

Article

Antimicrobial Activity of Ethyl (2-(Methylcarbamoyl)phenyl) carbamate and Its Mixed Ligand Ni(II) and Co(II) Complexes

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Abstract

The aim of this paper is to obtain ethyl (2-(methylcarbamoyl)phenyl)carbamate and its metal complexes as promising antimicrobial agents. The title compound was synthesized using the ring-opening of isatoic anhydride with methylamine and further acylation with ethyl chloroformate. All metal complexes were successfully obtained after mixing the ligand and dissolved in DMSO and water solutions of the corresponding metal salts and sodium hydroxide, in a metal-to-ligand-to base ratio 1:2:2. As a result, mixed ligand complexes of ethyl 2-(methylcarbamoyl)phenyl)carbamate and 3-methylquinazoline-2,4(1*H*,3*H*)-dione were obtained. The obtained complexes were characterized by their melting points, FTIR, NMR spectroscopy, and MP-AES. Then, the antimicrobial effect of the compounds against both Gram-negative and Gram-positive bacteria, yeasts, and fungi was studied. Only the Co(II) complex showed antimicrobial activity against almost all Gram-positive and Gram-negative bacteria. The cobalt complex exhibited promising antimicrobial activity against Gram-positive *Micrococcus luteus* with inhibition zones of 20 mm, *Listeria monocytogenes* (15 mm), *Staphylococcus aureus* (13 mm), as well as Gram-negative *Klebsiella pneumoniae* (13 mm) and *Proteus vulgaris* (13 mm). Given the potential of metal complexes as antimicrobial agents, understanding their cytotoxic effects is crucial for evaluating their therapeutic safety. To assess the in vitro biocompatibility of the experimental compounds, a range of cell viability assays was conducted using human malignant leukemic cell lines (LAMA-84, K-562) and normal murine fibroblast cells (CCL-1). The Ni(II) complex shows IC₅₀ = 105.1 μM against human malignant leukemic cell lines LAMA-84. Based on the reported results, it may be concluded that the mixed cobalt complex of 2-(methylcarbamoyl)phenyl)carbamate and 3-methylquinazoline-2,4(1*H*,3*H*)-dione can be attributed as a promising antimicrobial agent. Future in vivo tests will contribute to establishing the antimicrobial properties of this complex.



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Keywords: synthesis; metal complexes; anthranilic acid; antimicrobial activity; cytotoxic activity

1. Introduction

The membrane-like extracellular matrix formed by the attachment of bacterial colonies and extracellular polymeric materials, such as polysaccharides, nucleic acids, and proteins generated by bacteria during growth, is referred to as a biofilm, which is an organized population of bacteria [1]. EPS interacts with bacterial aggregates to give the biofilm cohesiveness and viscoelastic qualities [2]. Bacteria can therefore stick to the surfaces of both living and non-living things. Chronic persistent infections are largely caused by the production of pathogenic biofilms [3]. Researchers currently believe that bacterial biofilms are responsible for more than 80% of persistent illnesses [4].

Staphylococcus aureus, a drug-resistant pathogen, can lead to skin and soft tissue infections, as well as serious conditions like endocarditis, osteomyelitis, pneumonia, and other invasive diseases [5–10].

Listeria monocytogenes, a Gram-positive rod-shaped bacterium, is recognized for causing listeriosis upon infection [11]. This pathogen shows an extraordinary ability to withstand various external conditions, such as high salt concentrations and both acidic and alkaline environments [12]. It can multiply within a temperature range of 0–45 °C and remain viable under refrigeration for long periods, earning it the nickname “refrigerator killer” because of its potential danger to human health [13]. In human beings, *L. monocytogenes* infection can result in serious clinical consequences such as bacteremia, monocytopenia, meningitis, among others [14,15]. *L. monocytogenes* can penetrate the body’s defense mechanisms, leading to a death rate of 20–40% among those infected [16].

One of the main causes of nosocomial infections, including pneumonia, bacteremia, liver abscesses, and meningitis, is the gram-negative bacteria *Klebsiella pneumoniae*. Treatment options for *K. pneumoniae* infections are limited due to the recent rise in antibiotic resistance among isolates and the appearance of highly invasive hypervirulent strains [17,18].

Furthermore, it is thought that bacteria may adapt to severe settings by forming biofilms, which is a protected growth mode [19]. The biofilm protects bacterial cells against harmful factors such as high temperatures, a lack of nutrients, dehydration, and even antibacterial chemicals [20]. It also creates a stable internal environment for bacterial cell activity. The development of drug resistance depends on biofilm formation in addition to the diverse array of secreted virulence factors [21]. The microbial environment can benefit greatly from biofilm, which can enhance strain resistance by up to 1500 times [22].

Consequently, it is crucial to create new antibiofilm agents targeting *Klebsiella pneumoniae*, *L. monocytogenes*, and *S. aureus*.

Anthranilic acid analogues and their derivatives hold great promise for developing compounds for targeted antitumor activity, tackling metabolic issues related to diabetes [23,24], acting as antimicrobial and antiviral agents [25], and serving as biologically compatible anti-inflammatory agents [26,27]. Anthranilic acid is also capable of forming coordination complexes with various metals [28,29].

Some of the application areas of anthranilic acid and its metal complexes are highlighted in Figure 1.

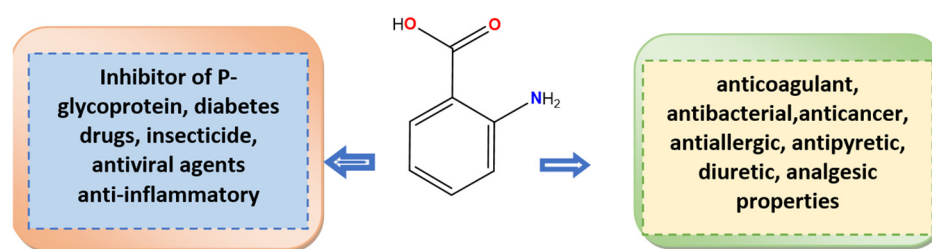


Figure 1. Anthranilic acid, its biological activities, and pharmaceutical applications.

