Synthesis, Characterisation and Antiamoebic Studies of Dioxovanadium(V) Complexes Containing ONS Donor Ligands Derived from *S*-Benzyldithiocarbazate

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Three new dioxovanadium(V) compounds, $[K(H_2O)][VO_2(sal\text{-}sbdt)]$ (**1**), $[K(H_2O)_2][VO_2(Clsal\text{-}sbdt)]$ (**2**), and $[K(H_2O)_2][VO_2(Brsal\text{-}sbdt)]$ (**3**) (where H_2sal-sbdt are the Schiff bases formed between salicylaldehyde and dithiocarbazates) have been synthesised, and the structures of **1** and **2** revealed by X-ray diffraction analyses. The ligands coordinate in the tridentate *ONS* fashion out of the enethiolate tautomeric form. The potassium counterions, coordinated to water molecules, the phenolate-O, the oxo groups on vanadium and, in the case of **1**, to the thiolate, provide interlinkages between the anions and thus a complex supramolecular network, further reinforced by intermolecular hydrogen bonds between oxo groups and water molecules. In vitro tests of the antiamoebic activity against the protozoan parasite *Entamoeba histolytica* showed comparable (**1** and **2**) or substantially better (**3**) amoebocidal action than metronidazole, a commonly used drug against amoebobiasis.

Introduction

Amoebobiasis, a world-wide disease due to protozoan parasites and affecting more than 10% of the world's population is responsible for approximately 100,000 deaths annually, placing it second to malaria in mortality of infectious diseases.[1,2] Nitroimidazole drugs such as metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] are presently the most effective antiamoebic medications. Side effects are, however, common:[3] mutagenic effects have been established for bacteria, and high doses in rodents can cause carcinoma.[4] As a result of this and also taking into account the possibility of the development of resistant strains of the amoeba against metronidazole, a search for new effective amoebicidal agents is required in order to provide a new dimension in the therapy of amoebiasis.

Schiff base and related metal complexes have experienced long standing applications in biology and medicine,[5,6] as well as in the catalysis of chemical and petrochemical processes.[7,8] Our interest in studies of complexes containing these ligands with sulfur and nitrogen functions arises from their significant antifungal, antiprotozoal, antibacterial, and anticancer activity.[9] More recently, an in vitro insulinmimetic potential of these compounds has been established.[10] Carcinostatic activities have been found for metal complexes of dithiocarbazoic acid and the Schiff bases derived from its *S*-methyl ester.[5] The ruthenium(II) and palladium(II) complexes of Schiff bases derived from *S*-alkyldithiocarbazates have also shown antiamoebic activity.[11,12] In this overall context, vanadium complexes are of specific physiological interest because of their redox activity (V^{IV}/V^{V}): The redox potential can be tuned by the choice of the ligand set so as to provide redox interaction with oxygen species such as peroxide and superoxide. The superoxide radical anion generated in the process $V^{V} + O_2^{2-} \rightarrow V^{IV} + O_2^{-}$ can trigger further physiological events.[13,14]

The potential of vanadium(V) complexes as antiamoebic agents has thus far only been marginally explored.[15] In order to obtain information on the biological activity of dioxovanadium(V) complexes with Schiff base ligands derived from dithiocarbazates, we report here on the synthesis, characterisation, structure elucidation and in vitro screening of the antiamoebic activity of VO_2^{+} complexes with an *ONS* donor set as represented in Scheme 1. In vitro

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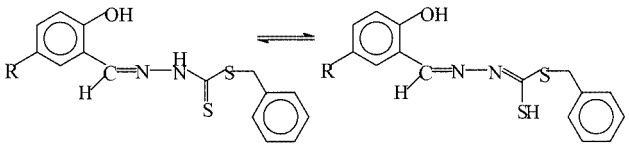
testing was carried out with the HM1:1MSS strain of *Entamoeba histolytica*.



Scheme 1. R = H: H_2sal-sbdt, R = Cl: H_2Clsal-sbdt, R = Br: H_2Brsal-sbdt

Results and Discussion

Synthesis and Thermogravimetric Properties

$[VO(acac)_2]$ reacts with the potassium salt of H_2Rsal-sbdt, H_2L (cf. Scheme 1) under mild basic conditions to give dioxovanadium(v) complexes, $K[VO_2L]$. The probable reaction pathway is given by Equations (1) to (3).

