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PAPER
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The role of atropisomers on the photo-reactivity and fatigue of diarylethene-based metal–organic frameworks

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New Triazoloquinoxaline Ligand and its Polymeric 1D Silver(I) complex

Synthesis, Structure, and Antimicrobial activity

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Abstract

The organic ligand 4-Benzyl-1-(N,N-dimethylamino)-[1,2,4]triazolo[4,3a]quinoxaline **1** (L) and its polymeric silver(I) complex, $[Ag_2L(NO_3)_2]_n$ (**2**), have been synthesized and characterized. The organic ligand **1** crystallizes in the triclinic space group P⁻1. The unit cell contains two parallel-stacked molecules. The complex $[Ag_2L(NO_3)_2]_n$ (**2**) crystallizes in the monoclinic space group $P2_1/n$. The structure contains two different silver(I) ions. Ag(2) is coordinated by three oxygens (involving two nitrate groups) and to a nitrogen of the triazole ring of **1**. These ligands form a strongly distorted tetrahedral, nearly planar coordination sphere. Ag(1) has an approximately tetrahedral geometry. It is bonded to one oxygen of a nitrate anion and a nitrogen of two different L; this aspect giving rise to an infinite chain structure. A final bond to Ag(1) involves the carbon of a phenyl group. It is more weakly bonded to the phenyl carbons on either side of this, so that the Ag(1)-phenyl bonding has aspects of an Ag-allyl bond. Ag(1) and Ag(2) participate in bonding to a common nitrate anion and alternate, the two distinct modes of bridging between them lead to a zig-zag chain structure. In addition to spectroscopic studies, the biological activities of the ligand and of the complex were scanned over a wide range of Gram positive and Gram negative flesh- and bone-eating bacteria. The results are discussed in comparison with well-known antibiotics.

Introduction

It is well recognised that the diffusion of increasingly drug resistant bacteria indicates a need for new antibiotics. The difficulty of designing new drugs can be overcame, as short-term measure, by modifying the characteristics of currently available antibiotics [1], by exploiting the fact that a lot of them can serve as metal ion ligands [2]. The bioavailability, membrane permeability [3] and distribution within the cell may thus be modified. Several groups have carried out work in this area; the present report concerns an apparently unique case which may be the precursor of a significant advance. A triazole ligand (**1**) which forms a complex with the Ag(I) ion (**2**) and, apparently, no other ion, to give a complex stable in aqueous media is reported. Closer investigation reveals that the complex contains Ag···π interactions with phenyl ring, and this presumably is the origin of its uniqueness. The role of Ag-N and Ag-O bonds in antimicrobial and antifungal activities have been explored [4], as well as the importance of Ag-C sigma bond [5], while the effect of Ag···π interaction in the same context has not yet been reported. We have checked the antibiotic activity of both the ligand **1** and the complex **2**. Whilst both the Ag(I) ion and the ligand **1** independently have antibacterial properties, *in vitro* the complex has a behavior that is more than the sum of its parts.

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Triazoles are an important class of heterocyclic compounds. In particular, fused 1,2,4-triazoles **3,4** (Scheme 1) may express antifungal,[6] bactericidal,[6,7] anxiolytic,[8,9] anticonvulsant [10] or herbicidal activities, [11] and can act as antidepressants[12]. Consequently, versatile and widely applicable methods for the synthesis of **3** and **4** are of considerable interest. Most methods are based on heterocyclic hydrazones or hydrazides as precursors.
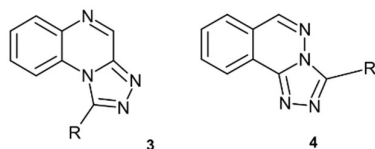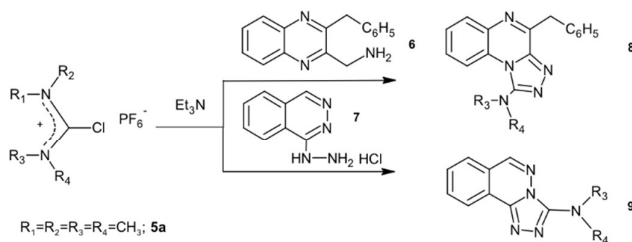

