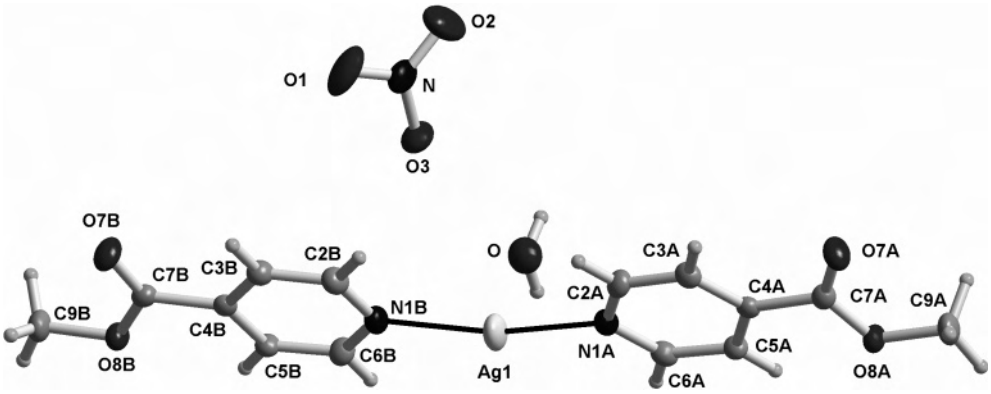

Figure 10. Thermal ellipsoid (50% probability level) plot of [Ag(methyl isonicotinate)$_2$](H$_2$O)(NO$_3$) (**11**). The N1A−Ag−N1B angle is 161.15(9)°.

[Ag$_2$(tetrazole)(PPh$_3$)$_2$]$_n$[27] and macrocyclic dinuclear gold(I) and silver(I) N-heterocyclic carbenes[28] also have antimicrobial activity. [Ag$_2$(2-bim)$_2$](ClO$_4$)$_2$ and [Ag$_2$(2-bim(Bz)-OH)$_2$](ClO$_4$)$_2$·EtOH (2-bim = bis(imidazol-2-yl)methane) were found to display antifungal activity when tested in vitro against the fungal pathogen *Candida albicans*,[5] and [Ag$_2$-(NH$_3$)$_2$(salH)$_2$] (salH$_2$ = salicylic acid) has antifungal and anticancer activity.[4]

In addition to the already large problem of wound healing, there is the related concern of resistance of pathogens to many current antibiotics. We therefore tested the Minimum Inhibitory Concentrations (MIC) of compounds **8**−**11** and silversulfadiazine (Aldrich) against 12 different pathogens clinically isolated from diabetic foot ulcers all resistant to

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Table 7. Selected Inter- and Intramolecular Silver Interactions in [Ag(ethyl isonicotinato)$_2$(NO$_3$)] (**10**) and [Ag(methyl isonicotinate)$_2$(H$_2$O)](NO$_3$) (**11**)[a]

	d (Å)			angle (deg)	
interactn	**10**	**11**	interactn	**10**	**11**
Ag1−N1A	2.203(3)	2.183(2)	N1B−Ag1−N1A	148.55(10)	161.15(9)
Ag1−N1B	2.203(2)	2.186(2)	N1B−Ag1−Ag1i	105.50(7)	
Ag1−Ag1^{i}	3.2429(5)		N1A−Ag1−Ag1^{i}	77.75(7)	
Ag1−O1C	2.573(3)		N1B−Ag1−O1C	122.80(9)	
			N1A−Ag1−O1C	87.06(8)	

[a] Symmetry transformations used to generate equivalent atoms: (i) $-x, -y + 1, -z + 1$.

