

Synthesis of New Bioactive Sulfur Compounds Bearing Heterocyclic Moiety and Their Analytical Applications

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Abstract

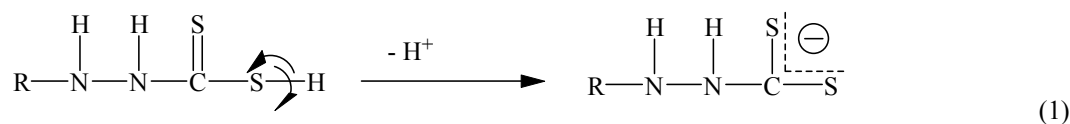
Some new bioactive sulfur compounds bearing heterocyclic nitrogen moieties such as 3-imino-2-thioxo-4,5-dihydro-thiazolidin-4-one (**3**), 3-imino-2-thioxo-3,4,5,6-tetrahydro-1,3-thiazine-4,6-(2H)dione (**4**), N-substituted-pyrazol-3,5-dione (**10**), 1,3-disubstituted-2-thioxo-pyrimidin-4,6-dione (**11**) and di-(3,5-diaminopyrazolin-1-yl)thioiketone (**13**) derived from dithioc formic acid hydrazide (**1**) and thiocarbonylhydrazide (**7**) were prepared via condensation of compound **1** or **7** with acyclic and cyclic oxo compounds (e.g. aldehydes and ketones) in 1:1 and 1:2 molar ratios, and addition of iso thiocyanate or treatment with active methylene compounds followed by ring closing reactions in different media (Schemes I & II). The biocidal effect of some compounds towards some bacteria and fungi was evaluated. Compound **4** was used as selective chelating agent for spectrophotometric determination of mercury (II) ions. The limit of detection (LOD) and quantification (LOQ) of the developed spectrophotometric method were found to be equal to 0.16 and 0.52 $\mu\text{g mL}^{-1}$ mercury (II), respectively. Compound **4** was also physically immobilized onto polyurethane foam (PUFs) and was successfully used as solid sorbent in packed column for removal of mercury (II) ions from wastewater.

Keywords: Heterocyclic compounds, Biocidal effects, Mercury (II) determination, Wastewater

1. Introduction

Recently, great attention has been oriented towards cyclic and acyclic heterocyclic systems containing nitrogen, oxygen or sulfur. Such class of compounds has wide applications as pharmaceutical drugs, biological activities, as anti HIV, anticancer, efficient plant protection and as analytical reagents for trace and ultra trace heavy metal determination and pre concentration in aqueous media (Ramadan, et al., 1993, PP. 291 -303; Piper, et al., 1997, PP. 377 -384; Abdel-Rahman, 2000, PP. 315 -357; Abdel-Rahman, 2001, PP. 18 -22; PP.195 -204, PP.410 -410; El-Gendy, et al., 2003, PP.2055 -2071; Gladis and Rao, 2004, PP. 60 -65; Yonetoku, et al., 2006; Sharma et al., 2006, PP. 1139 -1143; Kadi and El-Shahawi, 2009, PP.613 -620; Hamza et al., 2010, PP. 69 -74. The presence of proton at nitrogen and/ or sulfur atoms, provide these compounds with the ability to form metal complexes with heavy metal ions [13-17]. (Zaki et al., 1995, PP. 127 -138; Jozlowski, et al., 2002, pp. 677 -682; Pandey, et al., 2006, PP. 107 -109; Pulakesh, 2007, PP. 544 -547; Sadasivan and Alaudeen, 2007, PP. 1959 -1962).

The great electrophilicity of nitrogen atom compared to that of sulfur atom makes the latter more acidic and an active centre in the nucleophilic attack. The fact that, the sulfur anion formed is more stabilized by negative charge distribution as reported (Sbirna, et al., 2005, PP. 389 -392) as shown in equation (1):



Thus, in the case of dithioic acid hydrazides, the orientation of heterocyclization reactions will start from S on alkylation or from N on condensation (Sadasivan and Alaudeen, 2006, PP. 1145 -1148). The starting materials, dithioic formic acid hydrazide **1** and thiocarbohydrazide **7** possess two donor sites situated at -NH and / or =NH group in addition of sulfur (Sadasivan and Alaudeen, 2006, PP. 1145 -1148).

Recently, a series of cyclic and acyclic systems close to the title compounds has been used extensively in the literature as biocidal reagents (Sadasivan and Alaudeen, 2006, PP. 1145 -1148). Such compounds have been used as complexing agents for pre concentration, and subsequent determination of trace and ultra trace concentrations of toxic ions via both S and N as donor atoms (Hassanien, 2003, PP. 1987 -1997; Shindhu, et al., 2005, PP. 472- 474) as shown below:

<Figure 1>

Considering the biocidal and complexation properties of these compounds and as a part of our search for novel mono- and di-thiocarbohydrazide and alkyl, acyl derivatives, the present article reports the synthesis of heterocyclic nitrogen compounds containing sulfur using dithioic formic acid hydrazide (**1**) and thiocarbohydrazide (**7**) as starting materials via condensation with oxygenated compounds. Moreover, one of the compounds prepared by this methodology was tested as chelating agent for the pre concentration and subsequent determination of mercury (II) ion in water.