Scheme 1

However, these methods have restricted applicability and use reagents such as lead tetraacetate, [13,14] bromine, [14,15] or phosphorus oxychloride[13] which it is better to avoid. Syntheses involving chloramine T [16], (diacetoxy)iodobenzene[17,18] or electrochemical methods [19] have also been introduced. Recently, [20,21] a low toxicity and inexpensive reagent, copper dichloride, has been used for the preparation of 1,2,4-triazole derivatives[20-22] (Scheme 2).
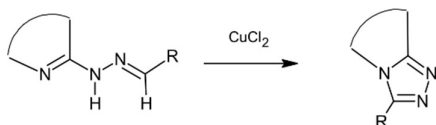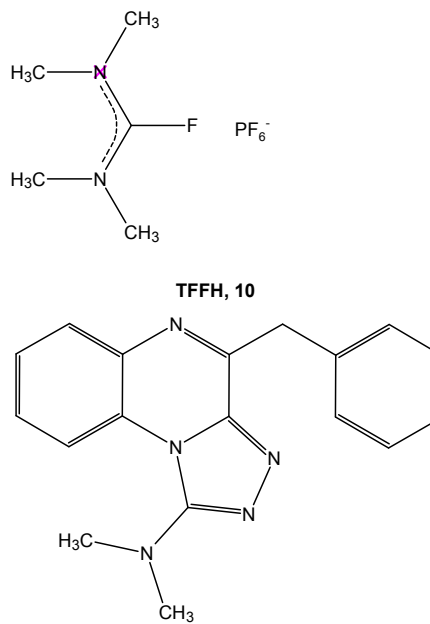

Scheme 2

We recently reported a new method for the synthesis of **8** and **9** in a single step, which involves the reaction of 3-benzyl-2-hydrazinoquinoxaline **6,** or 1-hydrazinophthalazine hydrochloride **7** with chlorouronium salts **5,** to afford the 1,2,4-triazole derivatives **8** and **9** in good yield and purity (Scheme 3) [23].

$R_1=R_2=R_3=R_4=CH_3$; **5a**

$R_1,R_2=R_3,R_4=(CH_2)_4$; **5b**

Scheme 3

In the present work we describe the synthesis of 4-Benzyl-1-(*N, N*-dimethylamino)-[1, 2, 4]triazolo[4,3-a]quinoxaline (**1**) using Tetramethylfluoroformamidiniumhexafluorophosphate (TFFH, **10,** Scheme 4)[24,25]. **1** reacts with silver nitrate to give the new complex $(Ag_2L(NO_3)_2)_n$ **2**, which has been appropriately characterized.

TFFH, 10

Scheme 4

The ligand, L, 1, studied in this work

We have made a detailed study of complexes of Ag(I) with several ligands [26], but the ligand **1** is particularly interesting because it does not seem to form complexes with common metal (we have not succeeded in preparing any complex in aqueous media, other than the complex with Ag(I)). Apart from the question of the specific interaction of **1** with the silver ion, there is a more important question about the role played by silver in bio-active systems. Since the antiquity, it has been known that metallic silver has an antibacterial action (although this aspect is controversial)[27]. At present, ceramic candles impregnated with colloidal silver are widely used in remote tropical areas to purify drinking water, and there is commercial production of broad-band silver additives which can be used for totally differing items, like food or clothing; in a completely different context, the silicone heart valve contains a silver-impregnated sewing ring, designed to

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reduce the incidence of prosthetic valve endocarditis [28]. The presence of silver has been found to make antibiotics orders of magnitude more effective against gram-negative bacteria[29,30]. Studies in this area have shown an enhanced antibiotic activity of the silver complexes by respect both the free ligand (antibiotic) or the silver ion itself. However, there is some uncertainty about the active species, attributable to two main problems. First, the biological environment may contain a variety of potentially complex-forming cations, that will be in competition with the silver ion for the antibiotic ligand. Second, unless there were a very large antibiotic enhancement it might be overlooked (the silver ion complex would normally be rather labile). Therefore, even if there were an enhancement due to the silver ion, the concentration of the silver complex present would be too low to be evident. With the lack of this evidence, possible small changes of the ligand that might enhance the relative stability of the silver complex have never been studied. Thirdly, the biological environment is often a reducing one, and it is possible that silver complexes may be reduced to metallic silver. This, of course, may have a bioactive effect, but usually this is attributed to the low concentration of Ag(I) ions arising from metallic silver. The present work is particularly relevant to the first of these problems. It seems that in aqueous media the ligand **1** forms water-stable complexes only with the silver ion. It is therefore possible to be confident that any observed enhancement of bioactivity results from the presence of the silver complex.

Results and discussion

Organic Ligand (**1**)

