The ligational behavior of an isatinic quinolyl hydrazone towards copper(II)- ions

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Abstract

Background: The importance of the isatinic quinolyl hydrazones arises from incorporating the quinoline ring with the indole ring. Quinoline ring has therapeutic and biological activities whereas, the indole ring occurs in Jasmine flowers and Orange blossoms. As a ligand, the isatin moiety is potentially ambidentate and can coordinate the metal ions either through its lactam or lactim forms. In a previous study, the ligational behavior of a phenolic quinolyl hydrazone towards copper(II)- ions has been studied. As continuation of our interest, the present study is planned to check the ligational behavior of an isatinic quinolyl hydrazone.

Results: New homo- and heteroleptic copper(II)- complexes were obtained from the reaction of an isatinic quinolyl hydrazone (HL) with several copper(II)- salts viz. Cl\textsuperscript{-}, Br\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, ClO\textsubscript{4}\textsuperscript{-}, SO\textsubscript{4}\textsuperscript{2-} and AcO-. The obtained complexes have Oh, T\textsubscript{d} and D\textsubscript{4h}- symmetry and fulfill the strong coordinating ability of Cl\textsuperscript{-}, Br\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-} and SO\textsubscript{4}\textsubscript{2-} anions. Depending on the type of the anion, the ligand coordinates the copper(II)- ions either through its lactam (NO\textsubscript{3}\textsuperscript{-} and ClO\textsubscript{4}\textsuperscript{-}) or lactim (the others) forms.

Conclusion: The effect of anion for the same metal ion is obvious from either the geometry of the isolated complexes (Oh, T\textsubscript{d} and D\textsubscript{4h}) or the various modes of bonding. Also, the obtained complexes fulfill the strong coordinating ability of Cl\textsuperscript{-}, Br\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-} and SO\textsubscript{4}\textsubscript{2-} anions in consistency with the donor ability of the anions. In case of copper(II)- acetate, a unique homoleptic complex (5) was obtained in which the AcO- anion acts as a base enough to quantitatively deprotonate the hydrazone. The isatinic hydrazone uses its lactim form in most complexes.

Background

Hydrazones and their metal complexes present numerous pharmaceutical applications as antimicrobial, anticonvulsant, analgesic, anti-inflammatory, anti-tubercular and antitumor agents [1-3]. Also, the metal complexes of hydrazones have potential applications as catalysts [4], luminescent probes [5] and molecular sensors [6]. The importance of the isatinic quinolyl hydrazones arises from incorporating the quinoline ring with the indole ring. Quinoline ring has therapeutic and biological activities whereas, the indole ring occurs in Jasmine flowers and Orange blossoms[1-3]. As a ligand, the isatin moiety is potentially ambidentate and can coordinate the metal ions either through the lactam or lactim forms. In general, this study is planned to investigate the ligational behavior of the studied hydrazone (Scheme 1); 3-[2-(4,8-dimethylquinolin-2-yl)hydrazono] indolin-2-one (HL) towards several copper(II)- salts (Cl\textsuperscript{-}, Br\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, ClO\textsubscript{4}\textsuperscript{-}, SO\textsubscript{4}\textsubscript{2-} and AcO\textsuperscript{-}). This work is an extension to our previous studies on the chelating ability of some quinolyl hydrazones [7-11].Scheme 1 Tautomeric forms of the isatinic hydrazone.

Results and discussion

Characterization of the hydrazone

The investigated hydrazone (HL) was prepared by a condensation reaction of 2-hydrazinyl-4,8-dimethylquinoline with isatin (indol-2,3-dione). The results of elemental analysis (Table 1) are in good harmony with the proposed formula. The IR spectrum (Table 2) showed very strong bands at 3203 and 1605 cm\textsuperscript{-1} which are assigned to ν(NH) and ν(C=N), respectively. The lactam nature of HL was supported by a very strong band at 1706 cm\textsuperscript{-1}; ν(C=O). On the other hand, the electronic absorption spectra of the hydrazone in DMF exhibit two intense bands at 268 and 391 nm characteristic for π-π* and charge transfer (CT) transitions. The latter band
Characterization of the isatinic complexes