2. Experimental

2.1 Reagents and materials

All chemicals and solvents used were of analytical reagent (A.R) grade quality and were used as received. Most of the chemicals were provided by Merck (Darmstadt, Germany). Doubly deionized water was used throughout. Low density polyethylene (LDPE) bottles, Nalgene were used and carefully cleaned first with hot detergent, soaked in 50% HCl (Analar), HNO_3 (2.0 mol L^{-1}), subsequently washed with dilute HCl (0.5 mol L^{-1}) and finally rinsed with distilled water. The sample solution was stored in LDPE bottles and stored at -20°C in a freezer. Stock solutions (0.1 \%w/v) of the steroid reagent were prepared in ethanol. A stock solution of HgCl_2 (1 mg/mL) was prepared by dissolving an accurate weight of the salt in doubly distilled water (100 mL). Britton – Robinson (B –R) buffers of pH 2-11 were prepared from the acid mixture of phosphoric acid, boric acid, acetic acid (0.04 mol L^{-1}) and adjusting the pH to the required value with sodium hydroxide (0.20 mol L^{-1}). A series of standard diluted mercury (II) solutions were then prepared in doubly distilled water.

2.2 Apparatus and measurements

A Perkin Elmer (Lambda EZ-210) double beam spectrophotometer ($190\text{-}1100 \text{ nm}$) with 1 cm (path width) was used for recording the electronic spectra of the compounds. A Perkin Elmer model RXI-FT-IR system 55529 was used for recording the IR spectra of the prepared compounds. A Bruker advance DPX 400 MHz model using TMS as an internal standard was used for recording the ^1H NMR spectra of the compounds on deuterated DMSO. A GC-MS-QP 1000-Ex model was used for recording the mass spectra of the compounds. Melting points were determined with an electro thermal Bibbly Stuart Scientific Melting Point SMPI (US). Molecular weights of the compounds were performed on Micro analytical center, Cairo University, Egypt. Microanalysis (Sulfur %) was performed by microanalytical center Ain-Shams University-Cairo-Egypt. A digital pH-meter (model MP220, Metter Toledo) was used for pH measurements.

2.3 Organic preparation

Mono-hydrazones (2a – d) and bis-hydrazones (5a and 5b)

A mixture of **1** and the selected aromatic / heteroaldehydes and / or cyclic diketones (1:1) and/ or 1,2-diketones (1:1 molar ratio) and a cyclic diketones (2:1 molar ratio) in ethanol- acetic acid (1:1, 100 mL) was refluxed for 1 h, cooled then poured onto an ice bath. The solid precipitate was collected and crystallized from the appropriate solvent to give **2a-d** and/ or **5a** and **5b**, respectively.

N¹-Arylidene-dithioic formic acid hydrazones (2 a-d):

2 a: This compound was crystallized from ethanol as yellow crystals. Yield = 80%, m.p. $150\text{-}151^\circ\text{C}$. $\text{C}_6\text{H}_7\text{N}_3\text{S}_2$ (185) Calcd.: S, 34.59. Found S, 33.95.

2 b: This compound was crystallized from ethanol as pale yellow crystals. Yield (85.2%), m.p. $105\text{-}106^\circ\text{C}$. IR: cm^{-1} = 3500 (OH), 3120 (NH), 1595 (C=N), 1380 (NCSN), 1185 (C-S), 1055 (- C-O-Me). $\text{C}_9\text{H}_{10}\text{N}_2\text{S}_2 \text{O}_2$ (242) Calcd.: S, 26.44. Found S, 25.58.

2c: This compound was crystallized from ethanol as pale yellowish crystals. Yield (90.05%), m.p. 220-221 °C. $C_{11}H_{14}N_2S_2O_3$ (286) Calcd.: S, 22.37. Found S, 22.18.

2d: This compound was crystallized from ethanol as faint yellow crystals. Yield (60.2%), m.p. 225-226 °C. UV, λ_{nm} (DMF): 380, IR: $\nu\text{ cm}^{-1}$ = 3315 (NH of indole), 3155 (NH of NHCS), 1686 (C=O), 1551 (C=N), 1432 (NCSN), 1190 (C-S), 826 (phenyl CH). $^1\text{H NMR}$ (DMSO): δ : 4.2 (s, 1H, SH), 6.8-7.2, 7.5-7.8 (each m, 4 H of aromatic CH), 10.2 and 14.5 (each s, 2H, NH of indole and acid hydrazide). MS, m/z (Int. %): 249 (0.0), 203 (M^+ -HCSH, 100), 157 (5.15), 145 (31.91), 131 (15.18), 102 (87.31), 90 (27.13), 77 (5.98). $C_9H_7N_3S_2O$ (237) Calcd.: S, 27.0. Found S, 26.59.