The room temperature single crystal X-ray structure analysis of the organic ligand, 4-Benzyl-1-(N,N-dimethylamino)-[1,2,4]triazolo[4,3a]quinoxaline, shows that it crystallizes in the triclinic system, space group P⁻1 and contains dimeric π-π stacked species in the unit cell. The dimeric unit is shown in Figure 1 and the collection data detailed in Table 2. The mechanism of dimerization is indicated in Figures 2 and 3. Figure 2 shows the packing of the dimeric units whilst the detail of Figure 3 indicates that the mechanism of dimerization is due to weak Van der Waals-bonding between the H atoms of dimethylamino groups and the π electron system of phenyl rings.
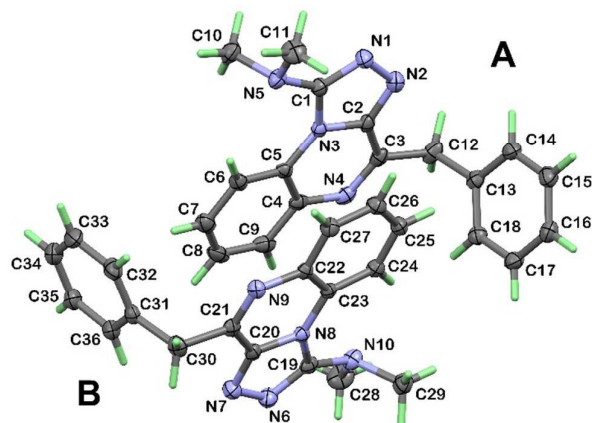
Figure 1 Labeling scheme and ellipsoids (30 % probability level) of the two ligand **(1)** molecules **A** and **B** in the asymmetric unit. The hydrogen atoms are not labeled for clarity in this plot.
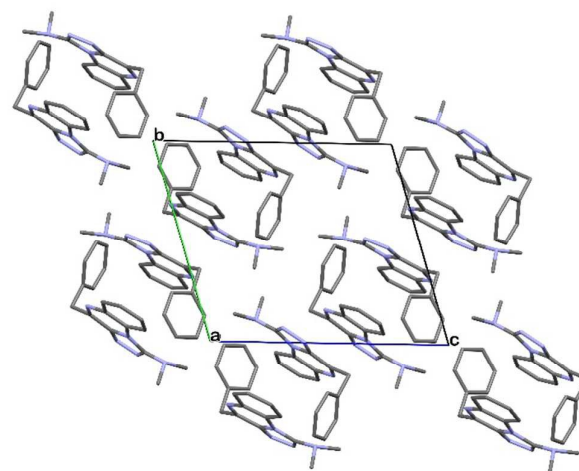
Figure 2 Packing scheme of the dimeric units of ligand **(1)** (view along [100]). The dimeric units are held together by π-stacking interactions.
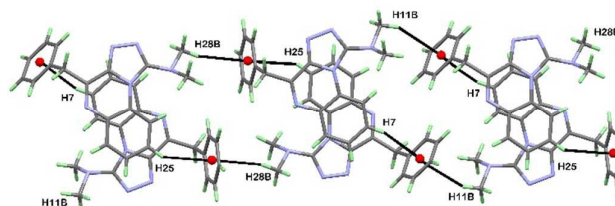
Figure 3 Contacts between some hydrogen atoms of the dimethylamino groups as well as protons bonded to an aromatic ring system with the π-electron system of the phenyl rings of neighboring molecules form van der Waals bonds in the structure of **(1)**. The distances between the hydrogen atoms and the

centroids (red balls) of the phenyl rings range between 2.707 Å (H7 – centroid) and 3.061 (H11B –centroid).
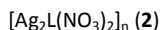
[Ag$_2$L(NO$_3$)$_2$]$_n$ (2)

A room temperature single crystal X-ray structure determination established that the complex [Ag$_2$L(NO$_3$)$_2$]$_n$ (2) crystallizes in the monoclinic system, space group P2$_1$/n (Figure 4 shows the content of the asymmetric unit). The structure contains two different silver(I) ions (Figure 4). Ag(2) is in a strongly distorted tetrahedral, nearly planar, environment, coordinated to three oxygen atoms of two nitrate groups and to one nitrogen atom of the triazole ring of the ligand. The silver-nitrogen bond and two of the silver-oxygen bonds are in a range often found in silver nitrate complexes with nitrogen-containing ligands (Ag(2)-N(1): 2.239 Å, Ag(2)-O(4): 2.338 Å, Ag(2)-O(1): 2.389 Å). These three ligand atoms form a triangular coordination around Ag(2). The fourth silver-oxygen bond much weaker than the two other ones, indicated by a significantly longer (Ag(2)-O5): 2.610 Å) distance. In addition to these four real bonds there are two further contacts with one oxygen (Ag(2)-O(2A): 2.82 Å) and one carbon atom (Ag(2)-C(16): 3.085 Å) significantly shorter than the sum of the van der Waals radii of silver and oxygen (3.24 Å), or silver and carbon (3.42 Å). Ag(1) has a much less distorted tetrahedral geometry. A nitrate oxygen links it to Ag(2) and it is also bonded to a nitrogen of two different ligand molecules thus forming a zig-zag 1D chain (Figure 5). However, particularly interesting is the observation that Ag(1) bonds to the carbon of a phenyl group (Figure 6). This is a long, but surely real, bond (Ag(2)-C(13)): 2.72 Å in comparison with 2.25 and 2.34 Å to nitrogens and 2.39 Å to oxygen. Similar Ag-C bonds are normally ca 2.5 Å.[31-34], but this is when there is no other bonding to Ag. The Ag-C distances to the carbon atoms C(14) and C(18) neighbouring C(13) in the phenyl ring show also weak interactions with Ag(1) (Ag(1)-C(14): 2.897 Å and Ag(1)-C(18): 3.002 Å); compared with the sum of Van der Waals radii of Ag and C of 3.42 Å, these contacts have to be considered as weakly bonding. Figure 4 illustrates the bonding situation described above. Some significant interaction involving these carbons is indicated, in which case the Ag-phenyl bonding is mediated through something akin to an allyl group (and Ag(1) six coordinate).