$$[VO(acac)_2] + K_2L \rightarrow [VOL] + 2\ acacK \quad (1)$$

$$2\ [VOL] + 1/2\ O_2 + 2\ MeOH \rightarrow 2\ [VOL(OMe)] + H_2O \quad (2)$$

$$[VOL(OMe)] + 2\ KOH \rightarrow K[VO_2L] + MeOK + H_2O \quad (3)$$

This reaction scheme is based on the fact that H_2sal-sbdt under aerobic conditions gives [VO(OEt)(sal-sbdt)] in ethanol which, in the presence of NaOH, is ultimately oxidised to $[VO_2(sal\text{-}sbdt)]^-$.[16] The orange-yellow compounds **1**−**3** are soluble in methanol, ethanol, acetonitrile, DMF, and DMSO. Lowering the pH of the methanolic solution of **1**−**3** with HCl dissolved in methanol gave brown, incompletely characterised complexes, which are also obtained on using one equivalent (instead of two) of KOH during the synthesis. As shown by, inter alia, thermogravimetric analysis, the complexes contain one (**1**) or 2 water molecules per formula unit (**2** and **3**). The loss of water in the temperature range 120−180 (**1**) and 120−220 °C (**2** and **3**) indicates covalently although not particularly strongly bonded water. On further heating of **1**, the anhydrous complex $K[VO_2(sal\text{-}sbdt)]$ loses the $SCH_2C_6H_5$ group between 180 and 320 °C, followed by the loss of an SCN group between 320 and 600 °C. The remaining residue decomposes to yield KVO_3 as the end product at ca. 670 °C. A very similar decomposition pattern had been observed previously for $[VO_2(acpy\text{-}sbdt)]$ (Hacpy-sbdt = Schiff base derived from acetylpyridine and *S*-benzyldithiocarbazate).[17] The anhydrous forms of **2** and **3** lose $SCH_2C_6H_5$ in the temperature range 220−390 °C, while thermal removal of SCN and other residues is not discernible in these complexes. However, the total weight loss corresponds to the remaining organic residue less one oxygen. The percent weight loss of the end product (i.e. KVO_3) in both complexes compares well with the expected values.

IR and Electronic Spectra

A list of selected IR and electronic spectroscopic data is presented in Table 1. The Schiff bases exhibit a strong band between 1030 and 1041 cm^{-1} due to the ν(C=S), thus indicating the thione (thiocarbonyl, cf. Scheme 1, left) nature of the uncoordinated ligands. This is further supported by the absence of an IR band at ca. 2500 cm^{-1} for ν(SH) and the presence of a band at ca. 3100 cm^{-1} for ν(NH) of the hydrazide moiety. The disappearance of both, the ν(C=S) and ν(NH) bands, in the spectra of the complexes suggests the thioenolisation of the C=S group (Scheme 1, right) and coordination of the enethiolate sulfur to vanadium. This is further supported by the appearance of a new band at 709−776 cm^{-1} due to the ν(C−S) stretch. A strong band associated with the ν(C=N) (azomethine) stretch appears at 1615−1622 cm^{-1} in the free ligands, and this band undergoes a bathochromic shift by 18−21 cm^{-1} in the complexes, thereby indicating the participation of the azomethine nitrogen in coordination. A broad band appears around 3400 cm^{-1} in the ligands and is assigned to the ν(OH) (phenolic) stretch. For the complexes, a broad band associated with water molecules governs this region. In addition, all complexes display two sharp bands at 888−930 and 921−954 cm^{-1}, corresponding to the ν_{sym}(*cis*-VO_2) and ν_{as}(*cis*-VO_2) modes, respectively.

The electronic absorption spectrum of **1** is dominated by an intense band at 392 nm, which is assigned to the $PhO^- \rightarrow V(d\pi)$ ligand to metal charge transfer (LMCT) band. Substitution by Cl or Br in the *para* position of the phenolate shifts this band to higher wavelengths. Two additional bands appearing in the UV region are due to intraligand transitions.

NMR Spectra

The coordination modes of the ligands were also confirmed by recording ^1H NMR spectra of the free ligands and the complexes. As presented in Table 2, all ligands display two sharp resonances in the δ = 10.25 to 13.45 ppm region, indicating the presence of NH (and thus the thionic nature of the free ligands) and phenolic OH protons. In contrast, the absence of these signals in the spectra of the complexes shows that bonding occurs through the phenolic oxygen and enethiolate sulfur atoms. A significant downfield shift of ca. 0.5 ppm for the azomethine (−CH=N−) proton signal in the complexes relative to the corresponding free ligands supports the coordination of the azomethine nitrogen atom. Aromatic and CH_2 protons resonate in the expected region in the spectra of the ligands and the complexes. These results are consistent with those previously obtained for dioxomolybdenum(vi) complexes.[18]

Table 3 contains ^{13}C NMR spectroscopic data of the ligands and complexes. Assignments of the signals are based on the chemical shifts and intensity patterns, and coordination induced shifts Δδ = [δ(complex) − δ(free ligand)] of the signals for carbon atoms in the vicinity of the coordinating functions. Δδ values are provided in parentheses in Table 3. A large Δδ was observed for the azomethine carbon