Bis - N-arylidene-dithioic formic acid hydrazones (5a, 5b):

5a: This compound was crystallized from methanol as pale yellow crystals. Yield (65.03%), m.p. 145-146 °C. $C_6H_{10}N_4S_4$ (266) Calcd.: S, 48.12. Found S, 47.95.

5b: This compound was crystallized from methanol as pale yellow crystals. Yield (80.1%), m.p. 190-191 °C. IR: $\nu\text{ cm}^{-1}$ = 3139 (NH), 1596 (C=N), 1350 (NCSN), 1213 (C-S), 793, 763 (phenyl CH). $C_{16}H_{14}N_4S_4$ (390) Calcd.: S, 32.82. Found S, 32.80.

N¹ - (Phenyl amino carbothia) dithioic formic acid hydrazides (6a)

Phenyl isothiocyanate (0.01 mmol) was added to a solution of **1** (0.01 mol) in DMF (50 mL) and refluxed for 20 min. After cooling, the reaction mixture was poured onto ice. The solid precipitate was collected and crystallized from DMF as deep yellow crystals. Yield (83.2%), m.p. 240-241 °C. IR: $\nu\text{ cm}^{-1}$ = 3195, 3103 (NH, NH), 1342 (NCSN), 1191 (C-S), 776 (phenyl CH). $^1\text{H NMR}$ (DMSO): δ = 7.2-7.4, 7.5-7.6 (each m, 10H, two phenyl), 8.1 (s, 1H, NH), 9.2 (s, 1H, SH), 10.8, 11.4 (each d, 2H, NH-NH). $C_8H_9N_3S_3$ (243) Calcd.: S, 39.5. Found S, 38.98.

N¹ - (4-Chlorophenyl amino carbothia) dithioic formic acid hydrazides (6b)

This compound was prepared by mixing 4-chlorophenyl isothiocyanate (0.01 mmol) with a solution of **1** (0.01 mol) in DMF (50 mL) and refluxed for 20 min. After cooling, the reaction mixture was poured onto ice and the solid precipitate was filtered and crystallized from DMF as deep yellow crystals. Yield (63.0%), m.p. 215-216 °C. IR: $\nu\text{ cm}^{-1}$ = 3180, 3165 (NH, NH), 1355 (NCSN), 1185 (C-S), 777 (phenyl CH), 624 (C-Cl). $C_8H_8N_3S_3Cl$ (277.5) Calcd. S, 34.65. Found S, 34.45.

3-Imino-2-thioxo-4, 5-dihydro-thiazolidin-4-one (3)

An equimolar mixture of **2a** and monochloroacetic acid with anhydrous sodium acetate (5 g) in ethanol (50 mL) was refluxed for 4 h. After cooling, the reaction mixture was poured onto ice and the resulting solid precipitate was collected and crystallized from acetic acid as yellow crystals. Yield (50.12%), m.p. 260-261 °C. IR: $\nu\text{ cm}^{-1}$ = 3110 (NH), 1710 (C=O), 1603 (C=N), 1356 (NCSN), 1480 (deformation CH_2). MS: m/z (Int.%): 227 ($M+2$, 5.18), 92 ($C_5H_4N_2$), 56 (100, C_2H_2NO). $C_8H_7N_3S_2O$ (225.11) Calcd.: S, 28.44 Found S, 28.01.

2-Thioxo-3-(2-oxoindolin-3-imino)-3,4,5,6-tetrahydro-1,3-thiazin-4,6-dione (4)

An equimolar mixture of **2d** and diethylmalonate was added to sodium ethoxide solution in absolute ethanol (0.2 mmol, 100 mL). The reaction mixture was refluxed for 4 h, cooled then poured onto ice-HCl. The produced solid was filtered and crystallized from THF as strong yellow crystals. Yield (70.12%), m.p. 280-281 °C. IR: $\nu\text{ cm}^{-1}$ = 3353 (OH of thiazine-4, 6-dione), 3056 (NH of indole), 1667, 1620 (2 C=O), 1607 (C=N), 1362 (NCSN), 1192 (C-S), 738 (phenyl CH). $^1\text{H NMR}$ (DMSO): δ = 3.2 (d, 2H, cyclic O- CH_2 -O), 6.2 (s, 1H, OH of 3-indole), 7.3-7.7 (m, 4H, of benzo-protons). MS, m/z (Int. %): 305 (0.0), 271 ($M-H_2S$, 100), 203 (13.11), 157 (51.15), 131 (13.08), 102 (78.34). $C_{12}H_7N_3S_2O_3$ (305) Calcd.: S, 20.98 Found S, 20.75.

Ketone thiocarbohydrazones (8a-d)

A mixture of compound **7** in hot water (10 mL) and the appropriate heteroaldehydes/ ketone (1:1 molar ratio) in ethanol-acetic acid (1:1, 50 mL) mixture was refluxed for 1 h, cooled and poured onto an ice bath. The produced solid precipitate was filtered and crystallized from ethanol to give **8a-d** as pale – yellow crystals.