The isatinic hydrazone (HL) has mainly two tautomeric forms (Scheme 1). Both rearrangements; the lactam-lactim forms are useful in explaining the different coordinating properties. The ligand was allowed to react with several copper(II)- salts viz. Cl⁻, Br⁻, NO₃⁻, SO₄²⁻, ClO₄⁻ and AcO⁻ in order to determine the effect of the anions on the formed products (Scheme 3). The obtained copper(II)- complexes reflect the strong coordinating power of SO₄²⁻, Cl⁻, Br⁻ and NO₃⁻ as compared to the weakly or non coordinating power of ClO₄⁻. This is consistent with the donor ability of the anions [12-14]; (DNₓ = 36.2, 33.7, 21.1 and 8.44 for Cl⁻, Br⁻, NO₃⁻ and ClO₄⁻, respectively). All complexes are mononuclear except complex 6 which is binuclear. The obtained complexes have the octahedral (O₆), tetrahedral (T₄) and square planar (D₄h) geometry; (Scheme 3) and reflect the non participation of the heterocyclic N- atom of the quinoline ring in the chelation. The isatinic hydrazone (HL) behaves as monoanionic or neutral NO⁻ donor which is consistent with steric properties of the more rigid ligand. Characterization of the obtained complexes was achieved via elemental and thermal analyses, magnetic and conductivity measurements as well as spectral studies.Scheme 3

Effect of anion on the copper(II) - isatinic complexes.

IR spectra of the complexes

The mode of bonding was studied by comparing the IR spectral bands of the metal complexes with those of the free ligand (Table 2). Inspection of the data revealed the following: (i) All complexes showed a broad band in the range 3463-3203 cm⁻¹ due to ν(OH) of the associated water or methanol molecules. (ii) The band at 1605 cm⁻¹ assignable to ν(C=N) in the free ligand was shifted to higher values indicating the participation of C=N of the hydrazone moiety in the chelation with π- electron

### Table 1 Analytical and physical data of the copper(II)- isatinic complexes.

| No. | Reactants (HL + metal salt) | Complex (F.W.) | Color | % Yield | m.p. (°C) | Elemental Analysis; % Found/(Calcd.) |
|-----|----------------------------|----------------|-------|---------|-----------|-------------------------------------|
|     |                            |                |       |         |           | C   H   N   M                      |
| 1   | Cu (ClO₄)₂.6H₂O           | [Cu (LH₂).H₂O] ClO₄.3H₂O | Red   | 60>     | 7.06      | 5.17  17.70                     |
| 2   | Cu (NO₃)₂.21/2H₂O         | [Cu (LH₂).NO₃]Cl₂.H₂O | Yellowish brown | 61> | 51.54  4.63  12.69  7.25  7.18 |
| 3   | CuCl₂.2H₂O                | [Cu (LH₂).Cl]Cl.2H₂O | Dark green | 30> | 54.10  4.12  16.44  7.62  7.54 |
| 4   | CuBr₂                     | [Cu (LH₂).Br]Br | Granulated greenish brown | 60> | 43.92  4.15  10.81  12.40         |
| 5   | Cu (OAc)₂.3H₂O           | [Cu (LH₂).OAc] | Bright greenish brown | 82> | 65.47  4.51  15.93  8.90  9.07 |
| 6   | CuSO₄.5H₂O               | [Cu₂ (LH₂).SO₄] | Dark orange | 64> | 47.12  4.69  11.13  12.70         |

