Synthesis, Characterization and Biological Evaluation of Novel Thiadiazoline Sulfonamides and Metal Complexes

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A B S T R A C T
In the present work, we reported a novel thiadiazoline sulfonamide derivatives and their metal complexes were efficiently synthesized based on N-phenyl 2-pyridine carbohydrazonoyl halide. Synthesized compounds were elucidated by elemental analysis and spectral data. The newly synthesized compounds were evaluated for their in vitro antimicrobial activity against Gram negative (Escherichia coli and Pseudomonas aeruginosa), Gram positive (Staphylococcus aureus and Bacillus subtilis) bacterial strains and fungal strain Saccharomyces cerevisiae. The minimum inhibitory concentration of the synthesized compounds against various microorganisms was also determined. The synthesized thiadiazoline sulfonamide derivatives and metal complexes had superoxide dismutase-like activity and inhibited superoxide radical generation. Moreover, these organic compounds induced a wide range of DNA and protein degradation processes, based on agarose gel and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analyses, respectively.

Key words: Hydrazonoyl, complexes, antimicrobial, DNA degradation, antioxidant

INTRODUCTION
The chemistry of hydrazonoyl halides has attracted wide interest due to their ability to undergo a wide variety of reactions that provide routes to a myriad of both heterocyclic and acyclic compounds (Sayed et al., 2014). In addition, diverse biologic activities, such as antiviral, antiarthropodal, antimicrobial, fungicidal, herbicidal, insecticidal, pesticidal, acaricidal and miticidal are associated with hydrazonoyl halides (Shawali, 2010; Kaugaris, 1972; Buzykin et al., 1981; Kukota et al., 1978; Strinadkin et al., 1985; Noguchi et al., 1973; Tozer et al., 1999). Sulfonamides contain a high density of hydrogen bond donor and acceptor sites, which allows them to coordinate to amino acid residues located at the active sites of enzymes (Tozer et al., 1999). Several sulfonamides of thiazole (Argyropoulou et al., 2009) and sulfathiazole complexes (Gandhi and Sekhon, 2010) have been synthesized and tested as antimicrobial agents.

Hydrazones are also useful for the synthesis of metal complexes as they easily form stable complexes with most transition metal ions (Karabocek et al., 2009; Despaigne et al., 2009). Hydrazones and their metal complexes have widespread application in technology and analytical chemistry (Tezcan et al., 2008; Mattson et al., 1947). Hydrazones are formed when hydrazines condense with aldehydes and ketones by the condensation of aldehydes (or substituted aldehydes) with phenylhydrazine (or substituted phenylhydrazine) and typically are crystalline compounds with sharp melting points (McMurry, 2003; Todeschini et al., 1998).

In the present study, a number of new heterocyclic compounds and complexes with different metals were prepared, as shown in Fig. 1. The antimicrobial, antioxidant and nuclease-like activities of thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide were studied.
Fig. 1(a-i): Synthesis of hydrazones, hydrazonoyl halides, thia diazolines and metal complexes, (a) 3a, (b) 3b, (c) 4a, (d) 7, (e) 9, (f) 12a, (g) 12b, (h) 13a-i and (i) 14

MATERIALS AND METHODS

Chemistry: All of the chemicals were purchased from Sigma-Aldrich or Fluka and used without further purification. Melting points were measured on an electrothermal Gallenkamp melting point apparatus and are uncorrected. The NMR spectra were recorded in dimethylsulfoxide (DMSO)-d6 with tetramethylsilane as an internal standard using a 300 MHz Varian Gemini spectrometer. The IR spectra were measured on Fourier Transform and Pye Unicam Infrared spectrophotometers using a potassium chloride wafer. Mass spectra were recorded on a GCMS-QP 1000 EX spectrometer at an ionizing potential of 70 eV. Electronic absorption spectra were recorded on a Perkin-Elmer Lambda 40 spectrophotometer. Elemental microanalyses were carried out at the Microanalytical Laboratory of Cairo University, Giza, Egypt. The identification of compounds from different experiments was confirmed by mixed melting points (mp) and superimposable IR spectra. Substances 4 (Zou et al., 2011) and 14 (Sayed and Wiggins, 2008) were prepared as previously described.