The anthranilic acid complexes with chromium [30], cobalt, nickel, and zinc [31,32] were obtained. Buvaylo et al. have obtained a new neutral Co^{II} complex with dimethylformamide (DMF) solvent molecules [33]. Recently, Cheng Xiao-Xue et al. used an azo-coupling reaction between 4-aminobenzoic acid and anthranilic acid, salicylaldehyde, and 2-naphthol to create nanostructured cobalt(II) metalorganic complexes [34]. Metal complexes of nano-anthranilic acid with various metal ions, including Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Mn(II), Bi(III), Al(III), and Cr(III), were successfully synthesized and characterized, as reported in reference [35]. El-Ajaily and Morad obtained cobalt(II) and copper(II) Schiff base complexes of salicylaldehyde and anthranilic acid [36]. Different variants of chelation [30–33] or bridge coordination [37] are present in the literature. The oxalate-bridged binuclear metal complexes with copper(II) and nickel(II) were synthesized by Saghatforoush et al. [37]. Preclinical pharmacological screenings for synthetic metal complexes' antibacterial, antifungal, anti-inflammatory, anticancer, DNA interaction, and antitumor properties have been a goal of researchers over the past few decades [38].

The biological properties and synthesis of new complexes with anthranilic acid were reported recently [39]. Mixed-ligand metal complexes of Co(II), Ni(II), Cu(II), Zn(II), Cd(II), and Hg(II) were synthesized using a Schiff base ligand *N,N'*-Bis-(4-dimethylaminobenzylidene)-benzene-1,4-diamine in combination with anthranilic acid [40]. The anthranilic acid, its derivatives, and their metal complexes were found to have anti-inflammatory properties [41,42], cytotoxicity [43], and antimicrobial activities [26,35,44–49]. They are also inhibitors of aldo-keto reductase enzymes [50].

Therefore, the current study aims to synthesize an anthranilic acid derivative, its nickel and cobalt complexes, and to study their antimicrobial properties.

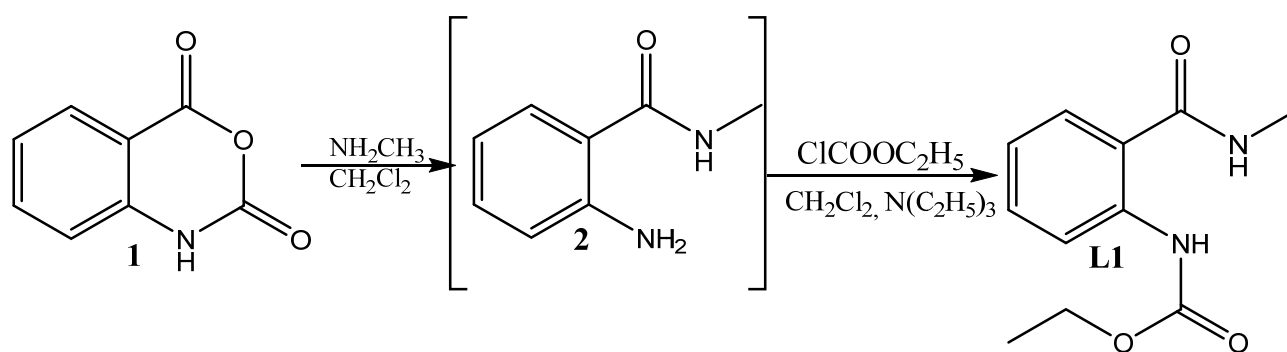
2. Results and Discussion

Anthranilic acid (Figure 1) and its analogs play a crucial role in numerous bioactive substances and medications, exhibiting diverse biological effects in the treatment of various diseases [51–54]. Functional groups, such as $-\text{COOH}$, $-\text{NH}_2$, $-\text{CONH}_2$, $-\text{COOCH}_3$, and $-\text{NHCOCH}_3$ assist conjugation for interaction with biological targets [55–57]. In order to determine the best pharmacophores, the functional groups serve as the sites for molecule customization and the analysis of the structure-activity connection of anthranilic acid-based libraries.

Esters could be found naturally in propolis, which demonstrates important biological activities, such as neuroprotective activity [58]. The ester functional group was also found to possess antiproliferative and antimetastatic effects on human lung adenocarcinoma cells [59]. The design and synthesis of prodrugs for nonsteroidal anti-inflammatory drugs has been a major focus of medicinal chemists, especially in the last ten years. Anti-inflammatory medications are among the most widely used drug classes. To shield the gastrointestinal tract from local irritation, the majority of the work put into creating ester prodrugs was focused on hiding the molecules' free acidic groups [60]. Some of them, such as salsalate, flurbiprofen axetil, etc., contain the ester functional group. Excellent analgesic

and anti-inflammatory prodrugs were shown by ester and amide derivatives of certain acidic anti-inflammatory drugs, including mefenamic acid, ketoprofen, and ibuprofen [61].

Esterification is regarded as one of the best methods for producing prodrugs of weak acids with a high yield (up to 89%) that does not require complicated setup, hazardous solvents, expensive reaction vessels, or complicated operating conditions [62]. Esters of weak acidic medicinal substances offer an ideal way to avoid the gastric acidic environment that restricts hydrolysis, as well as improving aqueous solubility, transcellular absorption (especially in the small intestine), and rapid metabolism to produce high concentrations of the active drug in the blood to meet desired bioavailability [63]. Thus, we decided to incorporate an ester residue in the anthranilic acid amide. To synthesize it, we used a previously reported procedure [64–67], which involves the ring-opening of isatoic anhydride **1** with methylamine to amide **2**, which further acylates to produce the ester of anthranilamide, (L1) (Scheme 1).



Scheme 1. Synthesis of the ligand L1.

The resultant compound **L1** was characterized by its melting point, FTIR, and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra. The structure of the synthesized compound was confirmed through spectral analysis, as detailed in Supplementary Figures S1–S3.

The interaction between the ligand and transition metal ions resulted in the formation of thermodynamically stable coordination compounds, with isolated yields ranging from 31% to 35%. The synthesized Ni(II) and Co(II) complexes exhibit characteristic green and purple colors, respectively. These complexes are air- and moisture-stable under ambient conditions and display limited solubility in common organic solvents such as ethanol, ethyl acetate, and tetrahydrofuran (THF), while being readily soluble in dimethyl sulfoxide (DMSO). The analytical results, including the yield percentages of the mixed ligand Ni(II) and Co(II) complexes, are shown in Table 1.

Table 1. Analytical and physical characteristics of coordination compounds.

