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

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ABSTRACT
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.

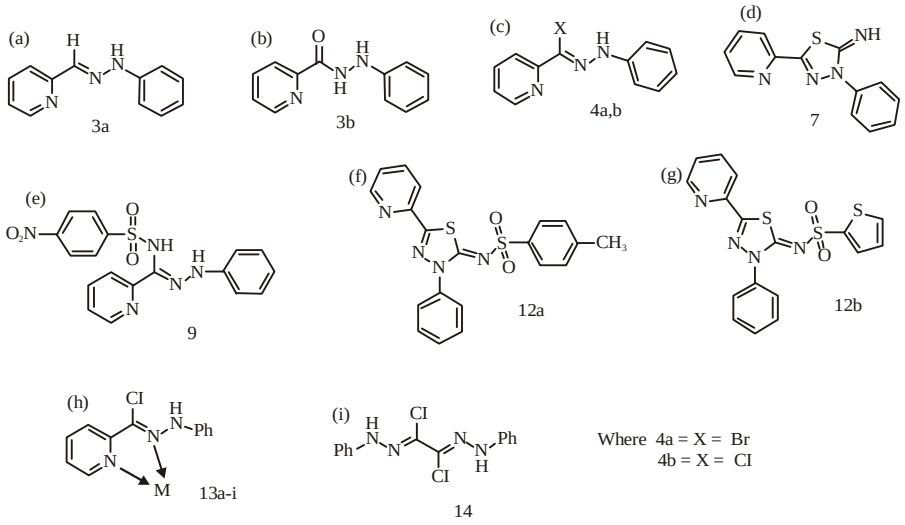


Fig. 1(a-i): Synthesis of hydrazone, hydrazonoyl halides, thiadiazolines and metal complexes, (a) 3a, (b) 3b, (c) 4a,b, (d) 7, (e) 9, (f) 12a, (g) 12b, (h) 13a-i and (i) 14

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)-d_6 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 (2.31g, 10 mmol) and the appropriate thiourea 5 (0.76 g, 10 mmol) 2 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/H_2O, Yellow brown solid, Yield (75%), mp 252°C, IR: 3298 (NH), 1608 (C = N), 695 (C-S-C) cm^{-1}, ^1H NMR (DMSO-d_6): 6.99-8.84 (m, 10H, ArH's, pyridinyl and NH). MS (EI) m/e (rel. int.), 254 (M^+, 60). Anal. Calcd for $C_{13}H_{10}N_4S$ (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 (SO_2), 1620 (C = N), 1542 (pyridinyl), 1481 (C = C) cm^{-1}. ^1H NMR (DMSO-d_6): 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 $C_{18}H_{15}N_5O_4S$ (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)-2-(4-methylphenylsulfonamide)-2 (3H)-1,3,4-thiadiazole (12a): Brown, Yield (66%), mp 228°C, IR: 1350, 1157 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6): 2.39 (s, 3H, CH_3), 7.24-8.01 (m, 13H, ArH's and pyridinyl) ppm. MS (EI) m/e (rel. int.), 408 (M^+, 25), Anal. Calcd for $C_{20}H_{16}N_4O_2S_2$ (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-(thiophene-2-yl)-2(3H)-1,3,4-thiadiazole (12b): Yellow green, Yield (70%), mp 183°C, IR: 1350, 1157 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6): 7.01-8.52 (m, 12H, ArH's, pyridinyl and thiophenyl) ppm. MS (EI) m/e

(rel. int.), 400 (M^+, 65), Anal. Calcd for $C_{17}H_{12}N_4O_2S_3$ (400.5): C, 50.98; H, 3.02, N, 13.99; Found: C, 51.01, H, 3.06, N, 13.78%.

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)Cl_2] (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.), 359 (M^+, 19), Anal. Calcd for $C_{12}H_{10}Cl_3N_3Ni$ (358.93): C, 39.89; H, 2.79, N, 11.63, Found: C, 39.91, H, 2.76, N, 11.65%.

[Mg(L)Cl_2] (13b): Yellow brown, Yield (65%), mp>300°C, IR: 3179 (NH), 1642 (C = N), 1558 (pyridinyl), 1434 (C = C), 1149 (N-N) cm^{-1}. ^{1}H NMR (dimethyl sulfoxide-d_6): 6.81-8.36 (m, 9H, ArH's and pyridinyl) and 10.59 (s, H, NH) ppm. m/z (%) = 324 (M^+, 75), Anal. Calcd for $C_{12}H_{10}Cl_3MgN_3$ (324.98): C, 44.09, H, 3.08; N, 12.85, Found: C, 44.01, H, 3.06, N, 12.81%.