Synthesis of 3-phenyl-5-(pyridine-2-yl)-1,3,4-thiadiazol-2(3H) imine (7): A mixture of 1-{chloro(pyridine-2-yl)methylene}-2-phenylhydrazine 4b (2.31 g, 10 mmol) and the appropriate thiourea 5 (0.76 g, 10 mmol) in EtOH (20 mL) and dimethyl formamide (DMF, 5 mL) was boiled under reflux for 15 h. The cold reaction mixture was then poured onto ice-cold hydrochloric acid with stirring. The solid that precipitated was collected. The resulting solids were filtered, washed with water several times and crystallized from MeOH/H2O, Yellow brown solid, Yield (75%), mp 252°C, IR: 3298 (NH), 1608 (C = N), 695 (C-S-C) cm−1, 1H NMR (DMSO-d6): 6.99-8.84 (m, 10H, ArH’s, pyridinyl and NH). MS (EI) m/e (rel. int.), 254 (M+, 60). Anal. Calcd for C13H10N4S (254.31): C, 61.40; H, 3.96; N, 22.03, Found: C, 61.38; H, 4.01; N, 22.11%.

Synthesis of sulfonamide derivatives (9) and (12a,b): A mixture of 4b or 7 (6 mmol) in anhydrous pyridine (10 mL) and the appropriate sulfonamide or sulfonyl chloride (6.3 mmol) was gradually added to the mixture. The reaction mixture was heated at 60°C for 4 h, then poured into ice water and acidified with 1N HCl. The solid product was filtered, washed well with water and crystallized from methanol.

Preparation of 1-{(4-nitrophenylsulfonamide) (pyridine-2-yl)methylene}-2-phenylhydrazine (9): Yellow brown, Yield (76%), mp 152°C, IR: 3263 (NH), 1350,1157 (SO2), 1620 (C = N), 1542 (pyridinyl), 1481 (C = C) cm−1, 1H NMR (DMSO-d6): 6.78-8.88 (m, 13H, ArH’s and pyridinyl) and 10.54 (s, H, NH), 11.02 (s, 1H, NH) ppm MS (EI) m/e (rel. int.), 397 (M+, 43), Anal. Calcd for C18H15N5O4S (397.08): C, 54.40; H, 3.80, N, 17.62; Found: C, 54.36, H, 3.88; N, 17.58%.

3-phenyl-5-(pyridine-2-yl)-1,3,4-thiadiazole-2 (3H) imine (12a): Brown, Yield (66%), mp 228°C, IR: 1350, 1157 (SO2) cm−1, 1H NMR (DMSO-d6): 7.24-8.01 (m, 13H, ArH’s and pyridinyl) ppm. MS (EI) m/e (rel. int.), 408 (M+, 25), Anal. Calcd for C20H16N4O2S2 (408.07): C, 58.80; H, 3.95; N, 13.72; Found: C, 58.75; H, 3.97; N, 13.91%.

3-phenyl-5-(pyridine-2-yl)-2-(4-methylphenylsulfonamide)-1,3,4-thiadiazole (12b): Yellow green, Yield (70%), mp 183°C, IR: 1350, 1157 (SO2) cm−1, 1H NMR (DMSO-d6): 7.01-8.52 (m, 12H, ArH’s, pyridinyl and thiophenyl) ppm. MS (EI) m/e
Synthesis of metal complexation of 1-{chloro(pyridine-2-yl)methylene}-2-phenylhydrazine (13a-i): Equimolar ratio of metal salt and 1-{chloro(pyridine-2-yl)methylene}-2-phenylhydrazine 4b in DMF were refluxed for 3 h and then poured into cold-ice water. The solid product was filtered, washed many times with water and then dried in an oven.

[Ni(L)Cl2] (13a): Pale green, Yield (80%), mp>300°C, IR: 3178 (NH), 1626 (C = N), 1573 (pyridinyl), 1488 (C = C), 1149 (N-N) cm⁻¹. MS (EI) m/e (rel. int.), 324 (M⁺, 75), Anal. Calcd for C₁₂H₁₀Cl₃Ni (324.93): C, 39.89; H, 2.79, N, 11.63, Found: C, 39.91, H, 2.76, N, 11.65%.

