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Synthesis and *in vitro* evaluation of novel rhodanine derivatives as potential antimicrobial activities

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ABSTRACT

3-cyclohexyl-2-thioxothiazolidin-4-one 1 was used as a precursor for the synthesis of novel bisrhodanine and rhodanine derivatives via its reaction with some electrophilic reagents. The novel synthesized compounds were characterized by IR, ^{1}H NMR and mass spectral studies. All the synthesized compounds were screened in vitro for their antibacterial and antifungal activities.

Keywords: Bisrhodanine, Rhodanine, Micheal addition, Antimicrobial activity

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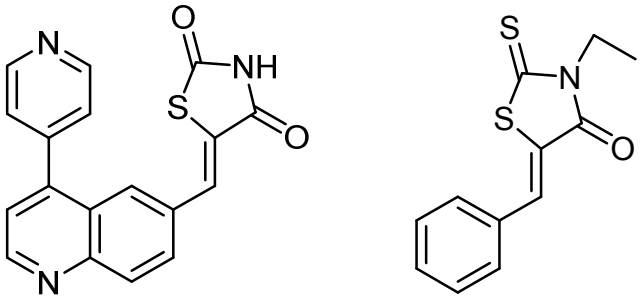
INTRODUCTION

Heterocyclic chemistry is of great importance to the medicinal chemists because the steady growth of interest in heterocyclic compounds is connected with their therapeutic activity. Further, the compounds containing the 2-thioxothiazolidin-4-one ring (rhodanine derivative) have demonstrated wide range of pharmacological activities, which include antimicrobial [1-3], antiviral [4], antidiabetic [5], anticancer activity [6], anti-inflammatory [7], antioxidant [8], anti-tubercular [3], anticonvulsant [9], and cytotoxic activities [10]. Additionally, rhodamine based molecules have been popular as small molecule inhibitors of numerous targets such as HCV NS5B protease[11], HCV NS3 protease [12], aldose reductase [13], β-lactamase [14], UDP-N-acetylmuramase/L-alanine ligase [15], fungal protein mannosyl transferase-1 (PMT1) [16], cathepsin-D [17], anthrax lethal factor protease [18], histidine decarboxylase [19], JNK-stimulating phosphatase-1 (JSP-1) [20] and phosphodiesterase (PDE-4) [21]. Among the thiazolidine derivatives, numerous compounds containing thiazolidine-2, 4-dione and rhodanine have been recognized as new potential anticancer agents. For example, GSK1059615 is a potent, reversible, ATP-competitive, thiazolidinedione inhibitor of PI3K α [22, 23]. Moorthy *et al*, have reported 5-benzilidene-3-ethyl rhodanine (BTR-1) (Fig. 1), 3-dimethyl-2-thio-hydantoin (ITH-1), 3-ethyl-2-thio-2,4-oxazolidinedione (ITO-1) and found that all the compounds induced cytotoxicity in a time and concentration dependent manner with an IC50 value of <10 μM and affected cell division by inducing a block at S phase, which finally led to the activation of apoptosis [24]. As a continuation of my research program to find out bioactive 4- thiazolidinone and rhodanine [25-29], the presence work is aim to synthesize novel rhodanine derivatives, starting from 3-cyclohexyl-2-thioxothiazolidin-4-one in order to evaluate their antimicrobial activity



GSK1059615 BTR-1

Fig. 1. Rhodanines with potential anticancer activity

MATERIALS AND METHODS

Starting materials and solvents were purchased from common commercial suppliers and were used without further purification. Melting points were determined in open capillary tubes and uncorrected. IR spectra (KBr) were measured on Shimadzu 440 spectrometer, ^{1}H NMR spectra were obtained in DMSO-d_6 on a Varian Gemini 300 MHz spectrometer using TMS as internal standard. Mass spectra were obtained on GCMS\QP 1000Ex mass spectrometer at 70 ev. Elemental analyses were carried out at Micro analytical Center of Cairo University, Egypt. Microbiology screening was carried out in the Regional Center for Microbiology and Biotechnology (RCMB), Antimicrobial unit test organisms, Al-Azhar University, Cairo, Egypt.