The fourfold coordinations of the silver atoms have been analyzed by calculating the τ4 [31] and the τ4' [32] geometry index.

$$\tau4 = (360° - (\alpha + \beta)) / (360° - 2\theta)$$

$$\tau4' = (\beta - \alpha) / 360° - \theta) + (180° - \beta) / (180° - \theta)$$

with α, β and β > α: largest occuring bond angles in the fourfold coordination sphere and θ = ideal tetrahedral angle, 109.5°. The values for ideal square planar and tetrahedral coordination spheres are 0 and 1, respectively.

The calculated geometry indices for the Ag(1) atom are τ4 ≈ 0.695 and a τ4' ≈ 0.646, the respective values for Ag(2) are τ4 ≈ 0.454 and a τ4' ≈ 0.371. It can be seen clearly that Ag(2) is closer to a distorted square planar arrangement, while the ligands around Ag(1) tend to form a distorted tetrahedron around the central atom.

As mentioned above the Ag$_2$L(NO$_3$)$_2$ units form 1D infinite zig-zag chains in the structure. The structure shows a relatively dense packing, which is due to the numerous polar interactions between the nitrates and protons of the ligand molecules of neighbouring chains. A section of the coordination polymer and the projection of the crystal structures along [001] is shown in Figure 5. Both views emphasize the folded, fishbone-type packing of the 1D infinite coordination polymers.

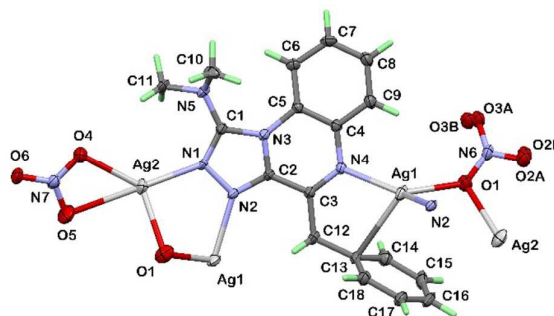**Figure 4** Labeling scheme and ellipsoids (30 % probability level) in the complex unit of the silver compound **(2)**. The hydrogen atoms are not labeled in this plot.
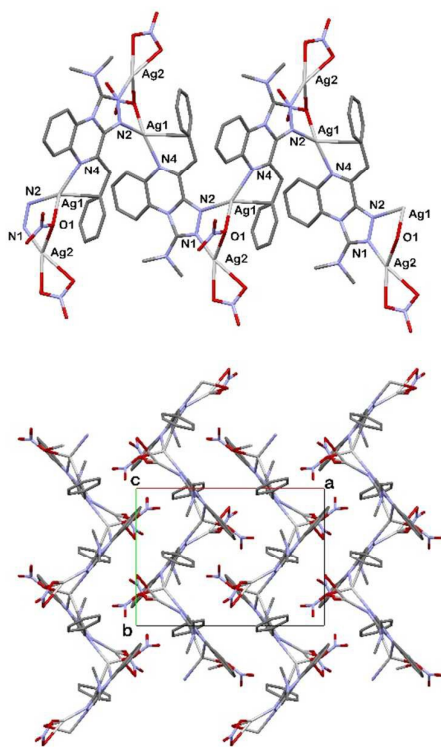
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their v.d.W. radii of 3.42 Å: Ag1 – C13: 2.717(8) Å, Ag1 – C14: 2.897(9) Å, Ag1 – C18: 3.002(9) Å, Ag2 – C16: 3.085(9) Å. The silver-oxygen distances Ag2 – O5 (2.610(7) Å) and Ag2 – O2A (2.82(2) Å) are rather long compared with the other Ag – O bonds and should better be termed ‚contacts' rather than bonds. For this drawing the coordinating atoms have been emphasized, all other atoms are shown as semi-transparent ellipsoids.

Another interesting aspect of the crystal structure of (**2**) is that it is accompanied by only a small distortion of the ligand. The heterocyclic backbone of this molecule is rigid; only the rotation around the sigma bond axes of the benzyl and the dimethylamino group, respectively, to the heterocyclic ring system allows some adaption to the requirements for a successful formation of complexes with metal atoms. Figure 7 illustrates the conformation of (**1**) in the crystal structures of the pure ligand and of the silver complex. It shows clearly that the major differences between the three molecules are mainly due to the rotation of the benzyl group.