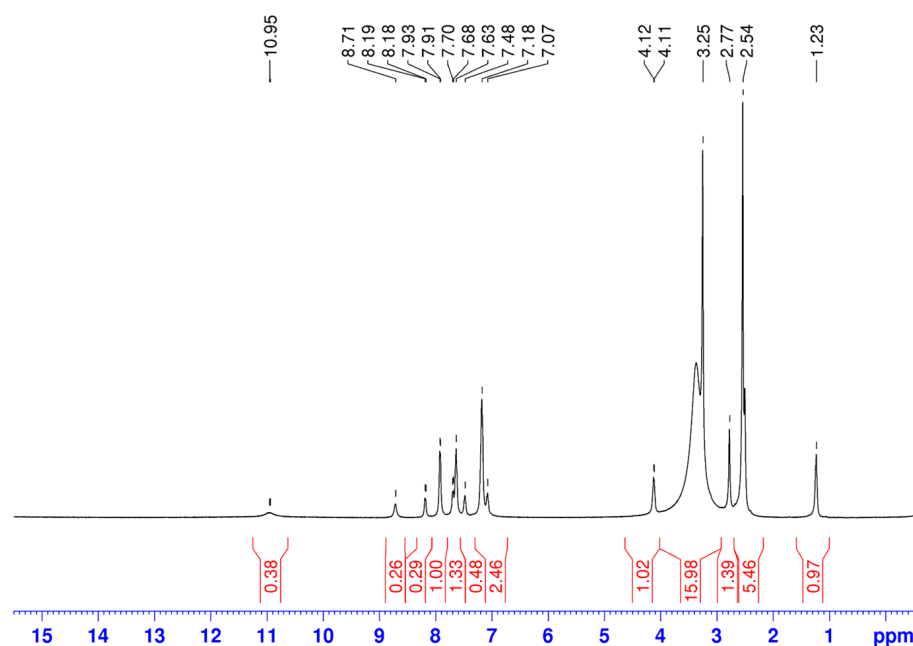
Complexes	Colour	Solubility	Yield (%)	Melting Point (°C)
L1	colorless	soluble in DMSO and CHCl_3	80	136–137
Ni(II)	bright green	insoluble in H_2O , THF, CH_3COCH_3 , EtOH, EtOAc and	31	245–247
Co(II)	purple	cyclohexane and soluble in DMSO	33	240–241

The ^1H -NMR data for ethyl (2-(methylcarbamoyl)phenyl)carbamate (L1) and its metal complexes are given in Table 2. However, in both ^1H and ^{13}C NMR spectra of the complexes, we observe the presence of additional, stronger signals than the signals of L1 (Figure 2).

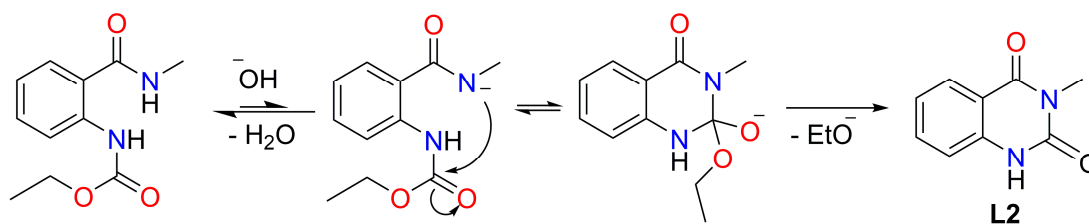
Table 2. ^1H -NMR data for L1, L2 and their mixed-ligand metal complexes of Ni(II) and Co(II).

Atom	δ (^1H) ppm (L1)	δ (^1H) ppm L2 [68]	δ (^1H) ppm Ni(II)	δ (^1H) ppm Co(II)
$\text{NH}(\text{COO})$	10.96 (s)		10.95 *	10.99 *
		11.43 (s)	-	11.43 **
NHCH_3	8.72 (q)		8.71 *	8.72 *
CH	8.19 (dd)		8.19 (d) *	8.19 *
		7.93 (d)	7.92 (d) **	7.92 **
CH	7.70 (dd)		7.69 (d) *	7.70 *
		7.64–7.66 (m)	7.63 **	7.64 **
CH	7.48 (ddd)		7.48 *	7.48 *
		7.17–7.21 (m)	7.18 **	7.18 **
CH	7.08 (ddd)		7.07 *	7.09 *
		7.17–7.21 (m)	7.18 **	7.18 **
NHCH_3	2.78 (d)		2.77 *	2.77 *
		3.26 (s)	3.25 **	3.25 **
CH_2	4.12 (q)		4.12 (q) *	4.12 *
CH_3	1.23 (t)		1.23 (t) *	1.24 *
DMSO	-	-	2.54	2.53

*—signals of L1 protons in the complex; **—signals of L2 protons in the complex. All signals in the complexes are broad singlets unless otherwise noted.

**Figure 2.** The ^1H NMR spectrum of a Ni(II) coordination compound.

Based on the spectral data, we anticipated that the metals formed a mixed complex with another byproduct ligand (labeled as L2). Roughly, the ratio can be estimated at 1:3 for L1 and L2, respectively. We deduced the structure of L2 from the chemical shift data for the ^1H and ^{13}C signals. The signals are completely consistent with 3-methylquinazoline-2,4(1H,3H)-dione [68]. The possible mechanism of L2 formation and the structure formula are given in Scheme 2.



Scheme 2. Reaction scheme for obtaining the second ligand 3-methylquinazoline-2,4(1H,3H)-dione (L2).

It should be mentioned that mixed ligand complexes of ethyl 2-(methylcarbamoyl)phenylcarbamate and 3-methylquinazoline-2,4(1H,3H)-dione were obtained for the first time.

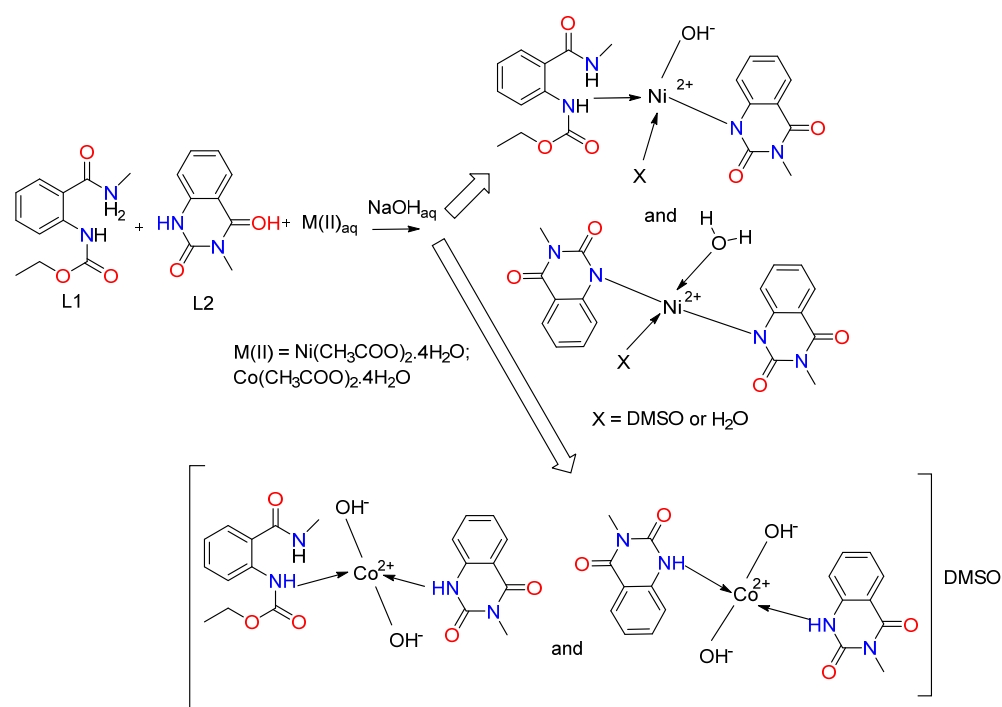
The lack of a signal for the proton of the amide group in L2 is observed when comparing the ^1H -NMR spectrum of the Ni(II) complex with the spectra of L1 and L2. This suggests that the nitrogen atom from this functional group is involved in the coordination to the metal center. The signal for the proton from the NHCH_3 group is moved to lower frequencies with 2.25 ppm in the ^1H -NMR spectrum of the Co(II) complex (Table 3). This demonstrates that the N-methyl group is involved in coordination with the metal ion. This fact is consistent with the information included in the infrared spectra.