Chemistry

Preparation of compounds (3, 4 & 5): general procedure:

To suspension of finally powdered potassium hydroxide (0.01 mol) in dry dimethylformamide (20 mL) 3-cyclohexyl-2-thioxothiazolidin-4-one **1** (0.01 mol) was added the reaction mixture was stirred for 30 min then, 4, 4'-diisothiocyanato-1, 1'-biphenyl (0.01 mol) was added in portions. The reaction mixture was stirred at room temperature for 12 h and then treated with ethyl 2-chloroacetate (0.01 mol), 2-chloroacetonitrile (0.01 mol) or 1-chloropropan-2-one (0.01 mol) and left to stirr at room temperature for another 12 h; the reaction mixture was poured into ice/water and acidified with 0.1 N HCl at pH 3-4. The resulting precipitate was filtered off, dried, and recrystallized from the proper solvent to give **3**, **4** and **5** respectively.

3,3''-([1,1'-biphenyl]-4,4'-diyl)bis(3'-cyclohexyl-2'-thioxo-2',3'-dihydro-3H,4'H-[2,5'-bithiazolylidene]-4,4'(5H)-dione) (3): Yield 52%, brown solid (DMF), mp 300-301ºC. IR spectrum, $\bar{\nu}$, cm^{-1}: 3034 (CH-arom.), 2977 (CH-aliph.), 1701 (C=O). ^{1}HNMR spectrum, δ, ppm: 1.23- 1.28 (m, 6H), 1.71- 1.76 (m, 6H), 1.85- 1.89 (m, 4H), 2.29- 2.33 (m, 4H), 4.11 (br, 4H, thiazolidinone), 4.48-4.57 (m, 2H), 7.44- 7.61 (m, 8H, ArH). Mass spectrum, m/z: 779 (M$^+$, 30.6%), 719 (21%), 689 (13%), 549 (16%), 491 (7%), 426 (6%), 374 (15%), 307 (100.0%), 283 (12%), 169 (9%), 141 (45%), 191 (34%). Found, %: C, 55.10; H, 4.20; N, 7.00. $C_{36}H_{34}N_4O_4S_6$. Calculated, %: C, 55.50; H, 4.40; N, 7.19.

5,5'-([1,1'-biphenyl]-4,4'-diylbis(4-aminothiazole-3(3H)-yl-2(3H)-yli- dene))bis(3-cyclohexyl-2-thioxothiazolidin-4-one) (4)*:*

Yield 30%, brown solid (DMF), mp 348-350ºC. IR spectrum, $\bar{\nu}$, cm^{-1}: 3356, 3276 (NH_2), 3021 (CH-arom.), 2968 (CH-aliph.), 1703 (C=O. ^{1}HNMR spectrum, δ, ppm: 1.19- 1.25 (m, 6H), 1.69- 1.77 (m, 6H), 1.81- 1.89 (m, 4H), 2.21- 2. 29 (m, 4H), 4.27-4.36 (m, 2H), 6.67 (br, 2H, thiazole), 7.72- 7.87 (m, 12H, ArH + 2NH_2). Mass spectrum, m/z: 778 (M^{+1}, 25%), 776 (20.1%), 771 (39.4%), 768 (28.2%), 761 (12.5%), 512 (100.0%), 417 (34%), 367 (37%), 203 (28%). Found, %: C, 55.40; H, 4.75; N, 10.60. $C_{36}H_{36}N_6O_2S_6$. Calculated, %: C, 55.64; H, 4.67; N, 10.81.

5,5'-([1,1'-biphenyl]-4,4'-diylbis(4-methylthiazole-3(3H)-yl-2(3H)-ylidene))bis(3-cyclohexyl-2-thioxothiazolidin-4-one) (5)*:* Yield 55%, white solid (DMF), m p 280-281 ºC. IR spectrum, $\bar{\nu}$, cm^{-1}: 2977 (CH aliph.), 1704 (C=O). ^{1}HNMR spectrum, δ, ppm: 1.18- 1.23 (m, 6H), 1.67- 1.75 (m, 6H), 1.81- 1.89 (m, 4H), 2.18 (b, 6H, CH_3), 2.28- 2.33(m, 4H), 4.30-4.41(m, 2H), 6.62 (b, 2H, thiazole), 7.28- 7.72 (m, 8H, ArH). Mass spectrum, m/z: 775 (M$^+$, 36.4%), 671 (100.0%), 620 (23%), 570 (14%), 514 (23%), 432 (45%), 319 (22%), 281 (22%), 207 (12%), 131 (54%). Found, %: C, 58.70; H, 4.70; N, 7.10. $C_{38}H_{38}N_4O_2S_6$. Calculated, %: C, 58.88; H, 4.94; N, 7.23.