Figure 5 Upper part: The Ag$_2$L(NO$_3$)$_2$ units form a one-dimensional infinite coordination polymer connected via Ag(1)-N(2) and Ag(1)-N(4) bonds and a N(1)-Ag(2)-O(1)-Ag(1) bridge. This figure shows only a section of this coordination polymer containing five Ag(1) nodes and four ligand molecules.

Lower part: Packing scheme of the complex (**2**), viewed along the c-axis. The coordination polymers extend along the monoclinic b-axis of the crystal structure of the silver complex. The Ag-C interactions have been omitted for these figures.

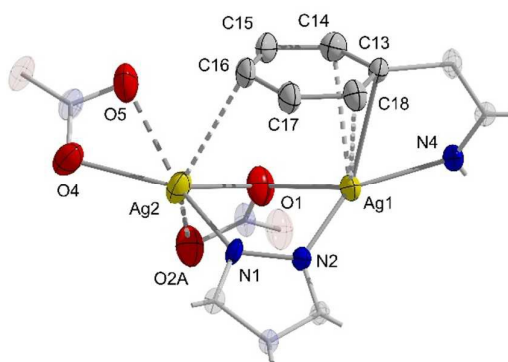**Figure 7** Comparison of the ligand conformations of the molecules **A** and **B** in the pure ligand structure (**1**) and in the Ag complex (**2**).

A key question is why Ag(I) should behave differently from the other ions tested [Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}]. There are two reasons that could serve as explanation. In silver complex (**2**) the ligand geometry is almost the same as in (**1**); this indicates that little distortion energy is required. Such a matching of geometric demands is rare and probably is unlikely to be found with alternative cations.

Perhaps more important is the observation that Ag(I) contains a bond to at least one carbon of the ligand phenyl group. Whilst Ag(I)-aryl bonds are well known and have been characterized crystallographically (Ag(I)-C$_6$H$_6$ is perhaps the best known example), they are usually formed without supporting bonding from other

Figure 6 Coordination spheres of the two silver atoms. Some silver-carbon distances are significantly shorter than the sum of

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ligands. In the present case, however, Ag(I) is also bonded to two nitrogens (of different ligands) and these presumably play a role in enabling silver-carbon bonding. Not that Ag-C bonding alone is insignificant; binding energies of above 150 kJ mol^{-1} have been reported for Ag(I)-benzene species [35,36]. Meanwhile, nitrogen containing ligands have been reported to selectively coordinate Ag$^+$ ions, also from water solution, because the ability of silver to establish Ag$^+$-π interactions[37]. The best evidence comes from molecules in which more than one aromatic ring is involved, where geometrical changes consequent upon Ag(I) bonding are evident [37]. It should be noted that although the bonding of the Ag(I) in the present species conforms to the general geometric pattern [38], its participation in extended bonding seems unusual. This interaction sets Ag apart from all of the other cations that we have studied and which failed to form complexes with **1**. Whether it is responsible for the enhancement of biological activities observed is impossible to say, although this possibility suggests a direction for future enquiry. Another contribution to the enhanced biological activity of the silver species is that the complex formed contains two silver ions, not one. These two ions are not independent but are linked, not only through the ligand, where they are bonded to adjacent nitrogens, but also through the oxygen of a nitrate group. As we have noted, Ag(1) is linked to a nitrogen of a separate ligand. This is the feature which leads to a polymeric structure in the solid but neither it nor the nitrate bridges can be expected to persist in aqueous solution. Clearly, the relationship between solid and solution structures of the complex becomes relevant. Here, the available relevant evidence comes from the NMR data, which indicate the persistence of either the complex or an immediately derived species in solution. In that, the complex contains two chemically different Ag(I) ions, and it cannot be assumed that they behave in the same way in the transition from solid to solution. So, it is possible that one Ag, probably Ag(1), remains complexed whilst the other is free in solution. Even so, the species present in the dilute aqueous solutions used in the actual measurements may differ from those in the NMR measurements and here the most relevant data perhaps comes from the biological measurements themselves, which indicate that the complex persists.