Table 8. Hydrogen Bond Data for [Ag(ethyl isonicotinato)$_2$(NO$_3$)] (**10**) and [Ag(methyl isonicotinate)$_2$(H$_2$O)](NO$_3$) (**11**)[a]

D−H···A	*d*(D−H) (Å)	*d*(H···A) (Å)	*d*(D···A) (Å)	−(DHA) (deg)
C2B−H2B···O2C (**10**)	0.95	2.51	3.239(4)	134
C3A−H3A···O1B^{ii} (**10**)	0.95	2.44	3.105(4)	127
C3B−H3B···O1A^{iii} (**10**)	0.95	2.48	3.187(4)	131
C6B−H6B···O1C^{i} (**10**)	0.95	2.44	3.144(4)	130
O−H1···O2^{i} (**11**)	0.814(18)	2.46(3)	3.073(4)	133(4)
O−H2···O3^{ii} (**11**)	0.839(18)	2.14(2)	2.948(3)	161(4)
C9B−H9B2···O^{iii} (**11**)	0.98	2.59	3.392(4)	139
C5A−H5A···O7B^{iv} (**11**)	0.95	2.36	3.090(3)	133
C5B−H5B···O7A^{v} (**11**)	0.95	2.40	3.123(3)	132
C6A−H6A···O2^{i} (**11**)	0.95	2.44	3.272(4)	146
C6B−H6B···O1^{i} (**11**)	0.95	2.43	3.109(4)	128

[a] Symmetry transformations used to generate equivalent atoms for **10**: (i) $-x, -y + 1, -z + 1$; (ii) $x + 1, y, z - 1$; (iii) $x, y - 1, z + 1$. Symmetry transformations used to generate equivalent atoms for **11**: (i) $x - 1, y + 1, z$; (ii) $x, y + 1, z$; (iii) $-x - 1, -y - 1, -z + 1$; (iv) $x, y + 1, z - 1$; (v) $x - 1, y, z + 1$.

at least 10 commonly used antibiotics. The results are displayed in Table 9, and we can note that for each bacterial strain there is at least one compound that has high, or very high, activity. In addition, in a toxicity bioassay all complexes were found to be nontoxic against daphnia at concentrations above 512 μg/mL overnight.

Moreover, we can note an overall higher activity than for the compounds described by us in an earlier paper even though the strains of the microorganisms were not the same. Even though, as shown here, the activity may vary between individual strains, there is an overall consistency that a compound active against one strain also shows activity against the other strains of the same bacteria. For comparison, we therefore now recapitulate the MIC values (μg/mL measured under identical circumstances) of the previous compounds against nonresistant strains of *S. aureus*, *P. mirabilis*, and *Ps. Aeruginosa*, respectively: [Ag(quinoxaline)]$_n$(NO$_3$)$_n$ (**12**), 16, 16, 256; [Ag(2,5-dimethylpyrazine)(NO$_3$)]$_n$ (**13**), 32, 64, 256; [Ag$_4$(3-aminopyridine)$_4$(NO$_3$)$_4$]$_n$ (**14**), 16, 128, 256; [Ag$_3$(2-aminopyridine)$_4$](NO$_3$)$_3$ (**15**), 32, 32, 8.[7] For the present compounds, **7** and **8** showed considerable activity against *Ps. Aeruginosa* (MIC values 2−8 μg/mL), compound