5,5'-(1,4-phenylenebis(methanylylidene))bis(3-cyclohexyl-2-thioxothia- zolidin-4-one) (7)*:* A mixture of compound **1** (0.01mol), terphthalaldehyde **6** (0.01 mol) and piperidine (0.01mol) in EtOH (50 mL) was heated under reflux for 1 h; the solid product which produced on heating was collected by filtration and recrystallized from acetic

acid to give a yellow solid. Yield 50%, mp 299-300ºC. IR spectrum, $\bar{\nu}$ cm^{-1}: 3021 (CH arom.), 2936 (CH aliph.), 1709 (C=O). ^{1}HNMR spectrum, δ, ppm: 1.23- 1.34 (m, 6H), 1.68- 1.72 (m, 6H), 1.82- 1.86 (m, 4H), 2.29- 2.33 (m, 4H), 4.85-4.98 (m, 2H), 7.78-8.09 (m, 6H, ArH + methylidene-H). Mass spectrum, m/z: 528 (M^+, 45%), m/z: 520 (28.5%), 501 (22%), 470 (11%), 458 (6%), 416 (24%), 386 (17%), 434 (5%), 316 (8%), 276 (11%), 249 (100.0%). Found, %: C, 58.99; H, 5.15; N, 5.10. $C_{26}H_{28}N_2O_2S_4$. Calculated, %: C, 59.06; H, 5.34; N, 5.30.

3-cyclohexyl-5-(4-fluorobenzylidene)-2-thioxothiazolidin-4-one (13)*:*
A mixture of compound 3-cyclohexyl-2-thioxothiazolidin-4-one **1** (0.01mol), 4-flourobenzaldehyde **12** (0.01 mol) and piperidine (0.01mol) in EtOH (50 mL) was heated under reflux for 1 h; the solid product was filtered and recrystallized from acetic acid to give a yellow solid. Yield 50%, mp 180-181ºC. IR spectrum, $\bar{\nu}$ cm^{-1}: 3016(CH-arom.), 2935 (CH-aliph.), 1708 (C=O). ^{1}HNMR spectrum, δ, ppm: 1.28-1.33 (m, 3H), 1.67- 1.70 (m, 3H), 1.81- 1.86 (m, 2H), 2.28- 2.33 (m, 2H), 4.84-4.92 (m, 1H), 7.37 (d, 2H, Ar-H), 7.70 (d, 2H, Ar-H), 7.72 (s, 1H, methylidene-H). Mass spectrum, m/z: 321 (M^+, 100.0%), 318 (19.1%), 311 (1.7%), 299 (33%), 241 (18%), 110 (7%), 99 (10 %), 53 (22%). Found, %: C, 59.31; H, 4.70; N, 4.00. $C_{16}H_{16}FNOS_2$. Calculated, %: C, 59.79; H, 5.02; N, 4.36.

5-benzylidene-3-cyclohexyl-2-thioxothiazolidin-4-one (15):
A mixture of compound **1** (0.01mol), ethyl 2-cyano-3-phenylacrylate **14** (0.01 mol) and piperidine (0.01mol) in 1,4-dioxane (30 mL) was heated under reflux for 1 h; the formed solid product was collected by filtration and recrystallized from acetic acid as a yellow solid. Yield 70%, mp 147-148ºC. IR spectrum, $\bar{\nu}$ cm^{-1}: 3054 (CH-arom.), 2936 (CH-aliph.), 1710 (C=O). ^{1}HNMR spectrum, δ, ppm: 1.24-1.33 (m, 3H), 1.67- 1.70 (m, 3H), 1.81- 1.86 (m, 2H), 2.26- 2.37 (m, 2H), 4.85-4.94 (m, 1H), 7.50- 7.71 (m, 6H, ArH + methylidene-H). Mass spectrum, m/z: 305 (M^{+1}, 66%), 291 (20%), 236 (13%), 218 (100.0%), 201 (12%), 116 (11%), 99 (14%). Found, %: C, 63.10; H, 5.40; N, 4.35. $C_{16}H_{17}NOS_2$. Calculated, %: C, 63.33; H, 5.65; N, 4.62.