Antimicrobial activity

The Minimum Inhibition Concentrations (MIC) of **1** and **2** were determined and compared with 17 antibiotics used for treatment of the infections reported in the experimental part; the obtained data are reported in Table 1. The last three rows of Table 1 demonstrate an independent behaviour of Ag(I) and compounds **1** and **2**. The complex under investigation, **2**, shows potent activity when compared with the activity of other known antibiotics. In one case (bold) it is the most powerful. This finding is in general accord with previous results: Nomiya *et al*[39] reported good activities for [Ag(imidazole)$_2$](NO$_3$), [Ag(1,2,4-triazole)]$_n$ and [Ag(tetrazole)]$_n$ against both *S. aureus* and *Ps. aeruginosa* (MIC 15.7, 7.9, 15.7 and 7.9, 7.9, 15.7 μg/ml) when compared to AgNO$_3$ (MIC 62.5 μg/ml for both bacteria). [Ag(imidazole)]$_n$ and {[Ag(L-histidine)]$_2$}$_n$ were equally active against *Ps. aeruginosa* and *S. aureus*(MIC 12.5 and 15.7 μg/ml), while [Ag(1,2,3-triazole)]$_n$ showed no activity against these bacteria[40].

Zhang and co-workers investigated [Ag((8-pyridin-3-yl)methylthio)quinoline]$^+$ with different counter ions, and higher activities were recorded for CF$_3$CO$_2^-$ against *S. aureus* and *Ps. aeruginosa* compared to NO$_3^-$ and CF$_3$SO$_3^-$ (MIC 0.25 and 0.06 μg/ml)[41]. This work suggests that the complexes function as ion pairs and that these differ, for instance, in their ability to penetrate the cell wall barrier.

Our research group recently reported on the antimicrobial activities of Ag(I) nicotinate compounds [42] where [Ag$_2$-μ-O,O'(2-aminonicotinium)$_2$-(NO$_3$)$_2$]$_n$ and [Ag(isonicotinamide)$_2$-μ-O,O'(NO$_3$)]$_2$ showed considerable activity against *Ps. aeruginosa* (MIC values 2-8 μg/mL), [Ag(ethyl nicotinate)$_2$](NO$_3$) against *S. aureus* (MIC 4-16 μg/mL) and *S. pyogenes* (MIC 2-4 μg/mL). [Ag(ethylnicotinate)$_2$](NO$_3$), [Ag(methylisonicotinate)$_2$(H$_2$O)](NO$_3$) and [Ag(ethylisonicotinate)$_2$(NO$_3$)] showed remarkable activities against *P. mirabilis* (MIC 1-16 μg/mL). With the ligand 4,5-diazafluoren-9-one we found considerable activity against *S. aureus*, *K. pneumoniae* and *P. mirabilis* (MIC = 6, 4, and 4 μg/ml, respectively).

The question to be addressed is whether there is evidence that the behaviour of the complex differs from that expected from the additive effects of Ag(I) and the ligand (that is, the `complete dissociation´ limit). The problem is complicated by the preliminary nature of the measurements, standard deviations are not available

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(the work involved in obtaining them is great and difficult to justify). Assuming that the presence of the bacteria or some extracellular enzyme and/or the bacterial by-product do not influence equilibria (i.e. that one component is not selectively extracted), the answer seems clear; the complex retains its identity. Ag(I), the ligand and the complex all exert significant anti-bacterial properties. With two exceptions, the complex either equals or out-performs the other two. The first exception is *S. enterica*, where Ag(I) is better than the complex by one, and perhaps two, orders of magnitude. Clearly, the complex is not acting solely as a source of Ag(I); if it were, an exception of this magnitude would not exist. It might also suggest that both the Ag(I) ions of the solid state compound remain coordinated in solution. The other exception is *M. luteus*, where the complex is the least effective of the three, by an amount that seems greater than any likely standard deviation. In one case, from the raw data, the complex seems the most effective inhibitor tested. Taken together, these data indicate that the complex retains its identity and is an effective anti-bacterial. To us this seems a significant conclusion. The addition of substituents to the ligand, with the possibility of enhancing the inhibitory effect, is clearly an attractive forward step.

Table 1. Minimum inhibitory concentration (MIC) for **1** and **2** against multi drug-resistant diabetic foot bacteria (those in the Table not given ATCC numbers) compared with a number of commercial antibiotics.

Antibiotic	Gram-positive bacteria			Gram-negative bacteria					
	M. luteus	S. aureus ATCC 6538p	S. aureus	E. coli ATCC 8739	E. coli	K. pneumonia	Ps. aeruginosa ATCC 9027	Ps. aeruginosa	S. enterica
	MIC (µg/ml)								
Amikacin	8	4	32	8	32	>256	4	12	>256
Gentamicin	16	16	16	4	16	32	12	24	64
Streptomycin	64	12	64	6	32	64	8	16	32
Amoxicillin	24	8	16	32	192	>256	8	256	>256
Ampicillin	16	4	12	24	64	>256	4	12	>256
Cephradine[i]	64	16	12	24	128	192	16	128	8
Cefuroxime[ii]	32	8	24	16	64	128	8	64	12
Cefoperazone[iii]	16	6	16	12	24	96	32	12	24
Cefepime[iv]	8	4	8	4	32	64	8	32	12
Imipenem	32	2	16	3	16	>256	8	>256	32
Meropenem	16	2	12	2	64	192	4	128	16
Azithromycin	16	12	24	12	32	64	12	128	96
Clarithromycin	24	16	32	8	24	32	8	96	48
Nalidixicacid[i]	32	24	64	4	16	128	8	64	32
Ciprofloxacin[ii]	24	4	48	6	32	32	4	48	32
Levofloxacin[iii]	16	3	32	8	24	16	2	32	128
Vancomycin	24	32	16	4	32	128	32	64	>256
(Ag(I))*	12	24	12	24	32	16	64	48	12
1	**4**	48	128	96	>256	128	24	32	>256
2	32	12	12	24	32	**8**	12	16	>256