Table 1. IR and electronic spectroscopic data

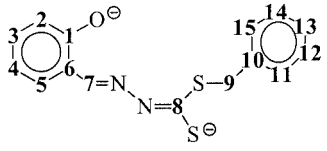
Compound	IR (cm^{-1})[a] ν(C=S/C−S)	ν(C=N)	ν (VO_2)	$\lambda_{max}(\varepsilon)$
H_2sal-sbdt	1041	1622		
[K(H_2O)][VO_2(sal-sbdt)] **1**	766	1601	921, 888	214.5(58300), 293.5(42910), 391.5(13370)
H_2Clsal-sbdt	1036	1618		
[K(H_2O)$_2$][VO_2(Clsal-sbdt)] **2**	697	1600	937, 902	234.5(43170), 294.5(31050), 401(10370)
H_2Brsal-sbdt	1030	1615		
[K(H_2O)$_2$][VO_2(Brsal-sbdt)] **3**	709	1595	954, 930	227(53030), 295(39998), 401(13180)

[a] ν(C=S) for the ligands and ν(C−S) for the complexes.

Table 2. ^{1}H NMR spectroscopic data

Compound	OH	NH	−CH=N−	CH_2	Aromatic
H_2sal-sbdt	13.40 (s, 1 H)	10.24 (s, 1 H)	8.55 (s, 1 H)	4.50 (s, 2 H)	6.89 (m, 2 H), 7.28 (m, 4 H), 7.40 (d, 2 H), 7.64 (d, 1 H)
[K(H_2O)][VO_2(sal-sbdt)] (**1**)			9.07 (s, 1 H)	4.40 (s, 2 H)	6.86 (q, 2 H), 7.23 (q, 1 H), 7.32 (t, 2 H), 7.43 (q, 3 H), 7.65 (d, 1 H)
H_2Clsal-sbdt	13.42 (s, 1 H)	10.51 (s, 1 H)	8.49 (s, 1 H)	4.51 (s, 2 H)	6.93 (d, 1 H), 7.33 (m, 4 H), 7.42 (d, 2 H) 7.61 (d, 1 H)
[K(H_2O)$_2$][VO_2(Clsal-sbdt)] (**2**)			9.09 (s, 1 H)	4.41 (s, 2 H)	6.86 (d, 1 H), 7.44 (m, 6 H), 7.79 (d, 1 H)
H_2Brsal-sbdt	13.45 (s, 1 H)	10.55 (s, 1 H)	8.47 (s, 1 H)	4.50 (s, 2 H)	6.86 (d, 1 H), 7.30 (m, 3 H), 7.41 (m, 3 H), 7.73 (d, 1 H)
[K(H_2O)$_2$][VO_2(Brsal-sbdt)] (**3**)			9.09 (s, 1 H)	4.41 (s, 2 H)	6.82 (d, 1 H), 7.33 (m, 3 H), 7.47 (m, 3 H), 7.91 (d, 1 H)

(C7), the carbon atom bearing the phenolate oxygen (C1) and the enethiolate sulfur (C8), thus confirming the coordination mode deduced from the IR and ^{1}H NMR spectra. Other signals appear at nearly identical positions in the free and complexed ligands (for numbering scheme see Scheme 2).



Scheme 2

The ^{51}V NMR spectra exhibit one strong resonance between δ = −465.9 and −468.0 ppm, well within the range of dioxovanadium(v) complexes where a soft thio functionality participates in coordination in addition to the *O* and *N* atoms.[19] The brown solid isolated by using only one equivalent of KOH during the synthesis of **1** gave a signal at δ = −404.6 ppm in addition to that of authentic **1** (δ = −468.0 ppm). Of the two resonances, the less intense resonance at δ = −404.6 ppm is close to the one reported for [VO(OEt)(sal-sbdt)] (δ = −399 ppm).[16] This further supports the proposed pathway for the formation of **1** through [VO(OMe)(sal-sbdt)] as shown by Equations (1) to (3).

Reactivity of 2 Towards HCl

Addition of methanol saturated with HCl gas to [K(H_2O)$_2$][VO_2(Clsal-sbdt)] (**2**) dissolved in MeOH leads to a darkening of the solution and a progressive drop in the intensities of the 400 and 295 nm bands. As the methoxo complex [VO(OMe)(sal-smdt)] (H_2sal-smdt is the Schiff base from salicylaldehyde and *S*-methyldithiocarbazate) exhibits very similar electronic absorptions (400, 307, and 238 nm),[20] the dioxo complex **2** apparently slowly changes