[Pb(L)Cl_2] (13c): Yellow green, Yield (71%), mp>300°C, IR: 3141 (NH), 1634 (C = N), 1573 (pyridinyl), 1434 C = C), 1150 (N-N) cm^{-1}. ^{1}H NMR (dimethyl sulfoxide-d_6): 6.89-8.31 (m, 9H, ArH's and pyridinyl) and 10.57 (s, H, NH) ppm. m/z (%) = 508 (M^+, 19), Anal. Calcd for $C_{12}H_{10}Cl_3N_3Pb$ (508.97): C, 28.27, H, 1.98, N, 8.24, Found: C, 28.22, H, 1.94, N, 8.29%.

[Cr(L)Cl_2](H_2O)$_6$ (13d): Pale green, Yield (76%), mp>300°C, IR: 3456 (OH), 3124 (NH), 1647 (C = N), 1543 (pyridinyl), 1434 (C = C), 1149 (N-N) cm^{-1}. MS (EI) m/e (rel. int.), 352 (M^+, 32), Anal. Calcd for $C_{12}H_{10}Cl_3CrN_3$ (352.93): C, 40.65, H, 2.84, N, 11.85, Found: C, 40.62; H, 2.82, N, 11.89%.

[Cu(L)Cl_2] (13e): Deep brown Yield (79%), mp>300°C, IR: 3078 (NH), 1651 C = N), 1545 (pyridinyl), 1451 C = C), 1164 (N-N) cm^{-1}. MS (EI) m/e (rel. int.), 363 (M^+, 20) Anal. Calcd for $C_{12}H_{10}Cl_3CuN_3$ (363.92): C, 39.37; H, 2.75, N, 11.48, Found: C, 39.34, H, 2.79, N, 11.48%.

[Mn(L)Cl_2] (13f) Brown, Yield (66%), mp>300°C, IR: 3068 (NH), 1620 C = N), 1572 (pyridinyl), 1434 (C = C), 1147 (N-N) cm^{-1}. MS (EI) m/e (rel. int.), 355 (M^+, 15), Anal. Calcd for $C_{12}H_{10}Cl_3MnN_3$ (355.93): C, 40.31, H, 2.82, N, 11.75, Found: C, 40.35, H, 2.81, N, 11.79%.

[Co(L)Cl_2] (13g): Brown, Yield (72%), mp>300°C, IR: 3070 (NH), 1650 C = N), 1589 (pyridinyl), 1488 (C = C), 1149 (N-N) cm^{-1}. MS (EI) m/e (rel. int.), 359 (M^+, 48), Anal. Calcd for $C_{12}H_{10}Cl_3CoN_3$ (359.93): C, 39.87, H, 2,79, N, 11.62, Found: C, 39.83; H, 2.81, N, 11.65%.

[Fe(L)Cl_3] (13h): 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 $C_{12}H_{10}Cl_4FeN_3$ (391.90): C, 36.59, H, 2.56, N, 10.67, Found: C, 36.61; H, 2.56, N, 10.69%.

[Zn(L)(OAc)$_2$] (13i): Brown, Yield (55%), mp 206°C, IR: 3433 (OH), 3101 (NH), 1639 (C = N), 1574 (pyridinyl), 1489 (C = C), 1150 (N-N) cm^{-1}. ^{1}H NMR (dimethyl sulfoxide-d_6): 1.98 (s, 6H, $2CH_3$). 7.02-8.33 (m, 9H, ArH's and pyridinyl) and 10.55 (s, H, NH) ppm. m/z (%) = 413 (M^+, 38), Anal. Calcd for $C_{16}H_{16}ClN_3O_4Zn$ (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* (*P. 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 l 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 5×10^4 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 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^{-5} M), NADH (4.7×10^{-4} 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 A_1 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:

Antioxidant activity (%) = 100 - (Absorbance of sample - Absorbance of blank)×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 designed sulfonamides (Argyropoulou *et al*., 2009) were prepared by heating the appropriate amine or other analogue with the select sulfonyl derivatives. By extending these principles, we reacted 4b with 8 in pyridine to obtain sulfonamide 9. The structure of 9 was confirmed by elemental analysis and by MS, IR and NMR spectral data, as shown in Fig. 2.