8a: Yield (85%), m.p. 182-184 °C. $C_6H_8N_4S O$ (184) Calcd.: S, 17.39. Found S, 17.11.

8b: Yield (90.02%), m.p. 185-186 °C. $C_6H_8N_4S_2$ (200) Calcd.: S, 32.01. Found S, 31.89.

8c: Yield (95.1%), m.p. 230-231 °C. $C_9H_{12}N_4S O$ (224) Calcd.: S, 14.28 Found S, 13.99.

8d: Yield (80.0%), m.p. 260-261 °C. UV, λ_{nm} (DMF): 420. IR: $\nu\text{ cm}^{-1}$ = 3353 (NH_2), 3150 (NH, NH), 1684 (C=O), 1552 (C=N), 1349 (NCSN), 776 (phenyl CH). The MS, m/z (Int. %): 235 (0.0), 203 (18.21), 157 ($C_8H_5N_3SO$), 131 (35.13), 102 (78.11), 90 (21.78). $C_9H_9N_3SO$ (235) Calcd.: S, 13.61 Found S, 13.41.

Formation of N^1, N^3 - di (iminoaryl)thioureas, (**9a-e**)

In hot ethanol, compound **7** (10 mL) was mixed with the appropriate heteroaldehydes or ketones (1:2 molar ratio) in ethanol-acetic acid (1:1, 50 mL). The reaction mixture was refluxed for 1h, cooled, and poured onto ice. The produced solid was recrystallized from isopropyl alcohol to give yellowish-crystals **9a-e**.

9a. Yield (80%), m.p.230-232 °C. $C_{17}H_{18}N_4SO_4$ (374) Calcd.: S,8.55 Found S,8.15.

9b. Yield (75.2%), m.p.195-196 °C. $C_{11}H_{12}N_6S$ (260) Calcd.: S,12.30 Found S,11,89.

9c. Yield (78.12%), m.p.196-197 °C. $C_{11}H_{10}N_4SO_2$ (262) Calcd.: S,12.21. Found S,11.88..

9d. Yield (75.05%), m.p.140-141 °C. $C_{11}H_{10}N_4S_3$ (294) Calcd.: S,32.65. Found S,32.45.

9e: Yield (80.0%), m.p.260-261 °C. IR: ν cm^{-1} = 3150, 3099 (NH, NH), 1647 (C=O), 1606 (C=N), 1354 (NCSN), 1211 (C-S), 769 (benzo- CH). MS, m/z (Int. %): 364(0.0), 203(17.27), 157 (85.15) , 131 (100), 102 (38.13). 90 (11.18). $C_{17}H_{12}N_6SO_2$ (364) Calcd.: S,8.79 Found S, 8.65.

1-(1H- 2-oxo-indol-3-hydrano) thioxo-2,3,4,5-tetrahydropyrazol-3,5-dione (**10**)

Compound **8d** (0.01 mmol) with dimethyl malonate (0.01 mmol) in solution of sodium ethoxide (0.02 mol, 100 mL) were refluxed for 4 h. After cooling, the reaction mixture was poured onto ice-HCl. The produced solid precipitate was filtered and crystallized from acetic acid as faint yellow crystals. Yield (60.0%), m.p.185 – 186 °C. IR: ν cm^{-1} = 3200-3080 (b, OH \rightleftharpoons NH, NH), 2890 (CH_2), 1693, 1670 (two C=O), 1348 (NCSN), 1185 (C-S), 780 (benzo - CH). MS, m/z (Int. %): 303(0.0), 247(M-56, CN_2O , 21.18), 203 (10.00), 147 (8.11) , 111 (12.12), 97 (12.12), 56 (100). $C_{12}H_9N_5SO_3$ (303) Calcd.: S,10.56; Found S, 9.96.

1,3-Di (1H- 2-oxo-indol-3-imino)-2- thioxobarbituric acid (**11**)

An accurate of compound **9e** (0.01 mmol) with diethyl malonate (0.01 mmol) in solution of sodium ethoxide (0.02 mol, 100 mL) were refluxed for 4 h. The reaction mixture was cooled, poured onto ice-HCl. The produced solid precipitate was filtered and crystallized from ethanol as deep yellow crystals. Yield (85.12 %), m.p.210-212 °C. IR: ν cm^{-1} = 3090 (NH), 2980 (CH_2), 1720, 1693, 1670 (C=O), 1600, 1595 (C=N), 1350 (NCSN), 1199(C-S), 777 (benzo- CH). $C_{20}H_{12}N_6SO_4$ (432) Calcd.: S,7.40 Found S, 7.45.

N, N-Di (acyl/thioacyl/amido)thiocarbohydazides (**12a-d**)

Carbon disulfide, phenyl isothiocyanate, adipoyl chloride or 4-methoxyphenyl chloride was added by dropwise addition to a solution of compound **7** (0.01 mmol) in DMF (20 mL). The reaction mixtures were refluxed for 1h, cooled and poured onto ice. The formed solids were filtered and crystallized from DMF to give yellowish crystals of **12a-d**, respectively. .