[Mg(L)Cl2] (13b): Yellow brown, Yield (65%), mp>300°C, IR: 3179 (NH), 1642 (C = N), 1558 (pyridinyl), 1434 (C = C), 1149 (N-N) cm⁻¹. MS (EI) m/e (rel. int.), 359 (M⁺, 19), Anal. Calcd for C₁₂H₁₀Cl₃MgN₃ (359.93): C, 39.91, H, 2.79, N, 11.63%.

[Co(L)Cl2] (13c): Brown, Yield (70%), mp>300°C, IR: 3055 (NH), 1733 (C = O), 1643 (C = N), 1573 (pyridinyl), 1488 (C = C), 1150 (N-N) cm⁻¹. MS (EI) m/e (rel. int.), 391 (M⁺, 65), Anal. Calcd for C₁₂H₁₀Cl₃FeN₃ (391.90): C, 36.59, H, 2.56, N, 10.67, Found: C, 36.61; H, 2.56, N, 10.69%.

[Fe(L)Cl3] (13b): Brown, Yield (70%), mp>300°C, IR: 3055 (NH), 1733 (C = O), 1643 (C = N), 1573 (pyridinyl), 1488 (C = C), 1150 (N-N) cm⁻¹. MS (EI) m/e (rel. int.), 391 (M⁺, 65), Anal. Calcd for C₁₂H₁₀Cl₃FeN₃ (391.90): C, 36.59, H, 2.56, N, 10.67, Found: C, 36.61; H, 2.56, N, 10.69%.

[Zn(L)(OAc)2] (13j): Brown, Yield (55%), mp 206°C, IR: 3433 (OH), 3101 (NH), 1639 (C = N), 1574 (pyridinyl), 1489 (C = C), 1150 (N-N) cm⁻¹. ¹H NMR (dimethyl sulfoxide-d₆): 6.81-8.33 (m, 9H, ArH’s and pyridinyl) and 10.59 (s, H, NH) ppm. m/z (%) = 413 (M⁺, 38), Anal. Calcd for C₁₂H₁₀Cl₃O₂Zn (413.01): C, 46.29; H, 3.88, N, 10.12, Found: C, 46.31, H, 3.82; N, 10.14%.

Antibacterial and antifungal activities: Antimicrobial studies of thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide were performed using the cup diffusion technique (Bauer et al., 1966). The test was carried out against the Gram-negative bacterial strains Pseudomonas aeruginosa and Escherichia coli (E. coli), the Gram-positive bacterial strains Bacillus subtilis (B. subtilis) and Staphylococcus aureus (S. aureus) and the fungal strain Saccharomyces cerevisiae (S. cerevisiae). The new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide were dissolved in DMSO at a concentration of 1 mg mL⁻¹. Luria-Bertani Agar (LBA) Medium (10 g bacto-tryptone, 5 g yeast extract, 20 g agar and 10 g NaCl in 1 de-ionized water) was made for inoculation and bacterial growth. An aliquot of the solution of the tested thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide equivalent to 100 mg was placed separately in cups cut in the agar. The LBA plates were incubated for 24 h at 37°C and the resulting inhibition zones were measured. From the inhibition zone diameter data analysis, the antimicrobial activity against the Gram-negative and Gram-positive bacteria and fungus were determined.

Minimum inhibitory concentrations (MIC): The MIC of the new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide was determined according to the standard procedures summarized in the CLSI/NCCLS methods (CLSI, 2006). In brief, various concentrations (0.5-50 μg mL⁻¹) of the new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide in DMSO were individually added to the sterilized LB broth solution. Test cultures (Gram-negative bacterial strains P. aeruginosa and E. coli, Gram-positive bacterial strains B. subtilis and S. aureus) were selected for the present study. All bacterial strains were grown in LB broth separately with 50 mL containing approximately 5×10⁶ colony-forming units of 18 h grown cultures of each organism to be tested. Microorganisms were inoculated and the final Optical Density (OD) of the test solution was kept as 0.5 and thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide were added to each well. Incubation was performed at 37°C for 24 h. The MIC was determined as the lowest concentration of the compound that inhibited visible growth.
carbohydrazonoyl halide were incubated at 37°C for 24 h. Respective blanks (culture and broth alone) were maintained accordingly. Following incubation, the turbidity of the growth medium was measured at 600 nm and the reduction in colony-forming units was calculated using the spread plate technique. The concentration at which the solution (OD = 0.5) turned turbid was considered the MIC of the test samples. Experiments were performed in duplicate.