Table 3. ^{13}C NMR spectroscopic data

Compound[a]	C1[b]	C6	C7[b]	C8[b]	C9	C10	C2 − C5/C11 − C15
H_2sal-sbdt	157.3	119.1	144.9	195.5	37.7	136.8	116.4, 119.7, 127.3, 127.4 128.5, 129.3, 132.3
[K(H_2O)][VO_2(sal-sbdt)] (**1**)	165.0, (7.7)	119.0	159.3, (14.4)	173.6, (−21.9)	36.3	137.7	117.6, 119.6, 127.0, 128.3, 129.0, 133.6
H_2Clsal-sbdt	156.0	121.0	142.6	196.2	37.6	136.9	118.3, 123.4, 125.4, 127.3, 128.5, 129.3, 131.6
[K(H_2O)$_2$][VO_2(Clsal-sbdt)] (**2**)	161.9, (5.9)	120.5	156.5, (13.9)	173.2, (−23.0)	34.6	135.9	119.0, 119.4, 125.3, 126.6, 127.3, 130.1, 131.3
H_2Brsal-sbdt	156.5	121.6	142.5	196.1	37.6	136.9	110.9, 118.7, 127.3, 128.3, 128.5, 129.3, 134.4
[K(H_2O)$_2$][VO_2(Brsal-sbdt)] (**3**)	163.8, (7.3)	121.3	158.0, (15.5)	174.6, (−21.5)	36.0	137.4	107.7, 126.7, 128.1, 128.8, 134.4, 135.5

[a] Numbering scheme see Scheme 2. [b] Coordination shifts are given in parentheses

to the corresponding methoxo complex. An increase of the amount of added HCl causes the disappearance of the 400 nm band, accompanied by a broadening and finally splitting of the 295 nm band into two components (310 and 282 nm); Figure 1. On the basis of ^{1}H and ^{51}V NMR spectroscopic studies, it has been suggested that there is an equilibrium between [VO(OEt)(sal-sbdt)] and [VO(OH)(sal-sbdt)] in $CDCl_3$.[16] A similar equilibrium may also be considered here, which should move towards the formation of [VO(OH)(Clsal-sbdt)] with the addition of more HCl. An oxo-hydroxo species has been generated on acidification of $K[VO_2(sal\text{-}inh)H_2O]$ (where H_2sal-inh is *N*-isonicotinamido-2-hydroxyphenylimine),[21] and even of the dimeric dioxo complex $[VO_2L]_2$ {where H_2L = *N*-[(*o*-hydroxyphenyl)methyl]-*N*′-(2-hydroxyethyl)ethylenediamine} in solution.[22] An oxo-hydroxo complex, viz. $[VO(OH)Tp(H_2O)]$ [Tp = *tris*(3,5-diisopropyl-1-pyrazolyl)borate] has recently been structurally characterised.[23] On allowing the above solution to stand overnight, or on addition of KOH dissolved in methanol, the solution acquires the original pattern, i.e. the reaction is reversible and gives the spectrum of **2**. The reversibility of such a reaction is an important observation in the context of the vanadate-dependent haloperoxidases, for which a hydroxo group in an apical position has been proposed on the basis of X-ray diffraction analysis.[24]

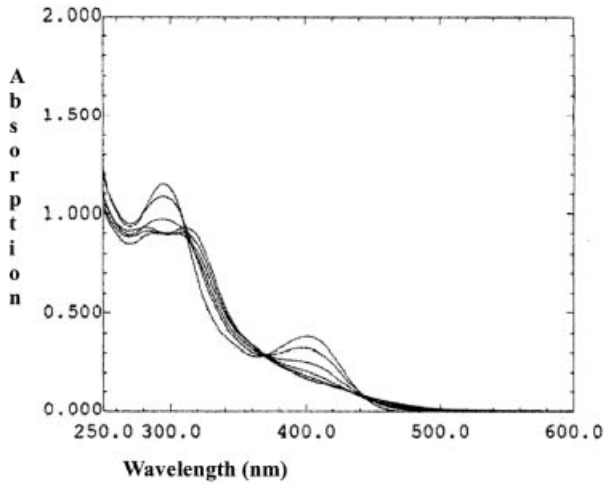


Figure 1. Electronic absorption spectra of the titration of compound **2** (maxima at 400 and 295 nm) with methanolic HCl, showing the formation of [VO(OH)(Clsal-sbdt)] (new bands at 310 and 282 nm)

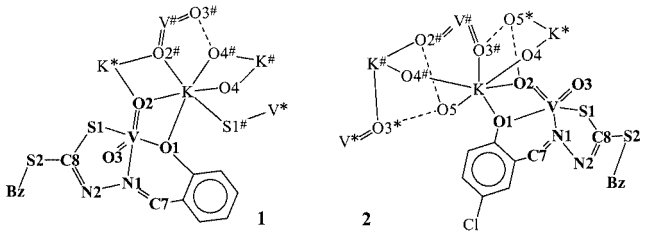


Figure 2. Schematic representations of the coordination environments of vanadium (bold) and potassium, and numbering schemes of **1** and **2**. O3 is the apical oxo group, O4 and O5 are water molecules; Bz = benzyl. # and * refer to atoms not belonging to the formula unit.