12a. Yield (75%), m.p.155-156 °C. $C_3H_6N_4S_5$ (258) Calcd.: S,62.01 Found S,61.75.

12b. Yield (80%), m.p.195-196 °C. $C_{15}H_{16}N_6S_3$ (376) Calcd.: S,25.55 Found S,25.27.

12c. Yield (78.1%), m.p.150-151 °C. $C_{21}H_{42}N_4SO_2$ (414) Calcd.: S,7.72. Found S,7.55.

12d. Yield (65.05%), m.p.165-166 °C. IR: ν cm^{-1} = 3350 -3080 (b, NH, NH), 1580 (CONH), 1335 (NCSN), 1189 (C-S), 1080 (-C-O-Me). MS, m/z (Int. %): 374 (1.15), 107 (15.31), 74 (100, CN_2SH_2). $C_{17}H_{18}N_4SO_4$ (374) Calcd.: S,8.55 Found S, 8.41.

Di (3,5- diaminopyrrolin-1-yl)thioketone, **13**

An equimolar mixture of **7** and malononitrile in DMF -EtOH (1:1, 100 mL, 1:1), was refluxed for 4h, cooled then poured onto ice. The solid formed was filtered and crystallized from ethanol to give **13** as deep -yellow crystals. Yield (90.2 %), m.p.110-112 °C. UV, λ_{nm} nm (DMF): 375 nm. IR: ν cm^{-1} :3300 (NH_2), 3100 (2 NH), 1580 (C=N), 1180 (C-S). MS, m/z (Int. %): 238 (M^+ ,5.0), 203 (25.01), 157 (12.11), 143 (18.18), 56 (100, CN_2O). $C_7H_{10}N_8S$ (238) Calcd.: S,13.44 Found S, 13.21.

2.4 Analytical procedures

2.4.1 Recommended Spectrophotometric determination of mercury (II)

In a series of volumetric flasks (25 mL), an appropriate concentration (0.2-2.0 $\mu g mL^{-1}$) of mercury (II) solution was allowed to react with the reagent **4** solution (1.50 mL, 0.05 %w/v). To the test solution, an approximate volume (5 mL) of Britton -Robinson buffer of pH 4-5 was added. The reaction mixture was completed with distilled water to the mark of the measuring flask (10 mL) and allowed to stand for 5 min before measuring the absorbance at λ_{max} 505 nm. The results were compared successfully with the concentration of mercury (II) determined with atomic absorption spectrometry.

2.4.2 Preparation of the immobilized reagent **4** polyurethane foams

The reagent **4** (0.1% w/v) in water-ethanol (1:1 v/v) was shaken with the PUFs cubes with efficient stirring for 30 min. The immobilized reagent PUFs cubes were squeezed and dried as reported (El-Shahawi, et al., 200, PP. 221-228). The retained reagent **4** onto the PUFs cubes was determined employing the equation:

$$a = (C_0 - C) \frac{V}{w} \quad (2)$$

where, C_0 and C are the initial and final concentrations (mol L^{-1}) of the reagent **4** in solution, respectively, v = volume of the reagent solution (liter) and w is the mass (g) of the PUFs sorbent.

2.4.3 Analysis of mercury (II) in water samples

Tap - and mineral water samples were collected from the laboratories of Chemistry Department, King AbdulAziz University, and local market of Jeddah city, KSA, respectively. The water samples were filtered through 0.45 μm cellulose membrane filter prior to analysis and stored in LDPE sample bottles (250 mL). The recommended general spectrophotometric procedure used to prepare the standard curve was followed. The concentration of mercury (II) ions was then determined following the recommended spectrophotometric procedure used for the preparation of the standard curve and employing the equation:

$$\text{Mercury (II) concentration} = C_{\text{std}} \times A_{\text{samp}} / A_{\text{std}} \quad (3)$$

where, C_{std} is the standard concentration and A_{samp} and A_{std} are the corrected absorbance of the sample and the standard at λ_{max} 505 nm, respectively.

Alternatively, the standard addition method was employed as follows: transfer known volume (5.0 mL) of the unknown water samples to the volumetric flask (25.0 mL) adjusted to pH.5-6 with B-R buffer (10 mL). An accurate volume (1.5 mL) of the reagent was added to the test solution and the reaction mixture was made up to the mark with distilled water. Repeat the same procedures after adding various concentrations (0.2-1.0 $\mu\text{g mL}^{-1}$) of mercury (II). Measure the true absorbance displayed by the test solutions before and after the addition of the standard (0.2-1.0 $\mu\text{g mL}^{-1}$) mercury (II) solution employing single wave spectrophotometry method. The concentration of mercury (II) was then determined via the calibration curve of the standard addition procedure.