**Determination of superoxide dismutase (SOD)-like activity**: The new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide were assayed for SOD enzyme-like activity (Bridges and Salin, 1981). The SOD-like activity of the thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide was assayed using phenazine methosulfate to generate superoxide anion radicals at pH = 8.3 (phosphate buffer). Reduction of nitro blue tetrazolium to form blue formazan was used as an indicator of superoxide production and was measured spectrophotometrically at 560 nm. The addition of phenazine methosulfate (9.3×10⁻⁵ M) to a solution of nitro blue tetrazolium (3×10⁻³ M), NADH (4.7×10⁻⁵ M) and phosphate buffer (final volume = 1 mL) led to a change in OD at 560 nm per 4 min. The reactions in blank samples and in the presence of the thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide were measured. For comparison, the activity of native horseradish superoxide dismutase (HR SOD) was also determined.

The superoxide radical scavenging ratio (%) was calculated using the following formula:

\[
\text{Superoxide radical scavenging ratio (\%) = } \left( \frac{\Delta A - \Delta A_1}{\Delta A} \right) \times 100
\]

where, A is the absorbance of the positive control and A₁ is the absorbance of the test samples.

**Estimation of antioxidant activities of the thiadiazoline sulfonamide derivatives using 2, 2-diphenyl-1-picrylhydrazyl (DPPH)**: The antioxidant activities of the new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide were tested according to the previously reported method (Gulcin, 2010). The purple-colored DPPH is a stable free radical, which is reduced to the yellow 2,2-diphenyl-1-picrylhydrazine when allowed to react with an antioxidant. Briefly, 5 mL of 0.3 mM DPPH ethanolic solution was added to 50 µg of the new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide samples in 100 µL DMSO and mixed vigorously. Negative controls were prepared by adding 100 µL DMSO and 5 mL of 0.3 mM DPPH ethanolic solution. Absorbance of the samples was measured at 517 nm after incubation at room temperature for 30 min in the dark. Ascorbic acid was used as an antioxidant standard. Determination of antioxidant activities of each new thiadiazoline sulfonamide derivative and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide using DPPH was carried out in triplicate. The percentage of antioxidant activity was determined using the following formula:

\[
\text{Antioxidant activity (\%) = 100 - (Absorbance of sample - Absorbance of blank) \times 100}
\]

**Agarose gel electrophoresis**: The new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide (20 mg) were each added to 1 µg of the DNA isolated from E. coli strain W3110 (Genthner et al., 1985). The samples were incubated for 1 h at 37°C. The DNA was analyzed using horizontal agarose gel electrophoresis. The electrophoresis was performed using 0.7% (w/v) agarose gels in TAE buffer [5 mM sodium acetate, 1 mM EDTA and 0.04 M Tris-HCl (pH 7.9)]. The agarose gels were stained with ethidium chloride (0.5 µg mL⁻¹) and the DNA was visualized on a UV transilluminator (Sambrook et al., 1989).

**Polyacrylamide gel electrophoresis**: Bovine serum albumin (BSA, 1 mg) was treated with each of the new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide (20 mg). The reaction mixtures were incubated for 1 h at 37°C. The protein samples were analyzed using vertical one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Laemmli, 1970).

**RESULTS AND DISCUSSION**

**Chemistry**: In continuation of our studies of hydrazonoyl halides and their reaction (Sayed et al., 2014), we treatment of 1-(chloro(pyridine-2-yl)methylene)-2-phenylhydrazine 4b with thiourea 5 in ethanol under heating gave a single isolated 3-phenyl-5-(pyridine-2-yl)-1,3,4-thiadiazol-2(3H)imine 7 via elimination of HBr and ammonia, as shown in Fig. 2. The structures of the final products 12a,b were elucidated, as shown in Fig. 4.