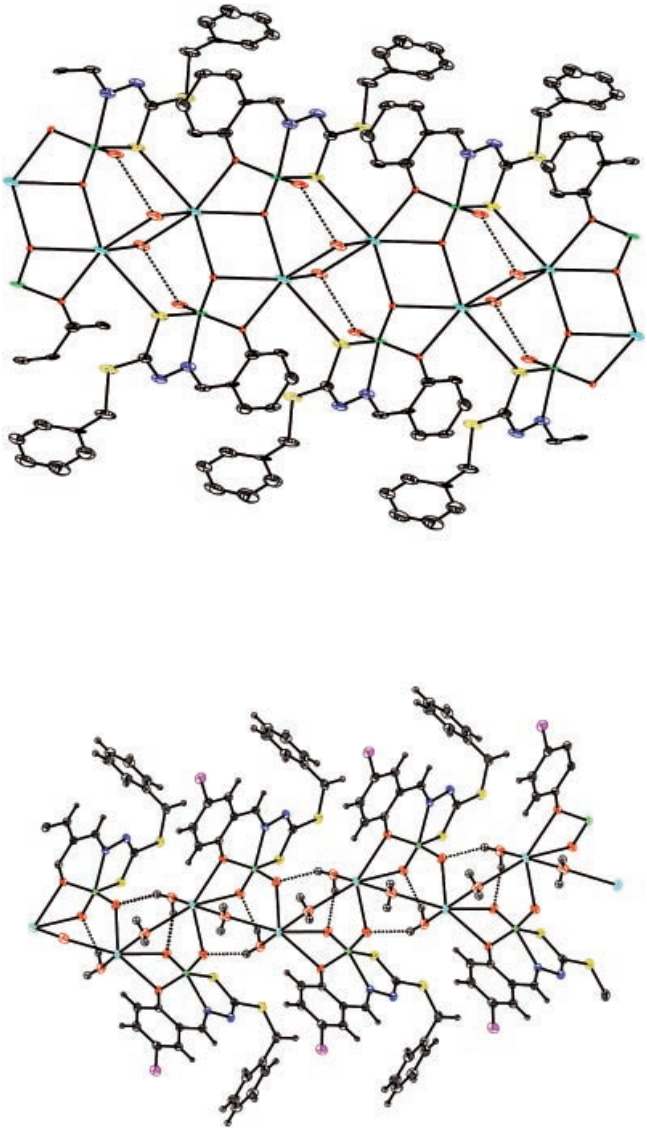


Figure 3. ORTEP drawings (50% probability level), representing sections of the supramolecular arrangements in **1** and **2**. Colour code: V (green), K (light blue), O (red), N (dark blue), S (yellow).

Description of the Structures

Schematic drawings of the coordination environments of vanadium and potassium in **1** and **2** are shown in Figure 2, ORTEP representations of the networks interlinking anions and cations in Figure 3. Table 4 provides a synopsis of selected structure parameters.

In compound **1**, vanadium is in a square pyramidal environment with one of the oxo groups (O3) in the apex and the second oxo group (O2) plus three atoms of the tridentate ligand (O1, N1, S1) forming the plane. Vanadium is 0.0953 Å above this plane. The distance to the nitrogen *trans* to O2, *d*(V−N1), is somewhat elongated with respect to the average V−N(azomethine) bond lengths.[17,25] While

Table 4. Selected bond lengths [Å] and angles [°] for **1** and **2** (#: refers to the coordination sphere of an adjacent cation or anion)

	$[K(H_2O)]$-$[VO_2(sal\text{-}sbdt)]$, **1**	$[K(H_2O)_2]$-$[VO_2(Clsal\text{-}sbdt)]$, **2**
V−O	11.881(6)	1.9040(10)
V−O	21.660(6)	1.6598(11)
V−O	31.606(6)	1.6223(11)
V−N	12.205(7)	2.1791(12)
V−S	12.370(3)	2.3716(4)
N1−N	21.405(9)	1.4046(16)
S1−C	81.783(8)	1.7370(15)
N2−C	81.251(10)	1.2923(18)
K−O	12.806(6)	2.7789(11)
K−O	22.769(6)	2.7935(12)
K−O2#	2.7586(6)	
K−O3#		2.7469(12)
K−O4	2.765(6)	2.8243(14)
K−O4#	2.751(6)	2.8342(14)
K−O5		2.7488(17)
K···S1#	3.467(3)	
O3···O4	2.887	
O2···O5		2.867
O3···O5		2.861
O1−V−S	1141.4(2)	143.04(4)
O1−V−O2	97.3(3)	96.36(5)
O1−V−N1	82.2(3)	83.23(4)
O2−V−S1	87.0(2)	87.36(4)
O2−V−N1	152.1(3)	151.91(5)
N1−V−S1	76.9(2)	76.98(3)
O3−V−S1	107.1(2)	104.67(4)
O3−V−O1	107.6(3)	109.04(5)
O3−V−O2	108.9(3)	107.68(6)
O3−V−N	197.7(3)	98.86(5)