Table 3. ^{13}C -NMR data for L1, L2 and their coordination compounds with Ni(II) and Co(II).

Atom	δ (^{13}C) ppm (L1)	δ (^{13}C) ppm (L2) [68]	δ (^{13}C) ppm Ni(II)	δ (^{13}C) ppm Co(II)
C=O(NCH ₃)	169.16	162.6	169.16 *	169.15 *
			162.66 **	162.65 **
C=O(NH)	153.37	150.8	153.37 *	153.40 *
			150.88 **	150.85 **
C	139.67	139.8	139.67 *	139.70 *
			139.91 **	139.83 **
CH	132.53	135.3	132.52 *	132.52 *
			135.28 **	135.28 **
CH	128.40	127.7	128.40 *	128.39 *
			127.71 **	127.72 **
CH	122.16	122.9	122.15 *	122.17 *
			122.82 **	122.86 **
C	120.03	114.1	120.02 *	120.08 *
			114.15 **	114.14 **
CH	119.05	115.5	119.05 *	119.05 *
			115.60 **	115.55 **
CH ₂ CH ₃	61.04	-	61.04 *	61.03 *
NHCH ₃	26.68	27.5	26.67 *	26.67 *
			27.46 **	27.46 **
CH ₂ CH ₃	14.85	-	14.85 *	14.84 *
DMSO	-	-	40.88	40.90

*—signals of L1 protons in the complex; **—signals of L2 protons in the complex. All signals in the complexes are broad singlets unless otherwise noted.

When comparing the ^1H -NMR spectrum of Ni(II) complex with L1 and L2's spectra, it is observed that one signal for the proton from the amide group in L2 is absent, which indicates the participation of the N-atom from this functional group in the coordination to the metal center. In the ^1H -NMR spectrum of the Co(II) complex, the signal for the proton from the NHCH_3 group is shifted to a lower frequency by 2.25 ppm (Table 3). This is an indication of the involvement of this group in coordination with the metal ion. The presented data is in agreement with the IR spectrum data. The aforementioned gives us reason to propose the following reaction scheme for the synthesis of metal complexes of ethyl 2-(methylcarbamoyl)phenylcarbamate (L1) and 3-methylquinazoline-2,4(1H,3H)-dione (L2) with Ni(II) and Co(II) (Scheme 3).



Scheme 3. Synthetic pathway for the preparation of mixed-metal complexes of ethyl (2-(methylcarbamoyl)phenyl)carbamate (L1) and 3-methylquinazoline-2,4(1H,3H)-dione (L2) with Ni(II) and Co(II).

Furthermore, the presence of a solvent molecule in the Ni(II) and Co(II) complexes was confirmed by the signals observed at 2.54 ppm and 2.53 ppm in the 1H NMR spectra, and at 40.88 ppm and 40.90 ppm in the ^{13}C NMR spectra, respectively.

The ^{13}C -NMR data for ethyl (2-(methylcarbamoyl)phenyl)carbamate (L1), 3-methylquinazoline-2,4(1H,3H)-dione (L2) and their coordination compounds are given in Table 3. The ^{13}C -NMR spectra of the Ni(II) and Co(II) complexes are almost identical.

Selected experimental IR spectral data (cm^{-1}) for the Ni(II)L and Co(II)L complexes, along with the free ligands L1 and L2, are summarized in Table 4. In the infrared spectrum of L1, the bands at 3345 cm^{-1} and 3258 cm^{-1} may refer to the stretching vibrations of the N-H groups of L1. In the infrared spectrum of L2, the band at 3165 cm^{-1} can be attributed to stretching vibrations of the N-H group [69]. The same band in the spectrum of the nickel (II) complex is missing, but for cobalt(II) complexes was shifted to higher frequencies by 23 and 24 cm^{-1} , respectively (the comparison is for L2). This showed that the NH group of the ligand L2 participates in the coordination of all complexes. In the IR spectra of L1 and L2, the bands at 1739 cm^{-1} and 1715 cm^{-1} can be attributed to stretching vibrations of C=O groups from the ligand L1 and L2, respectively. Comparing the infrared spectra of the complexes with that of L2 shows that the same band in the IR spectra of all complexes was observed at 1717 cm^{-1} and is not changed. This showed that the two C=O groups of the ligand L2 do not participate in the coordination with metal ions. In all infrared spectra, a broad band is observed at $3500\text{--}3400\text{ cm}^{-1}$, confirming the presence of a hydroxyl group from a water molecule. That is fully consistent with previously obtained mixed ligand complexes of anthranilic acid [70].

Table 4. Selected experimental IR spectral data (cm^{−1}) for Ni(II) and Co(II) complexes of ligands L1 and L2.

Assignment	L1	L2 [69]	Ni(II)	Co(II)
$\nu(\text{OH})$	-		3436	3442
$\nu(\text{NH}, -\text{C}(=\text{O})-\text{NH}-\text{CH}_3)$	3345		3345	3345
$\nu(\text{NH}, -\text{NH}-\text{C}(=\text{O})\text{OCH}_2\text{CH}_3)$	3258	3165	-	3189
$\nu(\text{C}_{\text{sp}^2}-\text{H}, -\text{Ph})$	3072		3060	3058
$\nu(\text{C}=\text{O})$	1739	1715	1718	1717
$\delta(\text{NH}) + \nu(\text{C}=\text{O}),$ $-\text{C}(=\text{O})-\text{NH}-\text{CH}_3)$	1664	1662	1666	1665
$\delta(\text{NH}) + \nu(\text{C}=\text{O}),$ $-\text{NH}-\text{C}(=\text{O})\text{OCH}_2\text{CH}_3)$	1633		1645	1645

The nickel and cobalt content in the complexes was determined using microwave plasma–atomic emission spectrometry (MP-AES). Based on the data obtained from ¹³C{1H} NMR spectroscopy, infrared (IR) spectroscopy, and elemental analysis, tentative average molecular compositions were proposed for the Ni(II) and Co(II) complexes, as presented in Table 5.