3. Results and Discussion

Heterocyclic systems containing endo- and exocyclic sulfur atom show a wide spectrum of potential applications. Thus, 3-imino - 2- thioxo-4,5-dihydro-thiazolidine-4-one (**3**) and 3-imino-2-thioxo-3,4,5,6-tetrahydro-1,3-thiazine-4,6(2H)dione (**4**) were obtained from condensation of dithioic formic acid hydrazide (**1**) with aldehydes to give the thiohydrazones **2** followed by heterocyclization with chloroacetic acid in ethanol - sodium acetate medium to give **3** or with dimethyl malonate in sodium ethoxide allows the formation of compound **4** [Scheme I].

Formation of compound **3** may be takes place via the nucleophilic attack of sulfide (S^-) to the electropositive carbon of chloroacetic acid ($\text{CH}_2\text{-Cl}$) followed by another nucleophilic attack of more nucleophilic nitrogen of thiohydrazone (N-H) to other electrophilic carbon of acetic acid ($-\text{COOH}$).

Compound **4** may be formed via nucleophilic sulfur atom of dithioic moiety on a more electrophilic carbon dimethyl malonate followed by heterocyclization via second nucleophilic nitrogen on the other electrophilic carbon. On the other hand, condensation of compound **1** with cyclic 1, 2- bicarbonyl compounds as biacetyl & benzil (2:1 by molar ratio) afforded the bis- compounds **5a** and **5b**, respectively. Addition of phenyl / p- chlorophenyl iso thiocyanate in warming DMF yielded N- (arylamino-carbothia) dithioic formic acid hydrazide **6** [Scheme I].

Thiocarbohyrazide (El-Gendy et al, 2001, PP. 376 -383; Rastogi and Yadav, 2005, PP. 448 -451) is one of the most important materials for building heterocyclic compounds containing sulfur and nitrogen (Hassanien, 2003, PP. 1987 - 1997; El-Gendy, et al., 2001, PP. 376 - 383). Thus, condensation of thiocarbohyrazide **7** with cyclic and a cyclic oxygenated compounds such as heteroaromatic aldehydes: pyrrole/furan/thiophene carboxaldehyde and cyclic hetero ketone e.g. indol-2,3-dione in boiling ethanol - acetic acid gave the mono hydrazone **8** (1:1 by moles) and/or the bis - hydrazone **9** (1:2 by moles) (Scheme II).

Heterocyclization of mono hydrazone **8d** was achieved via refluxing with dimethyl malonate in sodium ethylate furnished 1-[(2-oxoindol-3-ylimino) amino-thia]-2,3-dihydro-pyrazol-3,5-dione (**10**). Under the same experimental conditions, refluxing compound **9e** with diethyl malonate afforded 1, 3-di(2-oxoindol-3-ylimino)-2-thioxo-4,5-dihydro-pyrimidin-4,6-dione, **11**. (Scheme II). Addition of CS_2 and phenyl isothiocyanate to **7** in warming DMF yielded N, N- di (caramido) thioureas **12a** and **12b**, respectively, while

compounds **12 c** and **12 d** were isolated from careful treatment of compound **7** with adipoyl chloride and p-methoxybenzoyl chloride in warming DMF (Scheme II). Heterocyclization of compounds **12** failed because the high acidity with electronic symmetry of molecule in addition to the resonance stabilization of the conjugated anion may be formed (Rastogi and Yadav, 2005, PP. 448 -451). Treatment of acid hydrazide with malononitrile is one of the most important routes for the synthesis of poly functional amino heterocyclic systems (Schachtner, et al., 1999, PP. 335 – 341; El-Gendy, et al., 2001, PP. 376 -383; Hassanien, 2003, PP. 1987 -1997; Rastogi and Yadav, et al., 2005, PP. 448 -451; Burghate et al., 2007, PP. 103 -108). Thus, refluxing thio carbohydrazide **7** with malononitrile in ethanol – DMF afforded di (3, 5-diaminopyrazolin-1-yl) thio ketone, **13** (Scheme II). Compound **13** was also prepared by nucleophilic attack of the first primary NH₂ to the first cyano group followed by ring closing reaction of a second nucleophilic attack of secondary NH₂ to other cyano group.

3.1 Antibacterial activity

Recent literature survey has revealed the need of new compounds endowed with antimicrobial activity. Previous investigation has shown that, some of cyclic sulfur – nitrogen compounds have excellent antimicrobial activity (Burghate, 2007, PP. 103 -108). Therefore, in this study the cyclic sulfur compounds **2**, **5**, **6**, **8**, **9** and **12** were screened as antibacterial active agents using cup – plate diffusion method (Burghate et al., 2007, PP. 103 -108). The used bacterial organisms included both gram positive and gram negative strains: e.g. *Escherichia. coli*; *Bacillus subtilis*, *Staphylococcus. aureus*, *Pseudomonas .vulgaris* and *Shigella flexneri* in DMF. Streptomycin was used as standard antibiotic. The diameter of the inhibition zone in mm was measured at concentration of 100 µg mL⁻¹. The results are summarized in Table 1, where the compounds **6b** and **12 a**, showed high activity, the other compounds presented moderate or low antimicrobial activity compared to streptomycin. The activity of compounds **6b** and **12a** may be attributed to the thiourea and dithioic moieties in their structures, respectively as reported (Abuo-Rahma, et al., 2009, PP. 3879 -3886).