Sulfonamides 12a,b were prepared by heating the appropriate 3-phenyl-5-(pyridine-2-yl)-1,3,4-thiadiazol-2 (3H) imine 7 with sulfonyl chlorides 11 in pyridine for several hours to give 12a,b. The structures of the final products 12a,b were confirmed by elemental analysis and spectral data, as shown in Fig. 3.

The actual tautomer structures of compounds 3a, 4b and 13a-i were elucidated, as shown in Fig. 4.

As shown in Table 1 the electronic absorption spectra in DMF had, in each case, two bands in the regions 400-354 and 331-302 nm, characteristic of their hydrazo chromophore. The absorption pattern for compounds 3aA, 4bA and (13a-i) A is similar to that of a typical hydrazone chromophore (Shawali *et al*., 2002) and excludes the azo tautomeric forms 3B, 4B and (13a-i) B.

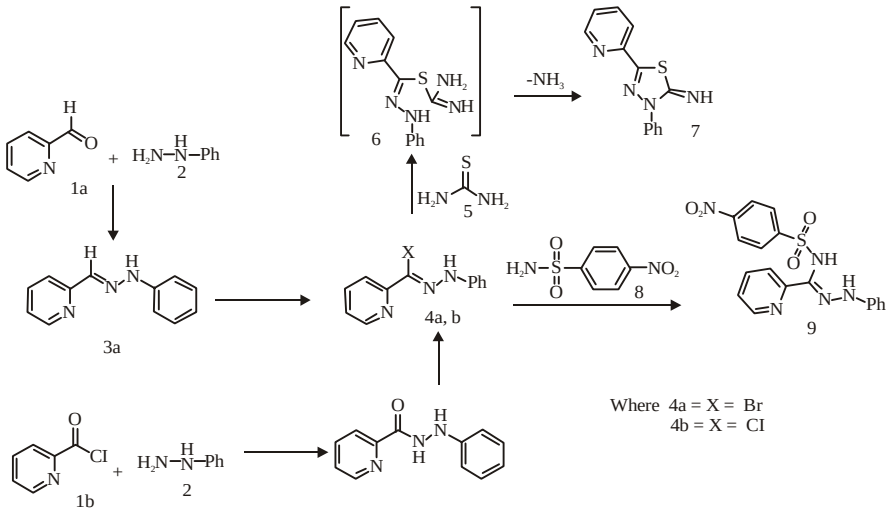


Fig. 2: Synthesis of hydrazonoyl 4, thiadiazoline 7 and sulfonamide 9

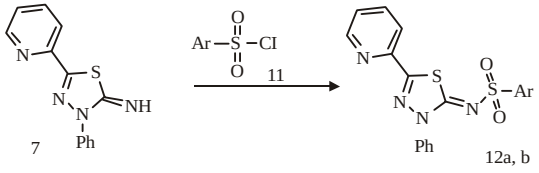


Fig. 3: Synthesis of 2-thiazoline sulfonamide 12a,b

Table 1: Electronic absorption spectra in dimethylformamide

Compound No.	λ_{max} (log ε)
3a	354 (4.83), 302 (4.80)
4a	355 (4.26), 314 (4.61)
13a	357 (4.62), 314 (4.75)
13b	360 (4.65), 311 (4.73)
13c	356 (4.62), 313 (4.75)
13d	360 (4.25), 311 (4.61)
13e	361 (4.80), 313 (4.93)
13f	360 (4.63), 312 (4.85)
13g	361 (4.60), 312 (4.82)
13h	355 (4.40), 314 (4.72)
13i	355 (4.57), 312 (4.89)

Table 2: Effect of thiadiazoline, sulfonamides derivatives and the metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide on some microorganisms

Parameters	*E. coli*	*B. subtilis*	*P. aeruginosa*	*S. aureus*	*S. cerevisiae*
Tetracycline	23	24	21	20	18
Sample 3a	14	11	16	12	13
Sample 4a	16	14	15	14	9
Sample 7	12	15	17	13	11
Sample 9	14	16	15	17	13
Sample 14	17	15	19	15	12
Sample 12a	18	19	20	17	14
Sample 12b	17	14	18	18	13
Sample 13i	21	19	21	19	14
Sample 13d	20	17	20	18	12
Sample 13e	19	21	19	14	11
Sample 13c	22	22	23	17	15
Sample 14f	20	19	21	14	13
Sample 13b	17	18	22	16	12
Sample 13a	18	15	19	14	15
Sample 13g	19	18	17	18	14
Sample 13h	17	20	22	16	13

Results expressed as zone inhibition in mm diameter

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 thiadiazoline 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.