Table 5. Elemental analysis data for the metal ions in the synthesized complexes.

Metal Complex	Composition *	Formulae	Mr g/mol	W(M)% calc./exp.
Co(II)	[L1.3L2.4(OH [−]).2Co].DMSO	C ₄₀ H ₄₈ N ₈ O ₁₄ SCo ₂	997.78	11.6/11.9 ± 0.6
Ni(II)	[L1.3L2.3H ₂ O.DMSO.2H ₂ O(OH [−]).2Ni]	C ₄₀ H ₄₆ N ₈ O ₁₃ SNi ₂	996.29	11.8/11.8 ± 0.6

* tentative average composition of complexes.

We assumed that the ligand in the complexes coordinates monodentately through the N-atom (Figure 3). We observe a combination of two complexes for the cobalt(II). In the first, two hydroxyl groups have been coordinated by the metal center, and both ligands L1 and L2 are engaged, while only ligand L2 is coordinated in the second one. Two hydroxyl groups are also linked to the cobalt ion. The charge of the metal center is offset by these groups. The complex's composition includes the solvent molecule DMSO, as indicated by the metal's elemental analysis data as well as the ¹³C{1H} NMR spectra. We consider the outer coordination sphere to be the location of DMSO.

We observe again a mixture of two complexes with different structures and compositions for the nickel(II). L1 is coordinated through the nitrogen atom of the ester group, and L2 is deprotonated and coordinated through the N donor atom in the first one. The third coordination site is occupied by a hydroxyl group, and the fourth coordination site is occupied by either a DMSO molecule or a water molecule (Figure 3). The second complex contains two deprotonated ligands, L2, coordinated to the metal ion through the nitrogen donor atoms, and one water molecule. The fourth coordination site can be occupied by either a DMSO solvent molecule or a water molecule. It should be mentioned that the structure of the complexes probably contains two bulky ligands and two smaller ones due to steric reasons.

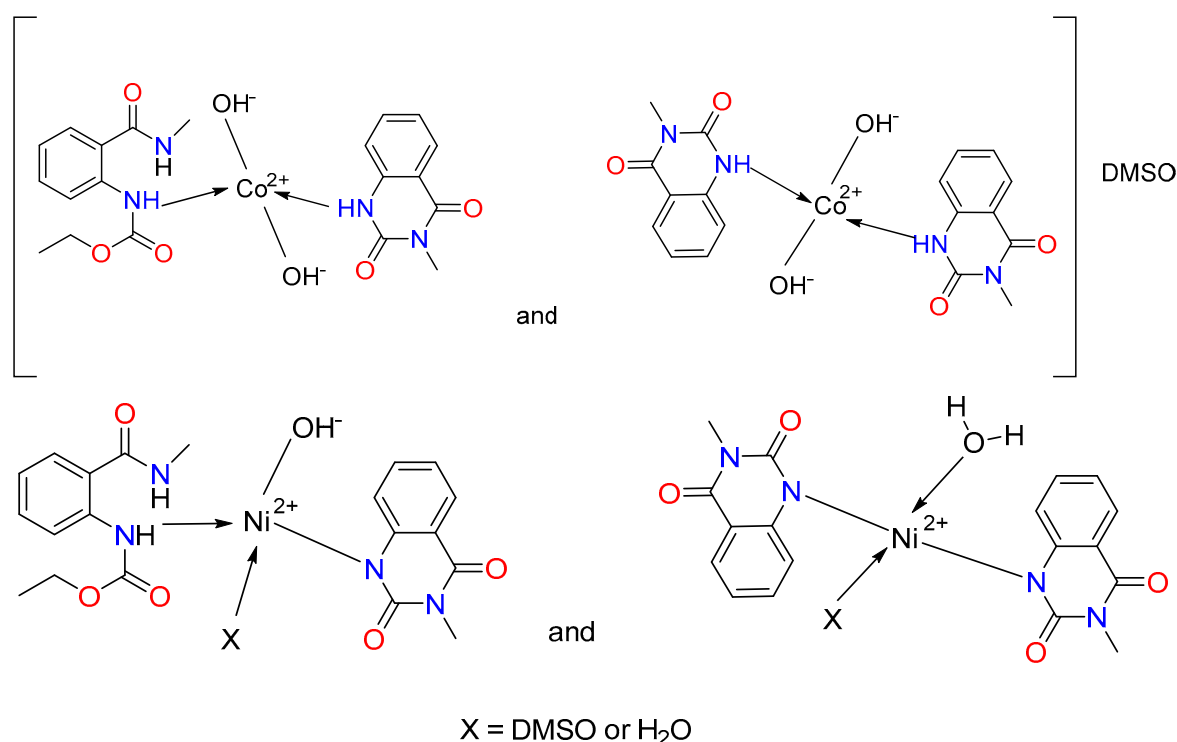


Figure 3. Proposed coordination modes of ligands L1 and L2 with Co(II) and Ni(II), illustrating the suggested donor binding sites.

The structure of the obtained complexes was similar to that presented for the Pd(II) complex of *N*-(2-benzoyl-4,5-dimethoxyphenethyl)-2-phenylacetamide [71]. The presented data are also consistent with previously synthesized Mn(II), Ni(II), Co(II), Cu(II), Zn(II), and Cd(II) complexes of anthranilic acid and pyridine-2-aldoxime in an alkaline environment [47]. The authors [47] found that nickel and cobalt complexes have a tetrahedral geometry and coordination number of 4, as we assumed for the Ni(II) and Co(II) complexes. Recently, Alwan reported the synthesis of mixed ligand complexes of Cu(II), Ni(II), Co(II), and Zn(II) with anthranilic acid [72]. Square planar geometry was suggested for the Co(II) and Zn(II) complexes, while we proposed tetrahedral geometries for the Ni(II) and Cu(II) complexes [72]. It should be noted that the complexes are soluble only in DMSO and partially in hot ethanol, similarly to our experiments. The tetrahedral geometry was also confirmed by Al-Noor et al. for Ni(II), Co(II), Fe(II), Zn(II), and Cd(II) mixed-ligands complexes containing anthranilic acid and amino acid L-alanine with Cu(II), Ni(II), Co(II), Mn(II), Fe(II), Zn(II) and Cd(II) [73]. The resulting complexes are insoluble in water but soluble in DMSO and DMF.