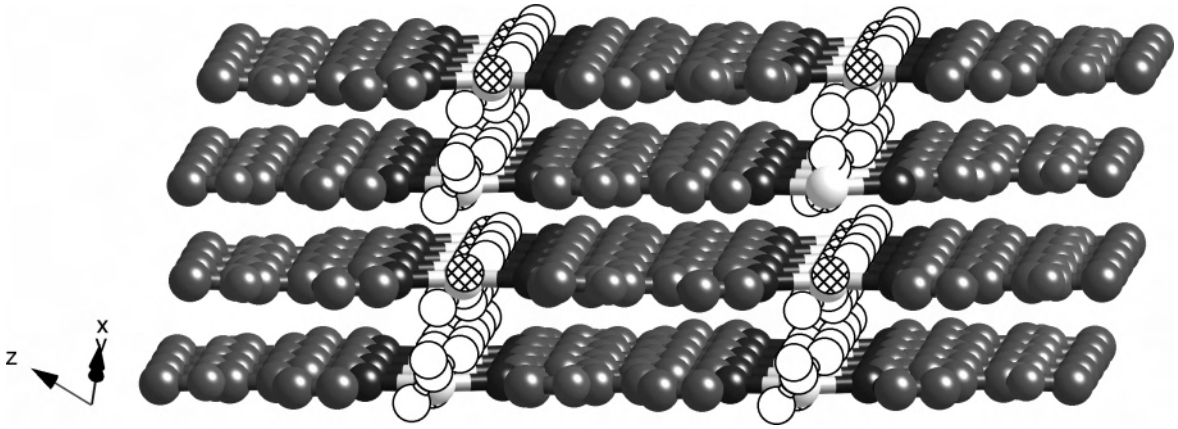

Figure 11. Packing in [Ag(methyl isonicotinate)$_2$](H$_2$O)(NO$_3$) (**11**). Hydrogen atoms have been omitted, nitrate groups are in white for clarity, and hatched spheres are water molecules.

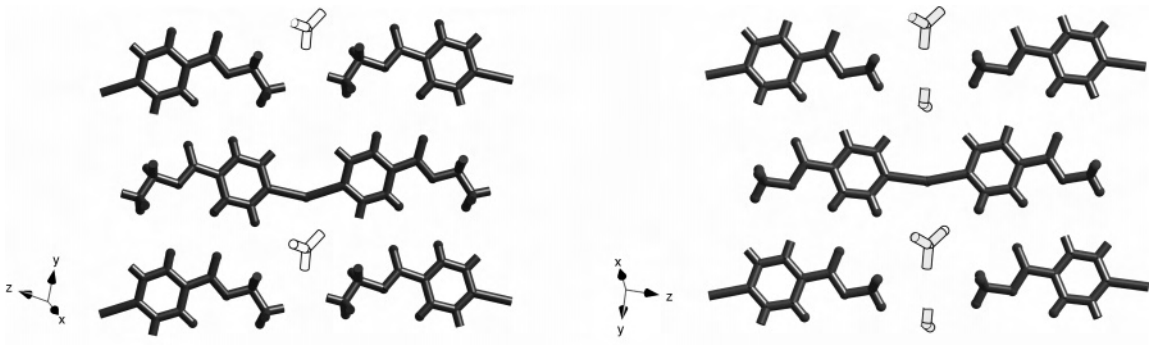

Figure 12. Comparison of the sheet formation in **10** (left, ethyl derivative) and **11** (right, methyl derivative + water of crystallization).

Table 9. Antibacterial Activities of Compounds **8**−**11** and the Prescription Drug Silversulfadiazine (SS) against 12 Different Pathogens Clinically Isolated from Diabetic Foot Ulcers All Resistant to at Least 10 Antibiotics

	MIC (μg/mL)											
	Gram-positive						Gram-negative					
	S. aureus 1	*S. aureus* 3	*S. aureus* 3	*S. pyogenes* 1	*S. pyogenes* 2	*S. pyogenes* 3	*P. mirabilis* 1	*P. mirabilis* 2	*P. mirabilis* 3	*Ps. Aeruginosa* 1	*Ps. Aeruginosa* 2	*Ps. Aeruginosa* 3
7	32	32	64	128	256	256	64	16	32	8	2	4
8	16	32	32	64	128	256	16	8	16	8	4	8
9	16	4	4	2	4	2	128	128	256	64	32	128
10	8	4	16	32	16	16	8	2	16	32	32	64
11	16	32	8	64	128	64	2	1	2	128	16	64
SS	4	8	8	32	64	32	16	16	32	16	8	16