Roman superscript numbers indicate the generation of the antibiotic.

* Ag(I) ions are known to be toxic and with a very short half-life in body fluids. They are also partially precipitated during the course of the bioassay.

Experimental

Material and instrumentation

Silver nitrate was purchased from Aldrich Company. All other chemicals were of analytical grade quality and were used as received. Elemental analyses for C, H and N were carried out using a Perkin-Elmer analyser; Ag(I) was determined with a Perkin Elmer Analyst 300 AAS atomic absorption spectrometer. Infrared spectra (IR) were recorded as KBr pellets on a Shimadzu 8300 series Fourier Transformer. NMR spectra were recorded on a Jeol 500 MHz spectrometer at ambient temperature (25°C). Melting points were obtained in open capillary tubes using a Gallenkamp Sanyo melting point apparatus and were uncorrected.

Abbreviation used in the text: TFFH = 1,1,3,3-tetramethylfluoroformamidinium hexafluorophosphate; DMF = dimethylformamide; Et$_3$N = triethylamine.

Synthesis

Ligand 1, 4-Benzyl-1-(N,N-dimethylamino)-[1,2,4]triazolo [4,3a]quinoxaline.

In the synthesis of this ligand, TFFH **10** (5 mmoles, 1.32 g) was added to a solution of 3-benzyl-2-hydrazinoquinoxaline **5** (5 mmoles, 1.25 g) and triethylamine (10 mmoles, 1.32 ml) in DMF (10 ml) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then poured into cold water. The solid formed was immediately filtered off and washed, first with water, then with saturated aqueous NaCl (30 ml) and then dried in a desiccator. The crude product was recrystallized from ethanol. The product was obtained as an orange solid in 64% yield (0.95 g), m.p. 166-167 ºC.

Analytical data (%):Calc.: C, 71.26; H, 5.65; N, 23.09 **Found:** C, 71.34; H, 5.57 ; N, 23.14. IR (KBr): 3100-2900 (C-H), 1620 (C=N), 1540-1500 (C=C aromatic), 1480 (-CH$_2$-), 1300 (C-N) cm^{-1}. ^{1}H NMR (DMSO-d$_6$): δ 2.93 (s, 6 H, 2 CH$_3$), 4.48 (s, 2 H, 1 CH$_2$), 7.20-7.27 (t t, 1 H, aromatic), 7.29-7.32 (m, 2 H, aromatic), 7.45-7.48 (d d, 2 H, aromatic), 7.59-7.64 (t d, 1 H, aromatic), 7.67-7.73 (t d, 1 H, aromatic), 7.96-7.99 (d d, 1 H, aromatic), 8.41-8.45 (d d, 1 H, aromatic) ppm. ms: m/z 303 (M+): 304.2

Synthesis of [Ag$_2$L(NO$_3$)$_2$]$_n$ (2)

To an aqueous solution, 10 ml, of silver nitrate (0.1 g, 0.59 mmol) a 15 ml ethanolic solution of the organic ligand (C$_{18}$H$_{17}$N$_5$), (0.3 g, ~1.0 mmol) was added dropwise with constant stirring over about 15 minutes. To the turbid solution, 2-3 drops of 0.1 M nitric acid solution were added with continuous stirring. The solution became clear and was repeatedly filtered until no more precipitate was noticed. The solution was allowed to stand for 3 weeks. Uniform, colourless to yellowish crystals suitable for X-ray measurements were collected and air dried. There was a yield of 0.075 g., about 40% with respect to the metal. Whilst the infrared spectra show little sign of complex formation, the same is not true for the NMR.

Calc.: C, 33.62; H, 2.66; N, 15.25; Ag, 33.56 **Found:** C, 33.57; H, 2.60; N, 15.37; Ag, 33.65. IR (KBr): 3100-2910 (C-H), 1625 (C=N), 1540-1500 (C=C aromatic), 1480 (-CH$_2$-), 1310 (C-N) cm^{-1}. ^{1}H NMR (DMSO-d$_6$): δ 3.03 (s, 6 H, 2 CH$_3$), 4.59 (s, 2 H, 1 CH$_2$), 7.21-7.27 (d, 1 H, aromatic), 7.29-7.55 (m, 2 H, aromatic), 7.56-7.88 (d, 2 H, aromatic), 7.59-7.64 (t, 1 H, aromatic), 7.64-7.73 (t, 1 H, aromatic), 8.07 (d, 1 H, aromatic), 8.54 (d, 1 H, aromatic) ppm.