The title compound and its metal complexes were synthesized as promising antimicrobial agents. Therefore, our main goal was to investigate their antimicrobial and antifungal activity.

The antibacterial activity against five Gram-positive, five Gram-negative, two yeasts, and three fungi was investigated using the agar diffusion technique. The sensitivity of harmful bacteria is revealed by the excellent diffusion method. As a result, a chemically sensitive organism will not grow close to the well where it was deposited. The area that does not expand is referred to as the clear zone or zone of inhibition. The size of the clear zone corresponds to the tested substance's inhibition. Table 6 provides a summary of the mean zones of inhibition in millimeters that were generated on the pathogenic bacteria that included L1 and its metal complexes.

Table 6. Antimicrobial effect of ethyl 2-(methylcarbamoyl)phenyl)carbamate and its metal compounds.

Tested Microorganisms	Inhibition Zones, mm		
	L	Ni(II)	Co(II)
<i>Candida albicans</i> NBIMCC 74	8	-	-
<i>Saccharomyces cerevisiae</i> ATCC 9763	9	-	-
<i>Aspergillus niger</i> ATCC 1015	8	9	9
<i>Aspergillus flavus</i>	8	-	-
<i>Penicillium chrysogenum</i>	9	-	-
<i>Rhizopus</i> sp.	8	8	-
<i>Fusarium moniliforme</i> ATCC 38932	8	8	-
<i>Salmonella enteritidis</i> ATCC 13076	-	-	11
<i>Salmonella typhimurium</i> NBIMCC 1672	-	-	11
<i>Klebsiella pneumonia</i> ATCC 13883	-	12	13
<i>Proteus vulgaris</i> ATCC 6380	-	-	13
<i>Bacillus amyloliquefaciens</i> 4BCL-YT--	8	-	10
<i>Enterococcus faecalis</i> ATCC 29212	-	-	9
<i>Staphylococcus aureus</i> ATCC 25923	-	-	13
<i>Listeria monocytogenes</i> NBIMCC 8632	-	-	15
<i>Micrococcus luteus</i> 2YC-YT	8	-	20

Our results illustrated that only the cobalt complex exhibited promising antimicrobial activity against Gram-positive *Staphylococcus aureus*, *Listeria monocytogenes*, *Micrococcus luteus*, and Gram-negative *Klebsiella pneumoniae* and *Proteus vulgaris*. This difference can be explained by different cell membrane permeability for both Gram-positive and Gram-negative bacteria, which is greatly influenced by several intricate factors, such as the membrane's zeta potential, lipophobicity, the thickness of the membrane and its surrounding layers, and the chemical composition of the antimicrobial drug [74].

The obtained results corresponded to those previously described by Shirode [70]. The author obtained mixed cobalt complexes of anthranilic acid semicarbazone and benzaldehyde and found that the cobalt complexes were moderately active against *Bacillus* sp. and *Staphylococcus* sp. In our experiments, mixed cobalt complexes with anthranilic acid ester amide showed similar activity to *Bacillus* sp. and *Staphylococcus* sp., but were also sensitive to *Listeria monocytogenes* and *Micrococcus luteus*. Results were confirmed by the results from cobalt complexes of a Schiff base ligand 2-ethyl-[3-(hydroxyl propyl)]-3, 1(4H) quinazoline-4-one-4 hydrazone [75] and mixed ligand complexes of anthranilic acid and tributylphosphine [76]. Results were also similar to the antimicrobial activity of bis(2-methoxy-6-[(2-methylpropyl) imino]methyl)phenolato) nickel (II) complex, which showed significant antibacterial activity against *E. coli* and *S. aureus* and a prominent antifungal activity over *C. albicans* and *C. tropicalis* [77] and In(III) complex of *N*-methyl-*N*-phenyl dithiocarbamate, active against *L. monocytogenes* (15 mm), *B. cereus* (16.67 mm), *E. faecalis* (14.67 mm), and *S. enterica* (14.67 mm) [78].

The findings of this study on the antimicrobial activity of ethyl 2-(methylcarbamoyl)phenyl)carbamate and its complexes highlight several potential applications in the field of antimicrobial agents. The cobalt complex demonstrated activity against a range of Gram-positive and Gram-negative bacteria, including clinically significant pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Micrococcus luteus* and *Klebsiella pneumoniae*. The complex could be a potential candidate for developing novel antibacterial drugs. Activity against *Listeria monocytogenes* and *Staphylococcus aureus* suggests potential use in disinfectants or coatings for hospital surfaces and medical devices to control the spread of these pathogens. The selective bacterial activity observed for the cobalt and nickel complexes suggests specific interactions between these metal ions and bacterial cells. Further research into these mechanisms may lead to the design of more effective and

specific antimicrobial agents. Given the activity against *Salmonella enteritidis* and *Salmonella typhimurium*, these complexes could be investigated for controlling bacterial contamination in the food industry, particularly in poultry and egg production. The observed resistance of fungi and certain bacterial strains provides a basis for understanding limitations and optimizing the structure of these compounds to enhance their antimicrobial spectrum.

The study's findings on the in vitro biocompatibility of the newly synthesized metal complexes suggest promising potential applications, particularly as anti-infective agents. Below are key avenues for future exploration:

1. Development of Safe Antimicrobial Agents

The negligible cytotoxicity and high IC₅₀ values (>100 µM) across diverse cell lines indicate that these compounds are biocompatible, making them ideal candidates for antimicrobial drug development with minimal risk of host toxicity.

2. Adjunctive Therapies in Infection Management

Given their selective activity against bacterial pathogens and lack of significant cytotoxicity, these compounds could serve as adjunctive agents in treating infections, particularly in immunocompromised individuals, without harming normal cells.

3. Biomedical Coatings and Materials

The combination of antimicrobial activity (especially in the cobalt complex) and biocompatibility makes these compounds suitable for use in coatings for medical devices, implants, and wound dressings to prevent biofilm formation and infection.

4. Research into Selectivity Mechanisms

The low cytotoxicity paired with antimicrobial activity opens avenues for studying mechanisms that confer selectivity toward bacterial cells over mammalian cells. Insights from this could inform the design of more targeted antimicrobial therapies.

5. Applications in Chronic Infections

For chronic myeloid leukemia (CML) or other conditions involving immunosuppression, where infections pose a significant risk, these biocompatible compounds could potentially be integrated into treatment protocols.