The new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide were incubated at 37°C for 24 h. Respective blanks (culture and broth alone) were maintained accordingly. Following incubation, the turbidity of the growth medium was measured at 600 nm and the reduction in colony-forming units was calculated using the spread plate technique. The concentration at which the solution (OD = 0.5) turned turbid was considered the MIC of the test samples. Experiments were performed in duplicate.

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\]

where, A is the absorbance of the positive control and A₁ is the absorbance of the test samples.

**Estimation of antioxidant activities of the thiadiazoline sulfonamide derivatives using 2, 2-diphenyl-1-picrylhydrazyl (DPPH)**: The antioxidant activities of the new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide were tested according to the previously reported method (Gulcin, 2010). The purple-colored DPPH is a stable free radical, which is reduced to the yellow 2,2-diphenyl-1-picrylhydrazine when allowed to react with an antioxidant. Briefly, 5 mL of 0.3 mM DPPH ethanolic solution was added to 50 µg of the new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide samples in 100 µL DMSO and mixed vigorously. Negative controls were prepared by adding 100 µL DMSO and 5 mL of 0.3 mM DPPH ethanolic solution. Absorbance of the samples was measured at 517 nm after incubation at room temperature for 30 min in the dark. Ascorbic acid was used as an antioxidant standard. Determination of antioxidant activities of each new thiadiazoline sulfonamide derivative and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide using DPPH was carried out in triplicate. The percentage of antioxidant activity was determined using the following formula:

\[
\text{Antioxidant activity (\%) = 100 - (Absorbance of sample - Absorbance of blank) \times 100}
\]

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**Polyacrylamide gel electrophoresis**: Bovine serum albumin (BSA, 1 mg) was treated with each of the new thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide (20 mg). The reaction mixtures were incubated for 1 h at 37°C. The protein samples were analyzed using vertical one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Laemmli, 1970).
Based on our review of the current literature, studies of the reaction of hydrazonoyl halides with different transition metals have not been performed. The general method used to prepare different transition metals is shown in Fig. 5. The designed metal complexes 13a-i were prepared by heating the hydrazonoyl (Ligand, L) with different metals in a 1:1 molar ratio in DMF for several hours. The level of purity of the ligand was verified by thin layer chromatography. During the course of this study we obtained complexes in good yields. Hydrazonoyl complexes 13a-i have an imine group (-N = CH-) that imparts chelating properties towards the central metal atom. The elemental analyses were in good agreement with the proposed composition of the ligand and its complexes. All of the complexes were stable at room temperature.

Preliminary identification of the formation of each ligand and its complexes was achieved using IR spectroscopy. In these complexes the IR spectrum of the C = N band undergoes a positive shift of 10-20 cm\(^{-1}\) that is attributed to coordination of the nitrogen atom. NMR spectra for 13a-i could not be recorded due to the paramagnetic complexes and poor solubility of the isolated products in the NMR solvents examined. All attempts to obtain a single crystal of the complexes suitable for X-ray crystallography failed. Finally, the mode of coordination of the ligand complexes was supported by thermogravimetric studies in which the weight of the remaining residues coincided with the weight of the metal.