### Table 2 Magnetic, conductivity, electronic and IR spectral data of the copper(II)- isatinic complexes.

| Complex | λ (nm) | µ eff B.M. | Conductance Ω⁻¹ cm⁻¹ mol⁻¹ | v (NH) | v (C = O) | v (C = N) | Others |
|---------|--------|------------|-----------------------------|--------|-----------|-----------|--------|
| HL      | 268, 391 | ——        | ——                          | 3203   | 1706      | 1605      | ——     |
| 1       | 396    | 1.80      | 95.0                        | 3287   | 1653      | 1627      | 1100; v(ClO₄) |
| 2       | 396    | 1.93      | 12.0                        | 3463   | 1692      | 1623      | 1387 and 1297; v(NO) |
| 3       | 391    | 1.87      | 37.0                        | 3235 br.| ——        | 1619      | ——     |
| 4       | 392    | 1.82      | 40.0                        | 3203 br.| ——        | 1621      | ——     |
| 5       | 453    | 1.77      | Zero                        | 3363   | ——        | 1608      | ——     |
| 6       | 450    | 1.40      | 15.0                        | 3273   | ——        | 1633      | 1135; v(50) |
delocalization. (iii) In most complexes, the band located at 1706 cm\(^{-1}\) due to \(\nu(C=O)\) of the free ligand disappeared indicating the participation of the lactim- form in the chelation. In contrast, the lactam- form participates in the chelation in case of the nitrato (2) and perchlorato (1) complexes as indicated by the shift of the above band to lower wave numbers; 1692 and 1653 cm\(^{-1}\), respectively. However, the greater lower value 1653 cm\(^{-1}\) for the perchlorate complex (1) may suggest mixed modes of bonding (lactim + lactam) [7]; Scheme 3. (iv) In complex 1, the strong broad band centered at 1100 cm\(^{-1}\) (antisymmetric stretch) and the sharp band at 621 cm\(^{-1}\) (antisymmetric bend) suggest uncoordinated ClO\(_4^-\) anion [14]. (v) For the binuclear sulfato complex (6), the chelating bidentate nature of the SO\(_4^{2-}\) group is indicated by the appearance of \(\nu_3(S-O)\) strong band at 1135 cm\(^{-1}\) characteristic for the high symmetry T\(_d\) (tetrahedral) point group. The nitrate complex (2) showed two bands at 1387 and 1297 cm\(^{-1}\) confirming the monodentate nature of the coordinated NO\(_3^-\) group; C\(_{2v}\) symmetry. (vi) Finally, the detection of the non ligand bands; \(\nu(M-O)\) and \(\nu(M-N)\) in the fingerprint region is more difficult and tentative.

**Conductivity and magnetic properties**

The recorded conductance for 10\(^{-3}\) molar DMF solutions of the complexes (Table 2) indicates that all complexes are non-conducting due to their neutrality (\(\Lambda = 17.0-1.9 \ \Omega^{-1} \ cm^2 \ mol^{-1}\)). The only exception is [Cu(L)(HL)(H\(_2\)O)\(_2\)]ClO\(_4\)3H\(_2\)O (1) which showed molar conductance of 95 \(\Omega^{-1} \ cm^2 \ mol^{-1}\), indicating its 1:1 electrolytic nature which is consistent with the IR spectra; \(\nu_3\) (Cl-O) at 1100 cm\(^{-1}\). In contrast, the halo-complexes (3 and 4) showed molar conductance of 37 and 40 \(\Omega^{-1} \ cm^2 \ mol^{-1}\), respectively indicating their partial electrolytic nature which is due to the replacement of the coordinated Cl\(^-\) and Br\(^-\) ions by DMF solvent molecules [4]. On the other side, the effective magnetic moments (\(\mu_{\text{eff}}\))
of the copper(II)- complexes (1-5) lie in the range 1.93-1.77 B.M. (Table 2) which is consistent with one unpaired electron and falls within the range reported for mononuclear copper(II)- complexes. However, the binuclear copper(II)- complex (6) exhibits lower $\mu_{\text{eff}}$ value at 1.4 B.M. indicating some metal—metal interaction.

**Mass and electronic spectra**

The mass spectra of complexes 3; [Cu (L) (H$_2$O) Cl].1/2 H$_2$O.½ MeOH (445.38) and 4; [Cu (L) (H$_2$O)$_3$ Br].½ H$_2$O (519.60) as representative examples showed their molecular ion and base peaks at m/e (447 and 156) and (519 and 45), respectively, confirming their suggested structures. On the other side, the electronic spectra of the complexes as DMF solutions showed a new intense broad band in the range 391-453 nm confirming its charge transfer (CT) nature (Figure 2 and Table 2). Therefore, the type of the d-d transitions can not be identified due to the strong CT bands tailing from UV region to the visible region. In general, the color of all complexes is dominated by the CT transition which obscured the weak d-d transition occurring in the same region; a phenomenon encountered with isatinic complexes [4]. Also, the electronic spectra of all complexes are nearly similar in terms of the position, intensity and shape of the bands (Figure 2 and Table 2).