6. Food and Environmental Safety

The safe profile of these compounds supports their application in controlling bacterial contamination in food and water, offering an alternative to traditional disinfectants that may have toxic side effects.

7. Foundation for Structural Optimization

While the current compounds are biocompatible, their activity could be further enhanced through structural modifications without compromising safety, leading to improved therapeutic potential against infectious agents.

These findings establish a solid foundation for advancing the development of these metal complexes in anti-infective applications while minimizing the risk of adverse effects on host cells. Further in vivo studies and formulation research will be critical to translating these promising results into practical applications.

Given the potential of metal complexes as antimicrobial agents, understanding their cytotoxic effects is crucial for evaluating their therapeutic safety. Finally, the in vitro effect of the metal complexes on cell proliferation and their biocompatibility was assessed in cellular test systems of leukemic (K-562, LAMA-84, bcr-abl+ chronic myeloid leukemia cell lines) and epithelial (CCL-1, normal murine fibroblast cells) origin. The results are presented in Table 7.

Table 7. In vitro cytotoxicity of the tested compounds [IC_{50} , $\mu M \pm SD$] against cell lines of different origin.

Compound	Cell Lines		
	K-562	LAMA-84	CCL-1
Co(II) complex	115.6 ± 8.2	120.7 ± 11.5	130.6 ± 10.5
Ni(II) complex	>200	105.1 ± 7.3	>200

Both complexes were shown to be devoid of any significant cytotoxic activity and exhibited a negligible effect on cell proliferation. The calculated half-inhibitory concentrations (IC_{50}) in all experimental models consistently exceeded 100 μM , indicating no observable cytotoxicity across the tested concentration range, irrespective of the cell line or the nature of the coordinating metal ion. These findings suggest that the compounds do not induce cellular damage or interfere with vital metabolic functions in mammalian cells, which is a critical prerequisite for their safe application in biomedical settings. Considering the potential application of the newly synthesized compounds as antimicrobial agents, their excellent biocompatibility profiles highlight a favorable therapeutic index. These characteristics position the complexes as promising candidates for further biological investigation, particularly in the development of antimicrobial agents with minimal adverse effects on host tissues.

3. Materials and Methods

3.1. Chemicals

All solvents and reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany). Melting points were measured on a Kruss M5000 melting point meter (A.Krüss Optronic GmbH, Hamburg, Germany). All solvents and reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany). Melting points were determined on a Boetius hot stage apparatus and are uncorrected. All the compounds were characterized by melting point, 1H -NMR, ^{13}C -NMR, IR, and MP-AES. IR spectra were determined on a VERTEX 70 FT-IR spectrometer (Bruker Optics, Ettlingen, Germany). $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker Avance III HD 500 spectrometer (Bruker, Billerica, MA, USA) at 500 MHz (1H -NMR) and 125 MHz (^{13}C -NMR), respectively. Chemical shifts are given in ppm and are not referenced additionally in order to facilitate the comparison with the data found in the literature. The NMR spectra were recorded at 298 K.

Microwave Plasma-Atomic Emission Spectrometry (MP-AES) Determination of Co and Ni in the Complexes

A total of 0.0200 g of sample was weighed on an analytical balance and dissolved with 65% nitric acid, p.a. (Chem-Lab NV, Zedelgem, Belgium). Blank solutions were prepared as well. After dilution, the concentration of Co and Ni was determined via MP-AES 4200 (Agilent Technologies, Santa Clara, CA, USA). Calibration standards were prepared from monoelemental standard solutions—1000 mg L^{-1} (Merck KGaA, Darmstadt, Germany). Conventional MP-AES operating conditions were used. Analytes were measured on three emission lines for estimation of potential spectral interferences, i.e., 341.476 nm, 352.454 nm, and 361.939 nm for Ni and 340.512 nm, 345.351 nm, and 350.228 nm for Co. Five replicates and 5 s measurements were applied for all lines.

3.2. Synthetic Methods Experimental Protocols

3.2.1. Synthesis of the Ligand—Ethyl (2-(Methylcarbamoyl)phenyl)carbamate L1

15 mmol methylamine was added to 10 mmol (1.63 g) isatoic anhydride in dichloromethane (30 mL), and the reaction mixture was stirred overnight at rt. The obtained anthranilamide

was treated with ethyl chloroformate (mL, 10 mol) in the presence of 10 mmol of $N(C_2H_5)_3$. In about 30 min, the reaction mixture was washed consequently with diluted HCl (1:4), Na_2CO_3 , and H_2O , then dried with anhydrous Na_2SO_4 , filtered on a short column filled with neutral Al_2O_3 , and concentrated (Supplementary Materials Figures S1–S4).

ethyl (2-(methylcarbamoyl)phenyl)carbamate (**L1**): white crystals, 80% yield, mp = 136–137 °C. 1H -NMR: 1.24 (t, $J = 7.1$, 3H, CH_2CH_3), 2.79 (d, $J = 4.4$, 3H, $NHCH_3$), 4.13 (d, $J = 6.8$, 2H, CH_2CH_3), 7.07–7.11 (m, 1H, Ar), 7.49 (t, $J = 7.8$, 1H, Ar), 8.2 (d, $J = 8.3$, 1H, Ar), 8.72 (broad s, 1H, $CONHCH_3$), 10.96 (s, 1H, $NHCOO$); ^{13}C -NMR: 169.17, 153.38, 139.7, 132.5, 128.4, 122.17, 120.04, 119.06, 61.05, 26.69, 14.86. IR (KBr, cm^{-1}): 3345 ($\nu(N-H)$), 3258 ($\nu(N-H, -NC(=O)O)$), 3116 ($\nu(Csp^2-H, Ph-)$), 3072 ($\nu(Csp^2-H, Ph-)$), 2985 ($\nu_{as}(Csp^3-H, CH_3-)$), 2951 ($\nu_{as}(Csp^3-H, CH_3-)$), 2913 ($\nu_{as}(Csp^3-H, >CH_2)$), 2880 ($\nu_s(Csp^3-H, CH_3-)$), 1739 ($\nu(C=O)$), 1664 ($\delta(-NH)+\nu(C=O)$), 1633 ($\delta(-NH)+\nu(C=O)$), 1605 ($\nu(C=C-C, Ph-)$), 1593 ($\nu(C=C-C, Ph-)$), 1554 ($\delta(-NH)$), 1530 ($\nu(C=C-C, Ph-)$), 1454 ($\nu(C=C-C, Ph-)$), 1409, 1390 ($\delta_s(-CH_3)$), 1364 ($\delta_s(-CH_3)$), 1333, 1248, 1218 ($\nu_{as}(N-CO-O-)$), 1167, 1149, 1122, 1114, 1063, 1049, 951 ($\nu_s(N-CO-O-)$), 870, 849, 820, 768, 753 ($\gamma(Csp^2-H, Ph-)$), 721, 669, 651, 536, 510. Raman (cm^{-1}) of **3**: 2939, 1730 (C=O), 1641, 1604, 1592, 1555, 1463, 1454, 1392, 1366, 1334, 1305, 1288, 1249, 1219, 1169, 1151, 1123, 1052, 1012, 915, 849, 822, 613, 395, 364.