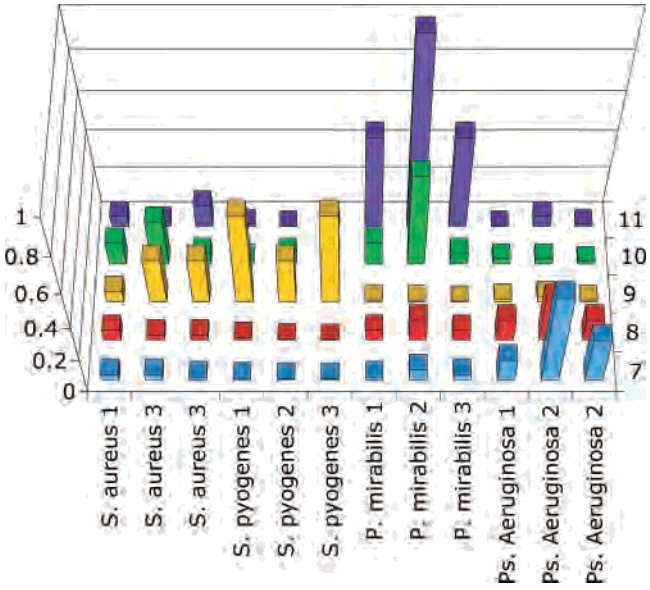

Figure 13. Comparison of the antibacterial effect of compounds **7**−**11** against different strains of bacteria. The height of the staples correspond to the inverted MIC values from Table 9; thus, a high staple means high activity.

9 against *S. aureus* (MIC 4−16 μg/mL) and *S. pyogenes* (MIC 2−4 μg/mL), and **9** and **11** against *P. mirabilis* (MIC 1−16 μg/mL). Compound **10** showed best activity against *P. mirabilis* (MIC 2−16 μg/mL). Only for *S. aureus* did silver sulfadiazine perform best. For reference we note that for silver sulfadiazine there are recently reported average MIC values of 64 μg/mL against *Ps. aeruginosa* and *S. aureus*[29] thus substantially higher than the average found in this study (10 μg/mL). This may certainly in part be caused by the "enhancement" by the DMSO solvent, as mentioned below, but likely the differences in bacterial strains will also be important.

A clearer comparison between the compounds may be obtained by looking at Figure 13, where the inverse of the MIC values of our compounds have been plotted. With no doubt, the different compounds show different activities against different types of bacteria; for example, [Ag(methyl isonicotinate)$_2$(H_2O)](NO_3) (**11**) has a high activity against *P. mirabilis* but very little activity against the other types. Besides the obvious interests in designing compounds selective for certain bacteria, this also proves that the effect of these compounds is not merely to control the levels of "free" Ag^+; the active species is most likely a silver−pyridyl coordination complex. The actual identity of these active compounds formed remains a question to be further studied; it cannot be taken for granted that they resemble the solid-state compounds obtained. For example, the high chloride ion concentration in physiological solutions makes mixed Ag−ligand−chloride complexes possible. This aspect will be further studied, by for example further NMR titrations.

(29) Neuwirth, C.; Martin, M. Joint conference between European Wound Management Association (EWMA), European Tissue Repair Society (ETRS), and Deutsche Gesellschaft für Wundheilung und Wundbehandlung e.V. (DGfW), Stuttgart, 2005, http://www.stuttgart2005.org/documents/poster_abstracts/Poster%20161−180.pdf (Nov 2, 2006).

It is also necessary to take into account that the distribution of these compounds dissolved in DMSO might enhance the activity, as reported by McCann et al.[5]

As far as structure−activity relations go, we have no clear indications that the substitution pattern on the pyridine ring is decisive; thus, the stoichiometry and coordination chemistry of the complex may be more important.