**Electron spin resonance spectroscopy**

The ESR spectra of [Cu(L)(HL)(H$_2$O)$_2$]ClO$_4$.3H$_2$O (1) and [Cu (L)$_2$].½ H$_2$O.½ MeOH (5); Figure 3 are largely different, however, the ESR spectral profile of complex 5 confirms its Td geometry. Generally, ESR spectra are useful for distinguishing square planar and tetrahedral geometries. The ESR spectral parameters were used to evaluate the bonding parameters $\alpha^2$ and $\beta^2$ which may be regarded as a measure of the in-plane $\sigma$- bonding and the in-plane $\pi$- bonding, respectively. The following simplified expressions were used to evaluate the bonding parameters [15,16]:

$$\alpha^2 = \left[ \frac{E(g_{||} - g_e)}{8\nu \beta^2} \right]$$

and

$$\beta^2 = \left[ \frac{E(g_{\perp} - g_e)}{2\nu \gamma^2} \right]$$

where $E$ is the transition energy, $\nu$ represents the electron spin-orbit coupling and $\gamma^2$ represents the out-of-plane $\pi$-bonding and was taken as a constant value. On the other hand, the super-exchange splitting constant $A_{11}$ was evaluated from the following expression:

$$\alpha^2 = \left( \frac{A_{11}}{P} \right) + (g_{||} - g_\perp) + \frac{3}{7}(g_\perp - g_e) + 0.04$$

where $P$ is the free ion dipolar term and its value depends on the type of the metal ion. Also, the $f$- factor ($f = g_{||}/A_{11}$) was evaluated to determine the extent of tetragonal distortion. The obtained ESR parameters are shown in Table 3.
Figure 3 The X-band ESR spectra of complexes 1 (Oh) and 5 (Td).

Table 3 ESR parameters

| Complex | $g_{11}$ | $g_{2}$ | $<g>$ | $G$ | $A^2$ | $\beta^2$ | $K^2_H$ | $(A_{11}) \text{cm}^{-1}$ | $f$ |
|---------|---------|---------|-------|-----|-------|----------|---------|-----------------|-----|
| (1); Oh | 2.174   | 2.076   | 2.109 | 2.033| 0.582 | 0.534    | 0.311   | $154 \times 10^{-4}$ | 141 |
| (5); Td | 2.187   | 2.052   | 2.097 | 3.716| 0.93  | 0.36     | 0.335   | $61 \times 10^{-4}$  | 362 |
In summary, the $T_d$- complex (5) with the lactim mode showed either higher values of $G$, $\alpha^2$, $K_{\Pi}^0$ and $f$ or lower values of $\beta^2$ and $A_1$ as compared to the $O_h$ complex (1) with the mixed mode (lactam + lactim) which is consistent with the greater discrepancy of the observed spectra.

**Thermal (TG-DSC) analysis**

The thermal degradation behavior of the investigated complexes was followed by the thermogravimetric (TG) and differential scanning calorimetric (DSC) techniques. The decomposition occurs in one or more steps according to the nature of each complex. Attempts to generalize the thermal degradation patterns were unsuccessful indicating that there is no simple relation or general trend for explaining these thermal degradations. However, the decomposition ends with the formation of Cu$_2$O in most cases. Inspection of the data revealed the following: (i) The perchlorato complex (1) decomposes in one strong endothermic step ($\Delta H = 391$ J/g at 272°C) to form Cu$_2$O as the end product, a phenomenon encountered with ClO$_4^-$ anions [14]. (ii) For the nitrato- (2) and sulfato (6)- complexes, the decomposition is not completed up to 800°C, indicating that the metal-ligand bonds are strong. This is consistent with the data extracted from IR. (iii) In contrast, for the other complexes (1, 4 &5), the decomposition process is ended with Cu$_2$O.

**Kinetic and thermodynamic parameters**

The activation energy ($E^*$) of decomposition of the complexes as well as the pre-exponential factor (A) were evaluated graphically using the Coats-Redfern equation:

$$\ln \left[ -\frac{\ln (1 - \alpha)}{T^2} \right] = -\frac{E^*}{RT} + \ln \left[ \frac{AR^2}{\phi E^*} \right]$$

where $\alpha$ is the fraction of sample decomposed at temperature $T$, A the pre-exponential factor, $\phi$ the heating rate, $E^*$ the activation energy and R the gas constant. A plot of $\ln [-(1 - \alpha)/T^2]$ against $1/T$ gives a straight line whose slope = $-E^*/R$ and its intercept = $\ln [AR/\phi E^*]$; a sample set (Figure 4). The activation entropy, enthalpy and free energy changes of the decomposition were calculated by the following equations:

$$\Delta S^* = 2.303 R \log (Ah/KT), \Delta H^* = -E^* + RT \text{ and } \Delta G^* = \Delta H^* - T\Delta S^*$$

where $h$, $K$ and $T$ are the Planck’s constant, Boltzmann’s constant and the mid temperature in the TG-thermogram, respectively. The calculated values of $A$, $E^*$, $\Delta S^*$, $\Delta H^*$ and $\Delta G^*$ for the decomposition steps are given in Table 4. Inspection of the data revealed the following remarks: (i) The pre-exponential factor (collision factor; A) showed a direct relation to the activation energy $E^*$ (ii) The non spontaneity and endothermicity of the degradation processes. (iii) The perchlorato complex (1); [Cu(L)(HL)(H$_2$O)$_2$]ClO$_4$.3H$_2$O which decomposes in one sudden step has abnormal higher values of A, $E^*$ and $\Delta H^*$ [7]. Also, it has a unique positive $\Delta S^*$ ~ 60 J mol$^{-1}$ K$^{-1}$ which is consistent with the formation of several species upon the sudden decomposition. (iv) In contrast, $\Delta S^*$ has negative values for all the other complexes indicating that the activated species are more ordered than the reactants [17-19].

**Biological activity**

The isatinic hydrazone and its copper(II)- complexes (1-6) were screened for both antibacterial and antifungal activities (Table 5). Inspection of the data revealed that all complexes lack the activity towards the fungus; Fusarium oxysporum. Also, the ligand and its complexes (1, 4 and 5) lack the antimicrobial and antifungal activities. On the other hand, only the chloro-complex (3) has high activity against Gram-positive bacteria (S. aureus & S. pyogenes) relative to the nitrato-complex (2). Also, the sulfato-complex (6) exhibited some activity towards Gram-negative bacteria (P. phaseolicola & P. fluorescens) as well as the Fungus; Aspergillus fumigatus. In general, the antibacterial results (Table 5) suggest low activity as compared to the standard references (control) and depend mainly on the type of the coordinated anion in consistency with our previous studies [14,20].

**Experimental**

**Materials**

The chemicals used in this investigation were of the highest purity available (Merck, BDH, Aldrich and Fluka). They included copper(II)- nitrate, perchlorate, sulfate, chloride, bromide and acetate as well as o-toluidine, ethyl acetoacetate, phosphorus oxychloride, hydrasine hydrate and isatin. Organic solvents were reagent grade chemicals and were used without further purification.

**Measurements**

Microanalyses were carried out on a Perkin-Elmer 2400 CHN elemental analyzer. Thermal analyses (TG-DSC) were carried out on a Shimadzu-50 thermal analyzer. Electronic spectra were recorded on a Jasco V-550 UV/ VIS spectrophotometer. IR spectra were recorded on a Bruker Vector 22 spectrometer using KBr pellets. ESR spectra were recorded on a Bruker Elexsys, E 500 operated at X-band frequency. Mass spectra were recorded either at 70 eV on a gas chromatographic GCMSQP 1000-EX Shimadzu mass spectrometer or Direct Inlet unit (DI-50) of Shimadzu GC/MS - QP5050A. $^1$H NMR spectra were recorded as DMSO-$d_6$ solutions on a
Varian Mercury VX-300 NMR spectrometer using TMS as a reference. Molar conductivity was measured as DMF solutions on the Corning conductivity meter NY 14831 model 441. Magnetic susceptibility of the complexes was measured at room temperature using a Johnson Matthey, MKI magnetic susceptibility balance. Melting points were determined using a Stuart melting point apparatus.

Figure 4 Evaluation of A and $E^*$ for the first stage of thermal decomposition.
Preparation of the hydrazone [9-11]
An ethanolic mixture of 2-hydrazinyl-4,8-dimethyl quinoline (0.01 mol) and isatin (0.012 mol) was refluxed for 1/2 h. The formed red compound was filtered off, washed with ethanol and crystallized from DMF. The results of elemental analysis, % yield and m.p°C are shown in Table 1.