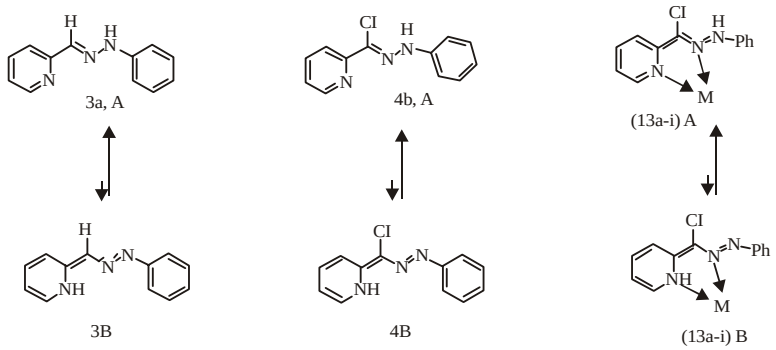
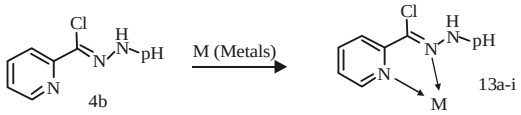


Fig. 4: Tautomer structures of compounds 3a, 4b and 13a-i



M (metals salt): a: $NiCl_2.6H_2O$, b: $MgCl_2.6H_2O$, c: $PbCl_2$., d: $CrCl_2.7H_2O$, e: $CuCl_2.4H_2O$, f: $MnCl_2.4H_2O$, g: $CoCl_2.4H_2O$, h: $FeCl_3$, i: Zn $(CH_3COO)_2$

Fig. 5: Synthesis of metal complexation 13a-i

Table 3: MIC of thiadiazoline, sulfonamides derivatives and the metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide (μg mL^{-1}) against some microorganisms

Parameters	*E. coli*	*B. subtilis*	*P. aeruginosa*	*S. aureus*
Tetracycline	20	25	25	15
Sample 3a	45	50	45	40
Sample 4	40	40	45	35
Sample 7	40	45	40	35
Sample 9	35	35	40	30
Sample 14	30	30	35	35
Sample 12a	30	35	30	35
Sample 12b	30	35	35	30
Sample 13i	30	30	30	25
Sample 13d	25	30	25	35
Sample 13e	30	35	35	30
Sample 13c	25	25	30	35
Sample 14f	30	40	35	30
Sample 13b	35	35	40	35
Sample 13a	35	30	35	30
Sample 13g	30	35	35	35
Sample 13h	25	30	35	30

The Zn complex with N-phenyl 2-pyridine carbohydrazonoyl 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 carbohydrazonoyl 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 carbohydrazonoyl halide series (Table 2). All of the metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide had high antibacterial activity compared with the positive control. The increased antimicrobial activity of the metal complexes with N-phenyl 2-pyridine carbohydrazonoyl 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 carbohydrazonoyl 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 carbohydrazonoyl 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 carbohydrazonoyl 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 carbohydrazonoyl 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 carbohydrazonoyl halide is shown in Table 4. The Zn, Fe, Cu, Ni, Mn and Cr complexes with the N-phenyl 2-pyridine carbohydrazonoyl 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%,

Table 4: Superoxide (SOD) like activity of thiadiazoline, sulfonamides derivatives and the metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide as antioxidant enzyme

	SOD like activity	
Parameters	Δ Through 4 min	Inhibition (%)
Control	0.698	-
HR SOD	0.157	77.5
Sample 3a	0.438	37.2
Sample 4a	0.372	46.7
Sample 7	0.385	44.8
Sample 9	0.364	47.9
Sample 14	0.406	41.8
Sample 12a	0.338	51.6
Sample 12b	0.307	56.0
Sample 13i	0.297	57.4
Sample 13d	0.341	51.1
Sample 13e	0.316	54.7
Sample 13c	0.384	44.9
Sample 13f	0.325	53.4
Sample 13b	0.355	49.1
Sample 13a	0.317	54.6
Sample 13g	0.358	48.7
Sample 13h	0.308	55.9

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 carbohydrazonoyl 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 carbohydrazonoyl 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 ability of the thiadiazoline sulfonamide derivatives and metal complexes with N-phenyl 2-pyridine carbohydrazonoyl 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 carbohydrazonoyl 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