the apical V=O bond, d(V−O3) = 1.606(6) Å is in the expected range, the basal d(V−O2) = 1.660(6) Å is widened as a consequence of covalent bonding contacts to two K^+ ions. Each potassium ion is thus coordinated to two basal oxo groups (O2, and O2# of a second vanadium centre), the phenolate-O (O1), and to two bridging water molecules (O4 and O4#). In addition, there is a weak bond of 3.467 Å to the S1 of the thiolate group (sum of covalent radii: 2.98, sum of van der Waals radii: 4.60 Å). The potassium ions form an endless zigzag chain via alternate $\{K(\mu\text{-}H_2O)\}_2^{2+}$ and $\{K(\mu\text{-}OVO)\}_2$ links. This chain is "layered" by the ligand fragments of the vanadate anion, with the benzyl rings attached to S2 forming the outer sphere. Adjacent anions in these layers are connected to each other through O2/O1−K−S1 links. Connection of two anions *across* the chain occurs through $\{K(\mu\text{-}VO_2)\}_2$ links; the apical oxo groups of the two $[VO_2(sal\text{-}sbdt)]^-$ anions oppose each other.

On the molecular level, the structure of **2** compares to that of **1**. In **2**, both the basal (O2) *and* the apical (O3) oxo groups of the VO_2 moiety are involved in binding to the K^+ ion. The resulting elongation of d(V−O3) = 1.622(1) is, however, less pronounced than that of d(V−O2) = 1.660(1) Å. The coordination environment of the counterion of **2** differs from that in **1** in that an additional − terminal − water molecule replaces the thiolate group. On the supra-molecular level, anions and cations again arrange so as to form a band structure with a central $\{K(H_2O)(\mu\text{-}H_2O)\}_n$ zigzag chain and an envelope formed by the ligands of the complex anion, with the hydrophobic benzyl fragments exposed to the outside. Additional linkages for the K^+ ion along the chain are bridging VO_2^+ moieties. Coordination of the K^+ ion to the phenolate-O group (O1), and H-bonds formed between both oxo groups (O2 and O3) and terminal H_2O molecule (O5) at the potassium ion provide interconnections between the anions *across* the chain.

Biological Activity

A quantification of drug action was obtained by determining the approximate concentration causing a 50% reduction in the number of cells of *E. histolytica* relative to a control. TYIS-33 medium was used as a control to estimate 100% live confluence of amoeba cells. The inhibition concentration (IC_{50}) of the ligands (H_2sal-sbdt**,** H_2Clsal-sbdt and H_2Brsal-sbdt, c.f. Scheme 1) and their complexes (**1**, **2**, and **3**) are indexed in Table 5. All the compounds screened for anti-amoebic activity in vitro had IC_{50} values of clearly less than 10 μM. IC_{50} value < 20 μM are usually considered significant in amoebicidal action.[26] The high activity of compounds **1**, **2**, and **3**, surmounting that of the ligands, showed that complexation of the organic ligands to vanadium substantially enhances the activity. This behaviour, which is particularly pronounced for H_2Brsal-sbdt (IC_{50} = 4.2) vs. complex **3** (1.35 μM), may be explained by Tweedy's theory,[27] according to which chelation reduces the polarity of the ligand due to partial sharing of its negative charge with the metal, favouring transportation of the complexes across the lipid layer of the cell membrane. In an earlier communication,[15] we had introduced dioxovanadium complexes with bidentate *ON* ligands, showing activity in the range of 2.4−9.6 μM as compared to metronidazole (IC_{50} 2.1 μM). The use of ligands containing a sulfur functionality, as in the case of **1**, **2**, and **3**, apparently improves the activity.

Table 5. In vitro screening for amoebicidal activity of the ligands and their vanadium complexes against an HM1:1MSS strain of *E. histolytica*

Compound	IC_{50} (μM)	S.D.[a]
H_2sal-sbdt	5.19	0.95
$[K(H_2O)][VO_2(sal\text{-}sbdt)]$ **1**	2.67	0.52
H_2Clsal-sbdt	3.57	0.57
$[K(H_2O)_2][VO_2(5\text{-}Clsal\text{-}sbdt)]$ **2**	2.23	0.43
H_2Brsal-sbdt	4.19	0.78
$[K(H_2O)_2][VO_2(5\text{-}Brsal\text{-}sbdt)]$ **3**	1.35	0.26
Metronidazole	2.05	0.31

[a] Standard deviation.

A similar observation holds for Pd^{II}, Pt^{II}, and Ru^{II} complexes of *SN* donor ligands derived from *S*-alkyldithiocarbazate,[11,12,28] as compared to the corresponding complexes with *ON* ligands, suggesting that sulfur attains a general

role in activity enhancement. This can be compared with the natural product allicin, a sulfinothioc acid [−S(O)−S−] derivative isolated from garlic (*Allium sativum*),[29] which is very active as an antimicrobial agent, possibly due to its specific interference with sulfhydryl enzymes. An additional important feature in these biologically active metal complexes may be imparted by the tridentate ligand system.[30] The binding of the chelated metals to the nitrogen bases of DNA and RNA, and the inhibition of DNA synthesis through the blockage of the enzyme ribonucleotide diphosphate reductase (RBD) have been suggested as possible mechanisms of action.[30,31] What is evident at this stage, however, is that the general mechanisms of operation of these compounds and their complexes, whether in their action as cytotoxic,[30] antibacterial or antifungal agents,[32−34] is still eluding researchers.