3.2.2. Synthesis of Ni(II) and Co(II) Complexes of Ethyl (2-(Methylcarbamoyl)phenyl) carbamate **L1**, M:L:OH[−] = 1:2:2

The Ni(II) and Co(II) complexes were synthesized by mixing a DMSO solution (5 mL) containing 0.0889 g (0.0004 mol) of the ligand (**L1**) with water solution of the respective metal salts: $Ni(CH_3COO)_2 \cdot 4H_2O$ 0.0498 g (0.0002 mol) or $Co(CH_3COO)_2 \cdot 4H_2O$ 0.0498 g (0.0002 mol). It should be mentioned that the solutions were alkalized with 5 mL of 0.0160 g (0.0004 mol) of NaOH solution. After stirring with an electromagnetic stirrer for 1–2 h, light green (Ni) and purple (Co) precipitates formed. These were filtered, washed with water, air dried, and further dried over silica gel in a desiccator for one week.

3.3. Microbiological Tests

3.3.1. Tested Microorganisms

Antimicrobial Activity Assay

The agar well diffusion method was used to assess AgNPs' antibacterial properties [25]. A tiny quantity of bacterial/yeast biomass was homogenized in five milliliters of sterile 0.5% NaCl to create the inocula. Five milliliters of sterile 0.5% NaCl were added straight into the cultivation tubes to create the fungal inocula. Before being used, the fungal inocula were filtered and put in fresh tubes after being agitated by vortex V-1 plus (Biosan, Riga, Latvia). A Thoma bacterial counting chamber (Poly-Optik GmbH, Bad Blankenburg, Germany) was used to count the number of fungal spores and viable cells. After that, suspensions were made with roughly 1×10^8 cfu.mL^{−1} for yeast/bacterial cells and 1×10^5 cfu.mL^{−1} for fungal spores. The inocula were then divided among LBG/MEA media that had been partially melted and tempered at 45–48 °C. They were then transferred in 18 mL to sterile Petri plates (d = 90 mm) (Gosselin, France). Six wells (d = 6 mm) per Petri plate were drilled after the inoculated agar medium had solidified at room temperature. Duplicates of 60 μ L of the extracts were then pipetted into the agar wells. The same conditions were used to incubate the plates.

The antimicrobial activity was determined by measuring the diameter of the inhibition zones around the wells on the 24th and 48th hours of incubation. Tested microorganisms with 18 mm or more inhibition zones were considered sensitive; inhibition zones from 12 to 18 mm were considered moderately sensitive, and inhibition zones up to 12 mm were considered bacteriostatic.

3.3.2. Culture Media

Luria-Bertani agar supplemented with glucose (LBG agar) (Laboratorios Conda S.A., Madrid, Spain) was used to cultivate bacteria. 50 g of LBG agar medium base was dissolved in 1 L of deionized water at pH 7.5 ± 0.2 .

To cultivate yeasts and fungi, malt extract agar (MEA) (Scharlab S.L., Barcelona, Spain) was utilized. One liter of deionized water (pH 5.6 ± 0.2) was used to dissolve 48 g of MEA medium base. Before being used, both culture media were prepared per the manufacturer's instructions and autoclaved for 20 min at 121 °C.

3.4. Cytotoxic Activity

A number of cell viability tests were conducted against normal murine fibroblast cells (CCL-1) and human malignant leukemic cell lines (LAMA-84, K-562) to assess the experimental compounds' *in vitro* biocompatibility. The German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany) was the source of all cell lines. The cell cultures were grown in RPMI 1640, a growth medium supplemented with 10% fetal bovine serum (FBS), 5% L-glutamine, and kept at 37 °C with 5% humidified CO₂.

Cell Viability Assay

A standard MTT-based colorimetric test was used to assess cell viability. In 96-well plates, exponential-phase cells were isolated and planted at a density of 100 µL/well for suspension cultures (LAMA-84 and K-562) and 1.5×10^5 for adherent cultures (CCL-1). Different concentrations of the experimental chemicals in the 400–6.25 µM concentration range were applied to cells and cultured with them. A filter-sterilized MTT substrate solution (5 mg/mL in PBS) was applied to each culture plate well following a 72-h exposure period.

The development of purple insoluble formazan precipitates was made in 1–4 h of incubation. The latter were dissolved in an isopropyl alcohol solution with 5% formic acid before the absorbance was measured using a Labexim LMR-1 microplate reader at 550 nm. After being blanked against MTT and isopropanol solution, the collected absorbance values were standardized to the mean of the untreated control (100% cell viability). The screened compounds' half-inhibitory concentrations against each tested cell line were determined, and semi-logarithmic "dose–response" curves were created. $p < 0.05$ was regarded as a statistically significant value.

4. Conclusions

Ethyl 2-(methylcarbamoyl)phenylcarbamate and its novel mixed complexes with 3-methylquinazoline-2,4(1*H*,3*H*)-dione were obtained. The novel complexes with Ni(II) and Co(II) were spectrally characterized by their melting points, FTIR, ¹H- and ¹³C-NMR spectra. Based on the spectral data obtained, we could conclude that the nitrogen atom is involved in the coordination with the metal center. From the studies on the antibacterial activity of mixed ligand complexes with Ni(II) and Co(II), we can summarize that the Co(II) complex showed promising antimicrobial activity against all Gram-positive and Gram-negative bacteria investigated. The cytotoxic properties of the novel complexes were also investigated. Based on the obtained results, it may be concluded that the mixed cobalt complex of 2-(methylcarbamoyl)phenylcarbamate and 3-methylquinazoline-2,4(1*H*,3*H*)-dione can be attributed as a promising antimicrobial agent.

By focusing on these applications, future research can tailor these compounds for targeted therapeutic and industrial uses, addressing the growing challenge of antimicrobial resistance.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/inorganics13080267/s1>, Figure S1: ¹H-NMR spectrum of the ligand L1, Figure S2: ¹³C-NMR spectrum of L1, Figure S3: DEPT spectrum of L1, Figure S4: FT-IR spectrum of L1.

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