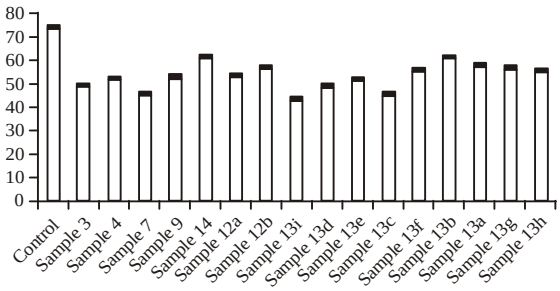


Fig. 6: Antioxidant activities of thiadiazoline, sulfonamides derivatives and the metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide using DPPH

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 carbohydrazonoyl 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 carbohydrazonoyl 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 carbohydrazonoyl halide 4a also exhibited a strong nuclease activity and degraded the DNA (Fig. 7, lanes 10-13). Metal complexes with N-phenyl 2-pyridine carbohydrazonoyl 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 only 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 carbohydrazonoyl halide had a strong degradation effect on the DNA *in vitro*. Metal complexes with N-phenyl 2-pyridine carbohydrazonoyl 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 carbohydrazonoyl 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 carbohydrazonoyl halide have significant nuclease activity toward the cleavage of genomic DNA in the absence of any external additives. Therefore, metal complexes

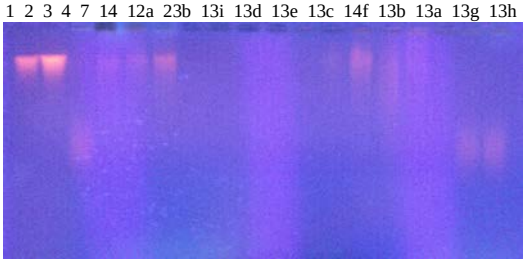


Fig. 7: Degradation effect of the new thiadiazoline, sulfonamides derivatives and the metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide on the DNA isolated from *E. coli* strain W3110, Lane 1: DNA, Lane 2: DNA in DMSO, Lane 3: Sample 3a, Lane 4: Sample 4a, Lane 5: Sample 7, Lane 6: Sample 9, Lane 7: Sample 14, Lane 8: Sample 12a, Lane 9: Sample 12b, Lane 10: Sample 13i, Lane 11: Sample 13d, Lane 12: Sample 13e, Lane 13: Sample 13c, Lane 14: Sample 14f, Lane 15: Sample 13b, Lane 16: Sample 13a, Lane 17: Sample 13g, Lane 18: Sample 13h

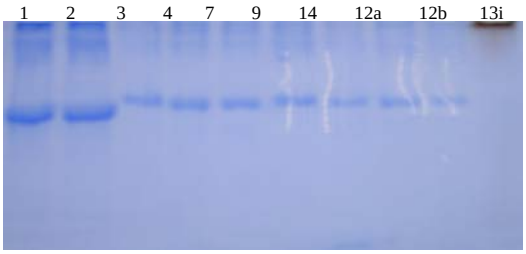


Fig. 8: Degradation effect of the new thiadiazoline and sulfonamides derivatives on BSA, Lane 1: BSA protein, Lane 2: BSA protein in DMSO, Lane 3: Sample 3a, Lane 4: Sample 4, Lane 5: Sample 7, Lane 6: Sample 9, Lane 7: Sample 14, Lane 8: Sample 12a, Lane 9: Sample 12b, Lane 10: Sample 13i

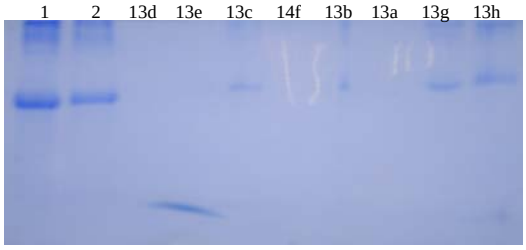


Fig. 9: Degradation effect of the new sulfonamides derivatives and the metal complexes with N-phenyl 2-pyridine carbohydrazonoyl halide on BSA, Lane 1: BSA Protein, Lane 2: BSA protein in DMSO, Lane 3: Sample 13d, Lane 4: Sample 13e, Lane 5: Sample 13c, Lane 6: Sample 14f, Lane 7: Sample 13b, Lane 8: Sample 13a, Lane 9: Sample 13g, Lane 10: Sample 13h

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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