**Biology:** Tetracycline, a broad-spectrum antibiotic was used as a positive control for the antimicrobial study. The results of the antimicrobial assessment of the thiadiazolone sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide against Gram-negative *E. coli* and *P. aeruginosa* and Gram-positive *S. aureus* and *B. subtilis* bacterial strains are shown in Table 2. The Pb complex with N-phenyl 2-pyridine carbohydrazonoyl halide 13c had maximal antimicrobial activity based on inhibition zone diameters against *P. aeruginosa* of 23 mm, *E. coli* and *B. subtilis* 22 mm, *S. aureus* 17 mm and *S. cerevisiae* 15 mm.
The Zn complex with N-phenyl 2-pyridine carbohydroxynol halide 13i had a wide spectrum of antimicrobial activity with inhibition zone diameters against E. coli and P. aeruginosa of 21 mm, B. subtilis and S. aureus 19 mm and S. cerevisiae 14 mm. The Cr, Cu, Co, Ni and Fe complexes with N-phenyl 2-pyridine carbohydroxynol halide 13d, 13e, 13g, 13a and 13h, respectively had considerable antimicrobial activity (Table 2). The thiadiazoline sulfonamide derivatives 3a, 4a, 7 and 9 series exhibited moderate antimicrobial activity with smaller inhibition zone diameters against the tested microorganisms compared to the metal complexes with N-phenyl 2-pyridine carbohydroxynol halide series (Table 2). All of the metal complexes with N-phenyl 2-pyridine carbohydroxynol halide had high antibacterial activity compared with the positive control. The increased antimicrobial activity of the metal complexes with N-phenyl 2-pyridine carbohydroxynol halide may be due to alterations in the structure as coordination and chelating tend to make metal complexes perform as more powerful bacteriostatic agents (El-Ayaan et al., 2009; Raman and Mahalakshmi, 2014). Moreover, coordination decreases the polarity of the metal ion, mostly because of the partial distribution of its positive charge with the donor groups within the chelate ring system formed during the coordination. This process increases the lipophilic nature of the central metal atom, which favors its more efficient penetration through the lipid layer of the microorganism, thus destroying them more aggressively.

The MIC of the thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydroxynol halide against different bacterial and fungal strains was established using the liquid dilution method. The lowest concentration at which no growth was observed was defined as the MIC value (Table 3). Comparison of the MICs (in µg mL⁻¹) of the thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydroxynol halide against susceptible microorganisms, using tetracycline as a standard is shown in Table 3.

The Cr, Fe, Pb and Zn complexes with N-phenyl 2-pyridine carbohydroxynol halide 13d, 13h, 13c and 13i, respectively had the highest activity against all of the bacterial and fungal strains examined (MIC = 25-35 µg mL⁻¹). The Co, Ni and Cu complexes with the N-phenyl 2-pyridine carbohydroxynol halide 13g, 13a and 13e, respectively with MICs of 30-35 µg mL⁻¹ had good inhibitory activity against all of the tested microorganisms. Furthermore, compounds 14, 12a and 12b having thiadiazoline sulfonamide derivatives exhibited potent inhibitory activity (MIC = 30-35 µg mL⁻¹) against all of the microorganisms. A possible explanation for this result is that the antibacterial activity of these compounds stems from the basic skeleton of the molecules as well as from the nature of the substituents such as oxygen, nitrogen and sulfur atoms.

The SOD-like activity of the thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydroxynol halide is shown in Table 4. The Zn, Fe, Cu, Ni, Mn and Cr complexes with the N-phenyl 2-pyridine carbohydroxynol halide, 13i, 13h, 13e, 13a, 13f and 13d, respectively, exhibited significant SOD-like activity with a percentage of inhibition of 57.4, 55.9, 54.7, 54.6 and 51.1%,
respectively. Thiadiazoline sulfonamide derivatives 12b and 12a displayed significant SOD-like activity (Table 4) with percentage of inhibition of 56.0 and 51.6%, respectively. In addition, Mg and Co complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide, 13b and 13g, respectively had moderate SOD-like activity (Table 4) with percentage of inhibition of 49.1 and 48.7%, respectively.

In the present study, the Zn, Fe, Cu, Ni, Mn and Cr complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide and the thiadiazoline sulfonamide derivatives 12b and 12a inhibited superoxide radical generation. Maintaining the balance between the rate of radical generation and the rate of radical scavenging is an essential part of biological systems. Therefore, it is suggested that the inhibition of superoxide radical generation by these metal complexes and thiadiazoline sulfonamide derivatives 12b and 12a is due to their free radical scavenging activity.

An antioxidant is defined as a substance that significantly delays or inhibits the oxidation process. A quick, simple and low-cost procedure for measuring the antioxidant activity of thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide involves the use of the free radical DPPH. DPPH is a stable free radical utilized to measure the antioxidant activity of tested compounds. The antioxidant effect of the thiadiazoline sulfonamide derivatives and metal complexes 13a-i by DPPH is shown in Fig. 6. The thiadiazoline sulfonamide derivatives with a higher antioxidant activity (>50%) were 4b (51.6), 9 (52.4), 14 (61.1), 12a (52.4), 13a (56.5) and 3a (48.4%). While the metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide with increased antioxidant activity were 13e (51.2), 13f (55.4), 13b (61.1), 13a (57.4), 13g (56.5) and 13h (55.5%), compared to the control (73.3%). Free radicals play an important role in inflammatory processes. Thus, compounds with possible antioxidant properties could play a crucial role against inflammation and lead to potentially effective drugs. Antioxidants that exhibit radical scavenging activity are attracting increased attention because they present interesting anticancer, anti-aging and anti-inflammatory activities (Raja et al., 2012, 2011). Therefore, compounds with antioxidant properties may offer protection against rheumatoid arthritis and inflammation.