3.2 Analytical application of compound 6-hydroxy-3-(2-oxoindolin-3-ylidene- amino)-2-thioxo-3,4,5,6-tetrahydro-1,3-thiazin-4(3H)-one, 4

3.2.1 Spectrophotometric determination of trace amounts of mercury (II)

On mixing the compound **4** abbreviated as HOTT with mercury (II) ions in the aqueous media of pH 4-5 and shaking, for 2-3 min, a red colored complex was developed. The absorption spectrum of the reagent showed one well defined absorption peak at 336 nm (λ_2) nm, while the spectrum of the complex **4** at the same pH showed one peak at 505nm (λ_2)nm (Fig. 1). Thus, in the subsequent work, the absorbance of the aqueous solution was measured at 505 nm against a reagent blank. Maximum absorbance of the produced colored complex was achieved at pH 4–5. In the aqueous solutions of pH < 4, the data revealed no complex formation between compound **4** and mercury (II) ions. In acidic pH, the equilibrium of the reagent moves to left, hence the quantity of the available dissociated species of the chelating agent **4** decreases and not able to form complex with mercury (II). The absorbance of the aqueous phase of pH ≥ 6 decreased due to the formation of non- colored complex species of mercury (II) ions e.g. hydroxo-species of mercury (II).

The influence of the concentration of compound **4** revealed that, a 2 mL of 5.1×10^{-4} mol L⁻¹ of the reagent was sufficient to react quantitatively (97–98%) with mercury (II) up to 10 µg mL⁻¹ in the aqueous layer. The molar absorptivity (ϵ) at λ_{max} 505 nm calculated from the absorbance measurement was found equal to 2.5×10^4 L mol⁻¹ cm⁻¹. The chemical structure of the produced mercury (II) complex species was determined by continuous variation method (Sawyer, et al., 1984) at various concentrations of the mercury (II) ions and reagent. The results revealed the formation of complex species of 1:2 molar ratio of mercury (II) to the reagent. Thus, the chemical structure of the developed colored species is most likely Hg (HOTT)₂.

3.2.2 Figure of merits

The values of LOD and LOQ of mercury (II) were determined employing the equations (Miller, 1994):

$$\text{LOD} = 3 \delta / b \quad (4)$$

$$\text{LOQ} = 10 \delta / b \quad (5)$$

where δ , is the standard deviation of the blank reading and b is the slope of the calibration plot. The LOD and LOQ values were found equal to 0.16 and 0.52 µg mL⁻¹ mercury (II), respectively. These values could be improved by immobilizing the reagent **4** onto PUFs sorbent in packed column for quantitative collection of trace and ultra trace amounts of mercury. The level of precision is suitable for the routine analysis of the mercury (II) in various types of water samples. The analysis of mercury (II) at the concentration levels of 1.0-15 µg mL⁻¹ was achieved with a recovery percentage of 97±2.9%, (n = 5). A satisfactory recovery percentage of various mercury (II) species spiked to the tested water samples was also achieved.

3.2.3 Effect of diverse ions

The selectivity of the developed method for the determination of $10 \mu\text{g mL}^{-1}$ of mercury (II) ions in the presence of a relatively high excess ($0.05\text{-}0.1 \text{ mg mL}^{-1}$) of some cations e.g. Li^+ , Na^+ , K^+ , Ca^{2+} , PO_4^{3-} , Al^{3+} , Fe^{2+} , Ni^{2+} , Co^{2+} , and Zn^{2+} and the anions MnO_4^- and CrO_4^{2-} , chloride, nitrate, sulfate and fluoride was investigated. The tolerance limit was defined as the concentration of the added foreign ion causing a relative error within $\pm 2\%$ of mercury (II) determination. The results revealed that, all the tested cations does not interfered even at 1:100 tolerable concentration of mercury (II) to the diverse ions, respectively. The interference of MnO_4^- and CrO_4^{2-} was eliminated by the addition of NaN_3 and sodium sulfite in HCl media (1.0 mol L^{-1}), respectively. Thus, the developed method could be extended for the analysis of mercury in various water samples.

3.2.4 Retention profile of mercury (II) onto reagent 6 loaded PUFs

In aqueous solution of pH 4-5, mercury (II) forms an orange – red colored complex species with compound **4**. Thus, the sorption profile of the aqueous solutions containing mercury (II) at pH 4-5 by the reagent **4** loaded PUFs was studied after shaking for 1h at room temperature. The amount of mercury (II) in the aqueous phase after equilibrium was determined spectrophotometrically (Marczenko, 1986). The %E and the D of mercury (II) sorption onto the PUFs decreased markedly at $\text{pH} < 4$ and maximum uptake was achieved at $\text{pH} \sim 4\text{-}5$. The high retention of mercury (II) at pH 4-6 is most likely attributed to the deprotonation of the reagent **4** and the available active sites on the reagent loaded PUFs membrane that enhanced the retention of analyte via “solvent extraction and/ or chelation mechanism” (El-Shahawi, et al., 2005, PP. 221 -228). At $\text{pH} > 6$, the sorption performance of the reagent loaded PUFs towards mercury (II) decreased markedly. This behavior is most likely attributed to the instability, hydrolysis, or incomplete extraction of the produced complex of mercury (II) –reagent **4** in the PUFs solid sorbent. These results suggested the use of the reagent **4** treated PUFs in packed column for removal of mercury from wastewater samples after percolation at $5\text{-}10 \text{ mL min}^{-1}$ flow rate. An acceptable removal percentage (97 ± 2.5) of mercury (II) was achieved.