The stoichiometry and geometry of the solid-state compounds may be important for two reasons. Either these compounds are similar to, or control (by the stoichiometry), the active species in vivo or they affect, by their solubility properties, the rate and amount of silver complexes in solution. In general, we can note that the compounds in this study are all mononuclear, with the exception of **7**. However, it is possible that **7**, when dissolved, will generate pyridyl type complexes similar to **8**−**11**. Comparing with the earlier study (compounds **12**−**15**), we see that these have higher nuclearity or are 1D coordination polymers. This will probably effect the solubility, and intuitively one would think that the coordination polymers should be less soluble since they have fairly large, partly covalent interactions extending through one dimension of the crystal (we note that the Silvazine active compound is a 1D coordination polymer). More clearly, we note that none of the compounds **12**−**15** can stoichiometrically form $Ag(pyridyl)_2^+$ compounds in solution, so if these complexes are indeed the active species we would expect a reduced activity by a factor of 2 for **12**−**14** and a factor of 3/2 for **15**. This would in fact bring the results for **12**−**15** in closer proximity to the results in this study, but on the other hand, as the $[AgL_2]/[Ag^+]$ ratio is dependent on the stability constant times the square of the ligand concentration, such species may seem less likely at concentrations corresponding to very low MIC values.

Conclusions

The five newly prepared silver(I) compounds with 1:2 and 1:1 metal to ligand stoichiometry have all high activities against several strains of multiresistant bacteria. Possibly, these or other compounds could be used to give a combination preparation with an increased broad antimicrobial activity spectrum. An evaluation the structure−activity relations on the basis of solution studies at biologically relevant conditions is needed as well as in vitro studies of their synergy with other antibiotics used in the field of wound treatment.

Experimental Section

Materials and Instrumentation. All chemicals and solvents were of analytical grade and used as received without further purification. All preparations and manipulations were performed under aerobic conditions.

X-ray crystallography. Crystallographic measurements were made on a Siemens Smart CCD diffractometer with graphite-monochromated Mo Kα radiation at 173 K. The structures were solved by direct methods and subsequent full-matrix least-squares refinement, including anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms were refined isotropically with use of geometrical constraints. The calculations were carried out with the SHELXTL program package.[30]

NMR. NMR analysis were made on Varian UNITY 400 and 500 MHz NMR spectrometers at 25 °C with DMSO-d_6 (99.8% D) as solvent. Chemical shifts are reported in ppm with the solvent as internal standard (CHD_2CD_3SO, $\delta(^1H) = 2.500$ ppm; $(CD_3)_2SO$, $\delta(^{13}C) = 42.900$ ppm). In the titration experiment 0.0050 g (0.033 mmol) of ethyl isonicotinate was dissolved in 0.8681 g (0.736 mL) of DMSO-d_6 to give a concentration of 0.038 mol·dm^{-3} and 0.0032 g (0.019 mmol) of $AgNO_3$ was dissolved in 0.8588 g (0.728 mL) of DMSO-d_6 to give a concentration of 0.026 mol·dm^{-3}. ^{1}H NMR was run on both solutions, and subsequently, the silver solution was added to the isonicotinate solution in portions of 3 × 200 μL and finally 85 μL giving the silver/ligand ratios 0.16, 0.32, 0.48, and 0.55.

$[Ag_2$-μ-O,O′(2-aminonicotinium)$_2](NO_3)_2$ (7). To an aqueous solution (20 cm^3) of $AgNO_3$ (0.34 g, 2.0 mmol) was added a 0.27 g, 2 mmol amount of 2-aminonicotinic acid in ethanolic solution (15 cm^3) with continuous stirring. A white precipitate was formed, boiled, and then filtered, and the clear filtrate was allowed to stand at room temperature for several days. Colorless needles of the complex suitable for X-ray measurement were collected and dried in air, with a yield ∼90% with respect to the metal. Anal. Calcd for $C_{12}H_{12}Ag_2N_6O_{10}$: C, 23.40; H, 1.96; N, 13.64; Ag, 35.02. Found: C, 23.56; H, 1.71; N, 13.57; Ag, 35.02.