Conclusion

Three new anionic dioxovanadium complexes containing an *ONS* ligand set have been prepared, where *O* is a phenolate, *N* an imine, and *S* a thiolate function from a ligand system obtained by condensation of salicylaldehyde and dithiocarbazate. The dianionic ligand coordinates out of its enethiolate tautomeric form. The potassium counterion is in a coordination environment provided by six *O* atoms (**1**) or five *O* atoms [plus a weak K···S(thiolate) bond] (**2**), from water, and phenolate plus the doubly bonded oxo groups on vanadium. The aquapotassium ions form zigzag chains interconnecting adjacent complex anions. The complexes exhibit anti-amoebal in vitro activity towards *Entamoeba histolytica* similar to or, in the case of **3**, better than metronidazole, a medication commonly used in amoebobiasis

Experimental Section

Materials and Measurements: V_2O_5 (Loba Chemie, India), benzyl chloride (Spectrochem, India), salicylaldehyde (E. Merck, India) and acetylacetone (Aldrich, U.S.A.) were used as obtained. 5-Chlorosalicylaldehyde,[35] *S*-benzyldithiocarbazate,[36] and [VO(acac)$_2$] [acac = acetylacetonate(1−)][37] were prepared according to methods reported in the literature. The ligands H_2sal-sbdt and H_2Brsal-sbdt were prepared as described.[38]

The Central Drug Research Institute, Lucknow, India, carried out elemental analyses. ^{1}H NMR spectra were obtained with a Bruker 200 MHz spectrometer, ^{13}C and ^{51}V NMR spectra with a Bruker AM-360 spectrometer at 90.56 and 94.73 MHz, respectively, in [D$_6$]DMSO with the usual parameter settings. All ^{51}V NMR spectroscopic values are referenced relative to $VOCl_3$ as external standard. UV/Vis spectra were recorded in acetonitrile and methanol on a UV-1601 PC UV/Visible spectrophotometer, IR spectra as KBr pellets using a Perkin−Elmer model 1600 FT-IR spectrometer. The Scientific Instrumentation Centre of the Indian Institute of Technology, Roorkee, carried out thermogravimetric analyses, in static air.

Crystal Structure Determinations: Data were collected on a Bruker SMART Apex CCD diffractometer using a graphite monochromator and Mo-K_α radiation (λ = 0.71073 Å) at 153(2) K. Hydrogen atoms were placed into calculated positions and included in the last cycles of refinement. Crystal data and details of the data collection and refinement are collated in Table 6. The program systems SHELXS 86 and SHELXL 93 were used throughout.[39]

CCDC-198451 (**1**) and CCDC-198450 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

The morphology of the crystals of **1** (thin plates) did not allow for a high quality data set ($R1$ = 0.068).

In vitro Tests Against *E. histolytica*: The ligands and their metal complexes were screened in vitro for antiamoebic activity against a (HM1:1MSS) strain of *Entamoeba histolytica* by the microdilution method.[40] *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously[41] in wells of 96-well microtiter plates (Costar). ca. 1 mg of the compounds subjected to the tests were dissolved in DMSO (40 μL), and culture medium was added so as to obtain a concentration of 1 mg/mL (corresponding to ca. 2.8 mM for the ligands and 2.2 mM for the complexes). Dissolution was facilitated by mild sonication in a sonicleaner bath for a few minutes. As evidenced by a comparison of UV/Vis spectra of the complexes dissolved in DMSO on the one hand, and DMSO + TYIS-23 medium on the other hand, the complexes preserve their integrity under biological conditions. Stock solutions were prepared by further dilution of the culture medium to 0.1 mg/mL. Under these conditions, the complexes are stable and no inhibition of amoeba occurs.[42,43] Twofold serial dilutions were made in wells of 96-well microliter plates in 170 μL of medium. Each test included metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae), and a blank (culture medium only). The control wells were prepared from a confluent culture by pouring off the medium, adding 2 mL of fresh medium and chilling the culture on ice to detach the organisms from the flask wall. The number of amoeba per mL was estimated with a haematocytometer, and trypan blue exclusion was used to confirm viability. Fresh culture medium was added to dilute the suspension to 10^5 organism/mL, and 170 μL of this suspension was added to the test and control wells so that the wells were completely filled (total volume 340 μL) An inoculum of $1.7 \cdot 10^4$ organisms/well was chosen in order to ascertain confluent − but not excessive − growth in the control wells. The plates were sealed with expanded polystyrene, secured with tape, placed in a modular incubation chamber and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h.