3.2.5 Validation

The proposed method was validated by the complete removal of the spiked mercury (II) onto tap and wastewater samples at a total concentration $\leq 15.0 \mu\text{g mL}^{-1}$. An acceptable extraction percentage ($95 \pm 3.5\%$, $n = 5$) of mercury was successfully achieved with the aid of the calibration plot and standard addition procedures. The plot of mercury (II) added to the tested water sample versus the amount of mercury (II) retained was linear with a slope of 0.998 and a correlation coefficient of $r = 0.999$ confirming the performance of the developed method for mercury (II) removal from water.

4. Conclusion

New bioactive sulfur compounds bearing heterocyclic moieties were prepared. Only one compound **4** was successfully used as selective chromogenic reagent for single wave spectro- photometric determination of mercury (II) in aqueous media. The presence of different tautomerism in the structure of compound **4** participates effectively on complex formation. The values of LOD and LOQ of the developed spectrophotometric were found equal to 0.16 and $0.52 \mu\text{g mL}^{-1}$ mercury, respectively. Moreover, the reagent **4** immobilized PUFs could be packed in column for on –line pre concentration and subsequent determination of mercury (II) ions at ultra trace concentrations.

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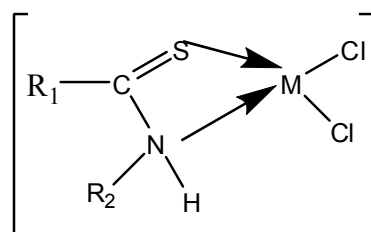
Table 1. Antibacterial activity of the prepared compounds

Compound No.	Inhibition zones (mm) *				
	E.c	B.s	S.a	P.v	S.f
1	16	15	17	15	18
2e	18	15	16	15	17
5	14	16	15	14	17
6b	23	21	21	22	24
7	15	15	15	15	15
8e	16	15	14	15	15
9e	14	15	14	15	16
12a	26	24	20	21	25
12b	22	21	20	22	24
Streptomycin	25	22	21	21	30

* *E. coli*; *B. subtilis*; *S. aureus*; *P. vulgaris* and *S. flexneri*.

Streptomycin: Reference antibiotics, Bristol-Myers Squibb, Giza, Egypt.

Highly active = inhibition zones > 19 mm; moderately active = inhibition zones 15 -19 mm and lethal active = inhibition zones 11-14 mm.



$R_1 = O$ -substituted heterocyclic

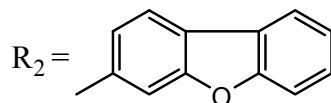


Figure 1.

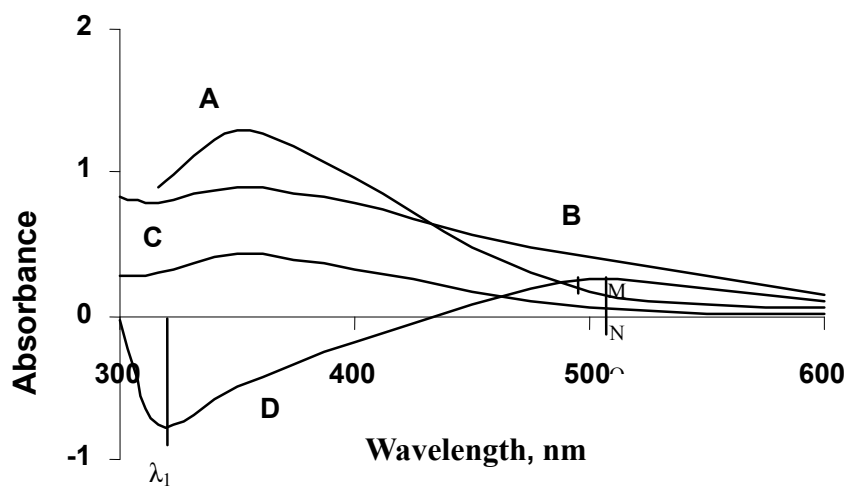


Figure 2. Absorption spectra of reagent 4 and its mercury (II) complex at pH 5-6. Curve A is the spectrum of the reagent blank (reference water); B is mercury (II) complex (reference, water); C is the excess of reagent (reference water) and D is mercury (II) complex (reference, reagent blank).