Synthesis of Complexes 8−11. To an aqueous solution (20 cm^3) of $AgNO_3$ (0.34 g, 2.0 mmol) was added 0.49 g, 4 mmol of isonicotinamide, 0.60 g, 4 mmol of ethyl nicotinate, 0.60 g, 4 mmol of ethyl isonicotinate, or 0.54 g, 4 mmol of methyl isonicotinate in ethanolic solution (15 cm^3) with continuous stirring. The clear mixtures were allowed to stand at room temperature for several days. Colorless crystals of $[Ag(isonicotinamido)_2$-μ-O,O′$(NO_3)]_2$ (**8**), colorless sheets of $[Ag(ethyl\ nicotinate)_2](NO_3)$ (**9**), colorless crystals of $[Ag(ethyl\ isonicotinato)_2(NO_3)]$ (**10**), and $[Ag(methyl\ isonicotinate)_2(H_2O)](NO_3)$ (**11**) suitable for X-ray measurement were collected and dried in air, with a yield ∼90% with respect to the metal. The compounds are sparingly soluble in DMSO at about 0.003−0.005 g/mL.

Anal. Calcd for **8**: C, 34.97; H, 2.44; N, 16.99; Ag, 26.17. Found: C, 34.84; H, 2.51; N, 17.09; Ag, 26.05. Calcd for **9**: C, 40.69; H, 3.84; N, 8.90; Ag, 22.84. Found: C, 40.73; H, 3.76; N, 8.97; Ag, 22.90. Calcd for **10**: C, 40.69; H, 3.84; N, 8.90; Ag, 22.84. Found: C, 40.55; H,3.88; N, 9.01; Ag, 22.67. Calcd for **11**: C, 36.38; H, 3.48; N, 9.09; Ag, 23.33. Found: C, 36.45; H, 3.35; N, 9.20; Ag, 23.43.

Determination of Minimum Inhibition Concentration (MIC). Antimicrobial activities of complexes **1**−**5** were determined according to the recommendations of NCCLS (1999), National Committee for Clinical Laboratory Standards, by the use of a broth microdilution method.[31] Minimum inhibitory concentrations (MICs) for the tested compound were conducted using 12 different bacterial pathogens clinically isolated from diabetic foot ulcers (Department of Vascular Surgery, Faculty of Medicine, Alexandria University, Alexandria, Egypt) and are all resistant strains for at least 10 antibiotics commonly used for diabetic foot ulcer treatment: *S. aureus* 1, 2, and 3 and *S. pyogenes* 1, 2, and 3 as Gram-positive bacteria; *P. mirabilis* 1, 2, and 3 and *Ps. Aeruginosa* 1, 2, and 3 as Gram-negative bacteria. The test materials were dissolved in DMSO

(30) *SHELXTL Structure Determination Programs*, 6.10; Bruker AXS Inc.: Madison, WI, 2001.

(31) *Performance standards for antimicrobial susceptibility testing.* NCCLS approved standard M100-S9; National Committee for Clinical Laboratory Standards (NCCLS): Wayne, PA, 1999.

to give a stock solution that was subsequently diluted in the growth medium to give final concentrations of 256, 128, 64, 32, 16, 8, 2, 1, and 0.5 μg complex/mL. A final concentration of 5% DMSO was present in all assays, a concentration which had no antibacterial effect on its own (a control treatment, with all the tested bacteria, using 10% DMSO showed no antibacterial activity). The highest concentration used was 256 μg/mL. The inoculum was 10^5 CFU/mL for bacteria. Bacteria were cultured in Mueller Hinton broth (MHB) for 24 h at 35 °C. The MIC value was corresponding to the lowest concentration that inhibited the bacterial growth. A toxicity bioassay against daphnia was conducted using standard methods.[32]

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Supporting Information Available: Crystallographic information files (CIF) and NMR data for **7**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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