The differences in chemical shifts between ligand and complex are to be noted; these differences are important because they permit the composition of solutions to be determined (the shifts are not concentration-dependent).

Although the silver complex is the only one we have successfully prepared, we have explored the coordination of **1** with a variety of transition and other metals. An ethanolic solution of the ligand was added to aqueous solutions of several, representative, simple metal salts MCl$_2$ and M(NO$_3$)$_2$ [M = Mn, Co, Ni, Cu, Cd, Zn] by changing both temperature and reaction stoichiometry. In all cases, the only product was an essentially quantitative recovery of the organic ligand

X-ray crystallography

X-ray crystallographic data were collected on an Enraf-Nonius 590 Kappa CCD single crystal diffractometer with graphite monochromator using MoKα (λ = 0.71073Å). The intensities were collected at room temperature using a φ-ω scan mode; the crystal to detector distance was 40 mm. Further details are given in Table 2. The cell refinement and data reduction were carried using Denzo and Scalepak's programs[43]. Multiscan absorption corrections were applied to all data sets using the program SORTAV[44]. The

crystal structures were solved by direct methods, which revealed the positions of all non-hydrogen atoms, and subsequently refined by full matrix least square cycles based on F^2, both using the SHELX package[45]. The temperature factors of all non-hydrogen atoms were refined anisotropically, before hydrogen atoms were introduced in a riding model with C-H = 0.96Å and refined isotropically. The data collection up to 50° in 2theta has been skipped by the operator of the diffractometer since there have been only a very small number of significant reflections in the range between 45-50 °; probably due to the room temperature measurement. Low temperature data collection were temporarily not available for these measurements.The Molecular graphics were prepared using the Diamond 3.0 program [46]. Crystallographic and refinement data are summarized in Table **S1**. Tables **S2** and **S3** list selected bond distances and angles for compounds **1** and **2**, respectively.

Biological measurements.

Determination of Minimum Inhibitory Concentration (MIC).

Antimicrobial activities of the organic ligand **1** and complex **2** were determined according to the recommendations of the National Committee for Clinical Laboratory Standard (NCCLS) 1999, by the use of the broth microdilution method. Bacteria used in this investigation were of two categories; standard bacteria from the American Type Culture Collection (ATCC) and clinical bacteria, all multi-drug resistant, isolated from diabetic foot ulcers by swabbing techniques. Minimum inhibitory concentrations (MICs) for the tested compound were measured using 9 different bacterial strains, three standard American Type Culture Collection (ATCC, namely: *Staphylococcus aureus* ATCC 6538p, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027 and 6 multidrug resistant (MDR) clinical virulent bacteria (isolated from diabetic foot ulcers), two Gram-positive; *Micrococcus luteus, Staphylococcus aureus* and four Gram negative, *Escherichia coli; Klebsiella pneumoniae; Pseudomonas aeruginosa* and *Salmonella enterica*. All clinical bacteria were resistant to at least 10 commonly used antibiotics of choice for diabetic foot ulcers.

The test materials were dissolved in DMSO to give a stock solution which was subsequently diluted in the growth medium to give final concentrations of 256, 128, 64, 32, 16, 8, 2, 1, and 0.5µg/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 antimicrobial activity). Bacteria were cultured in Mueller Hinton broth (MHB) for 24 h at 35°C. The MIC value was that corresponding to the lowest concentration that inhibited bacterial growth. A toxicity bioassay against *Daphnia magna* was conducted using standard methods (US-EPA, 1999).

Conclusions

There have been many studies on the biological activity of metal-ligand combinations. So, the question of whether it is possible to enhance or make more selective the action of an antibiotic by complex formation has been much studied. A besetting problem is the question if trace concentrations of other metal species could distort the experimental data by competing with the chosen metal in complexing with the ligand. The system explored in the present work, with his specificity for Ag(I), is a rare case in which such complexities are absent. The complex studied shows a clear activity against a wide range of bacterial species. Since chemical modification of the ligand is a real possibility, it would be both possible and valuable to extend the present investigation.

The structural analysis underlined the importance of the Ag^+-π bonds in the complex formation, and it will be of importance to check for the existence of this bonding in all related species that may be prepared. The structure of the complex **2** and the solution data suggest that the silver ion may be capable of a succession of weak bonding interactions along a molecular framework which enable it to gain a unique access to cellular structure and which lead to eventual cell death. That is, the Ag^+-π and dispersion interactions involving the silver ion in biological systems could prove to be of real importance.

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