After incubation, the growth of amoebae in the plates was checked with a low power microscope. The culture medium was removed by inverting the plate and shaking gently. The plate was then immediately washed once in sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly, and the plates were not allowed to cool in order to prevent the detachment of amoebae. After drying at room temperature, the amoebae were fixed with methanol and, when dry, stained with 0.5% aqueous eosin for 15 min. The stained plates were washed once with tap water and then twice with distilled water and allowed to dry. A 200 μL portion of a 0.1 M sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader. The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells, and plotted against the logarithm of the dose of the drug tested. Linear

Table 6. Crystal data and structure refinement for $[K(H_2O)][VO_2(sal\text{-}sbdt)]$ (**1**) and $[K(H_2O)_2][VO_2(Clsal\text{-}sbdt)]$ (**2**)

Empirical formula	$C_{15}H_{14}KN_2O_4S_2V$	$C_{15}H_{15}KN_2O_5S_2ClV$
Formula mass [g mol^{-1}]	440.4	492.9
Crystal system, space group	monoclinic, $P2_1/c$	monoclinic, $P2_1/c$
Unit cell dimensions	17.282(12)	6.2843(2)
a [Å]	7.309(5)	37.3447(11)
b [Å]	14.162(10)	8.7230(3)
c [Å]	94.933(12)	107.2450(10)
β [°]	1782(2)	1955.13(11)
Cell volume [Å^3]	4	4
Z	1.642	1.675
Calculated density [g cm^{-3}]	1.047	1.100
Absorption coefficient [mm^{-1}]	896	1000
F(000)	0.40 × 0.15 × 0.10	0.8 × 0.5 × 0.3
Crystal size [mm]	2.37 to 25.05	1.09 to 32.57
θ range for data collection [°]	$-13 \leq h \leq 20, -8 \leq k \leq 8,$	$-8 \leq h \leq 9, -31 \leq k \leq 56,$
Limiting indices	$-16 \leq l \leq 16$	$-13 \leq l \leq 12$
Reflections collected/unique	9280/3133	28486/7088
R(int)	0.2204	0.0286
Parameters refined	216	260
Goodness of fit on F^2	0.786	1.174
R indices, $[I > 2\sigma(I_0)]$: $R1$, $wR2$	0.0685, 0.1445	0.0359, 0.0947
R indices (all data): $R1$, $wR2$	0.1654, 0.1741	0.0408, 0.1010
Largest diff. peak/hole [e·Å^{-3}]	0.727/−0.710	0.605/−0.544

regression analysis was used to obtain the best-fitting straight line from which the IC_{50} value was determined.

Preparation of Complexes: Spectroscopic data are collated in Table 1 (IR and UV/Vis), Table 2 (^{1}H NMR), and Table 3 (^{13}C NMR) in the Results and Discussion section.

$[K(H_2O)][VO_2(sal\text{-}sbdt)]$ (1): H_2sal-sbdt (0.604 g, 2 mmol) was dissolved in methanol (30 mL) containing KOH (0.056 g, 1 mmol), and $[VO(acac)_2]$ (0.53 g, 2 mmol) was added to this solution. The reaction mixture was refluxed on a water bath under aerobic condition for 6 h. After cooling to room temperature, 1 mmol of additional KOH was added, and the solution was kept for 2 days at ambient temperature to allow for slow evaporation of the solvent. The yellow precipitate thus obtained was filtered, washed with cold methanol and finally recrystallised from methanol to afford 0.40 g (45%) of crystalline complex **1**. $C_{15}H_{14}KN_2O_4S_2V$ (440 g·mol^{-1}): calcd. C 40.91, H 3.18, N 6.36; found C 40.89, H 3.23, N 6.42. ^{51}V NMR ([D$_6$]DMSO): δ = −468.0 ppm.

$[K(H_2O)_2][VO_2(Clsal\text{-}sbdt)]$ (2), and $[K(H_2O)_2][VO_2(Brsal\text{-}sbdt)]$ (3): Complexes **2** (0.439 g, 50% yield) and **3** (0.45 g, 42% yield) were prepared analogously to **1**, replacing H_2sal-sbdt by H_2Clsal-sbdt or H_2Brsal-sbdt. Both complexes were also recrystallised from methanol. Data for **2**: $C_{15}H_{15}ClKN_2O_5S_2V$ (492.5 g·mol^{-1}): calcd. C 36.55, H 3.05, N 5.69; found C 36.67, H 3.11, N 5.53. ^{51}V NMR: δ = −467.3 ppm. Data for **3**: $C_{15}H_{15}BrKN_2O_5S_2$ (537 g·mol^{-1}): calcd. C 33.52, H 2.79, N 5.21; found C 33.38, H 2.83, N 5.16. ^{51}V NMR: δ = −465.9 ppm.

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