The degradation effect of 20 mg of the thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide on DNA in vitro is shown in Fig. 7. The negative control (DNA only) and the positive control DNA in DMSO did not undergo degradation during the incubation period (Fig. 7, lanes 1 and 2). The thiadiazoline sulfonamide derivatives 12a-14 completely degraded the DNA (Fig. 7, lanes 7-8). In addition, metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide 13d and 13e mimicked a nuclease and completely degraded the DNA (Fig. 7, lanes 11 and 12). Metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide 4a also exhibited a strong nuclease activity and degraded the DNA (Fig. 7, lanes 10-13). Metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide 13a, 13g and 13h induced considerable degradation (Fig. 7, lanes 16-18). On the other hand, thiadiazoline sulfonamide derivatives 7 and 9 and metal complex 13c had weak degradation effects on the DNA (Fig. 7, lanes 6, 7 and 13). It is clear that the metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide had a strong degradation effect on the DNA in vitro. Metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide have attracted special attention as endonuclease mimics. Different mechanisms for DNA cleavage have been assumed, including hydrolytic effects or oxidative pathways. Hydrolytic cleavage directly breaks down the phosphodiester bond but does not result in sugar damage (Youssef et al., 2012; Ibrahim et al., 2011). The phosphodiester backbone of DNA is stable and resists hydrolytic cleavage. Metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide exhibit nuclease activity. This is a valuable feature for application as a chemotherapeutic agent in anticancer treatments. This result is consistent with that of analogous metal complexes that catalytically cleave target DNA in the absence of any external reductant (Arjmand et al., 2013; El-Ayaan et al., 2009).

The present study demonstrates that metal complexes with N-phenyl 2-pyridine carbohydrayzonoyl halide have significant nuclease activity toward the cleavage of genomic DNA in the absence of any external additives. Therefore, metal complexes...
with N-phenyl 2-pyridine carbohydrazonoyl halide can be used as promising anti-tumor agents in vivo to inhibit DNA replication in cancer cells and to prevent tumor growth. More work in vivo must be carried out to elucidate their precise role and to understand the exact pathway of this metal complex series in vivo.

Additional biochemical studies were performed to evaluate the effects of thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide on BSA as a high molecular weight biotic compound. The effects of the tested compounds on BSA are shown in Fig. 8 and 9. As a control, BSA and BSA in DMSO were used (Fig. 8 and 9, lanes 1 and 2).

Thiadiazoline sulfonamide derivatives 3a, 4a, 7, 9, 14, 12a and 12b partially degraded the BSA (Fig. 8, lanes 3-9, respectively). Metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide 13i, 13d, 13f and 13a completely degraded the BSA (Fig. 8, lane 10 and Fig. 9, lanes 3, 5 and 7). The metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide 13e, 13c, 13b, 13g and 13h had strong degradation effects on BSA compared to the control (Fig. 9, lanes 4, 5, 7, 9 and 10). Metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide interact with nucleophilic molecules, including DNA and proteins. In the present study, the metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide degraded DNA and protein in vitro. Metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide may also interact with DNA, forming inter and intra-strand adducts and hindering DNA replication, thus leading to cell cycle arrest and apoptosis.

CONCLUSION

In conclusion, several new thiadiazoline sulfonamide derivatives and metal complexes were prepared based on N-phenyl 2-pyridine carbohydrazonoyl halide. These organic compounds exhibited antimicrobial and antioxidant activities. In addition, the newly synthesized compounds degraded both DNA and protein, based on horizontal and vertical gel electrophoresis, respectively.

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