Preparation of the metal complexes
Methanolic solutions of the metal salt and the ligand were mixed in the mole ratio 1:1 and/or 1:2; M:L and refluxed for 6-10 hours depending on the nature of the isolated complexes. The resulting precipitates were filtered off, washed with methanol then ether and finally air-dried. The results of elemental analysis, % yield and m.p°C are shown in Table 1.

Antimicrobial and antifungal activities
The standardized disc-agar diffusion method was followed to determine the activity of the synthesized compounds against the sensitive organisms Staphylococcus aureus (ATCC 25923) and Streptococcus pyogenes (ATCC 19615) as Gram-positive bacteria, Pseudomonas fluorescens (S 97) and Pseudomonas Phaseolicola (GSPB 2828) as Gram-negative bacteria and the Fungi Fusarium oxysporum and Aspergillus fumigatus. The antibio tic chloramphenocol and Cephalothin were used as standard references in case of Gram-negative and Gram-positive bacteria, respectively whereas Cycloheximide was used as a standard antifungal reference. The tested compounds were dissolved in DMF which have no inhibition activity to get concentration of 2 mg/mL.

The test was performed on medium potato dextrose agar (PDA) which contain infusion of 200 g potatoes, 6 g dextrose and 15 g agar. Uniform size filter paper disks (3 disks per compound) were impregnated by equal volume (10 μL) from the specific concentration of dissolved tested compounds and carefully placed on inoculated agar surface. After incubation for 36 h at 37°C in case of bacteria and for three days at 25°C in case of fungi, inhibition of the organisms which evidenced by clear zone surround each disk was measured and used to calculate mean of inhibition zones.

Conclusion
The present study is planned to check the effect of the counteranions on the isolated complexes as well as the ligatdal behavior of the isatinic hydrazone ligand. The effect of anion for the same metal ion is obvious from either the geometry of the isolated complexes (Oh, Td and D4h) or the various modes of bonding. Also, the obtained complexes fulfill the strong coordinating ability of Cl−, Br−, NO3− and SO42− anions as compared to the ClO4− anion in consistency with the donor ability of the anions [12]. In case of copper(II)- acetate, a unique homoleptic complex (5) was obtained in which the AcO− anion acts as a base enough to quantitatively deprotonate the hydrazone. The isatinic hydrazone uses its lactim form in most complexes.

Authors’ contributions
HSS conceived the study, participated in the sequence alignment and drafted the manuscript. GAE and BAE participated in the design of the study, analysis of the data and modification of the manuscript. HSS and MAM isolated and characterized the complexes. FIH prepared and characterized the organic ligand. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Table 4 Thermodynamic and kinetic parameters of the copper(II)-isatinic complexes.

| Complex | Stage | T (K) | A × 10^-9 sec^-1 | E* | ΔH* | ΔG* | -ΔSmol^-1 K^-1 | -ΔSimol^-1 K^-1 |
|---------|-------|-------|------------------|-----|------|------|---------------|---------------|
| 1       | 1st   | 592   | 1.709 x 10^7     | 71.952 | 67.038 | 31.487 | -60.053       |
| 2       | 1st   | 355   | 1.486            | 5.942  | 2.996  | 28.082 | 70.667         |
|         | 2nd   | 475   | 5.627            | 9.123  | 5.18   | 34.644 | 62.029         |
| 4       | 1st   | 590   | 9.601            | 10.914 | 6.017  | 41.059 | 59.393         |
| 5       | 1st   | 602   | 14.053           | 12.281 | 7.284  | 41.235 | 56.398         |

* E*, ΔH* and ΔG* are in kJ mol^-1 while -ΔSmol^-1 K^-1 in J mol^-1 K^-1.

Table 5 The biological activity of HL and its copper(II)- complexes.

| Organisms | % inhibition |
|-----------|--------------|
|           | Gram - positive bacteria | Gram - negative bacteria | Fungus |
| S. aureus | S. pyogenes | P. Phaseolicola | P. fluorescens | Aspergillus fumigatus |
| HL        | –           | –               | –           | –               | –               |
| (2)       | 7           | –               | –           | –               | –               |
| (3)       | 24          | 22              | –           | –               | –               |
| (6)       | –           | –               | 9           | 22              | 18              |

*Complexes 1, 4 and 5 lack the biological activity.
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