3-cyclohexyl-5-(2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (19)*:* A mixture of compound **1** (0.01mol), isatin **18** (0.01 mol) and piperidine (0.01mol) in EtOH (50 mL) was heated under reflux for 1 h; the obtained solid product was collected by filtration and recrystallized from acetic acid as yellow solid. Yield 65%, mp 324-325ºC. IR spectrum, $\bar{\nu}$ cm^{-1}: 3334(NH), 2927 (CH-aliph.), 1688 (C=O, indol), 1701(C=O; thiazolidinone). ^{1}HNMR spectrum, δ, ppm: 1.25-1.32 (m, 3H), 1.66-1.70 (m, 3H), 1.79- 1.85 (m, 2H), 2.27- 2.33 (m, 2H), 4.81-4.89 (m, 1H), 6.94 (d, 1H, ArH), 7.10-7.42 (m, 2H, ArH), 8.80 (d, 1H, ArH), 11.21 (s, 1H, NH, exchangeable with D_2O). Mass spectrum, m/z: 345 (M^{+1}, 45%), 321 (18.6%), 301 (100%), 216 (9%), 151 (16%), 122 (22%), 77 (12%). Found, %: C, 58.95; H, 4.20; N, 8.05. $C_{17}H_{16}N_2O_2S_2$. Calculated, %: C, 59.28; H, 4.68; N, 8.13.

2-(3-cyclohexyl-4-oxo-2-thioxothiazolidin-5-ylidene)-1H-indene-1,3(2H)-dione (21)*:* A mixture of compound **1** (0.01mol), ninhydrine **20** (0.01 mol) and piperidine (0.01mol) in EtOH (50 mL) was heated under reflux for 1 h; the solid product which produced was filtered and recrystallized from DMF to give a brown solid. Yield 70%, mp >350 ºC. IR spectrum, $\bar{\nu}$ cm^{-1}: 2937 (CH-aliph.), 1715(C=O, thiazolidinone), 1690 (br, 2C=O, indene). ^{1}HNMR spectrum, δ, ppm: 1.23- 1.27 (m, 3H), 1.67- 1.85 (m, 3H), 1.90-1.99 (m, 2H), 2.20- 2.47 (m, 2H), 4.63-4.78 (m, 1H), 7.22-7.97 (m, 4H, Ar-H). Mass spectrum, m/z: 357 (M^+, 52%), 352 (21.6%), 302 (100.0%), 292 (11%), 268 (9%), 201 (7%), 167 (17%), 146 (23%), 121 (21%), 108 (34%), 96 (33%). Found, %: C, 60.30; H, 4.15; N, 3.60. $C_{18}H_{15}NO_3S_2$. Calculated, %: C, 60.48; H, 4.23; N, 3.92.

3-cyclohexyl-5-(2-hydroxybenzylidene)-2-thioxothiazolidin-4-one (23)*:* A mixture of compound **1** (0.01mol), salicylaldehyde **22** (0.01 mol) and piperidine (0.01mol) in EtOH (30 mL) was heated under reflux for 1 h; the solid product which produced was collected by filtration and recrystallized from acetic acid yield a yellow solid. Yield 65%, mp 253-254ºC. IR spectrum, $\bar{\nu}$ cm^{-1}: 3398 (OH), 3032 (CH arom.), 1701 (C=O). ^{1}HNMR spectrum, δ, ppm: 1.21- 1.26 (m, 3H), 1.65- 1.86 (m, 3H), 1.96- 1.99 (m, 2H), 2.22- 2.41 (m, 2H), 4.79-4.86 (m, 1H), 7.31- 8.15 (m, 5H, Ar-H + methylidene-H), 10.33 (s, 1H, OH). Mass spectrum, m/z: 319 (M^+, 19.4%), 301 (100.0%), 289 (9.8%), 261 (21%), 227 (33%), 218 (32%), 197 (22%), 150 (11%), 101 (3%), 97 (5%). Found, %: C, 59.90; H, 5.40; N, 4.20. $C_{16}H_{17}NO_2S_2$. Calculated, %: C, 60.16; H, 5.36; N, 4.38.

3-cyclohexyl-5-((2-hydroxynaphthalen-1-yl)methylene)-2-thioxo-thiazolidin-4-one (25):
A mixture of compound **1** (0.01mol), 2-hydroxy-1-naphthaldehyde **24** (0.01 mol) and piperidine (0.01mol) in EtOH (50 mL) was heated under reflux for 1 h; the solid product which produced on heating was filtered and recrystallized from dioxane to give a yellow solid. Yield 60%, mp 271-272ºC. IR spectrum, $\bar{\nu}$ cm^{-1}: 3400 (OH), 3051 (CH-arom.), 2933 (CH-aliph.), 1703 (C=O). ^{1}HNMR spectrum, δ, ppm: 1.25- 1.27 (m, 3H), 1.67- 1.86 (m, 3H), 1.91- 1.97 (m, 2H), 2.19- 2.42 (m, 2H), 4.79-4.84 (m, 1H), 7.22-8.92 (m, 6H, ArH + methylidene-H), 12.00 (s, 1H,OH, exchangeable with D_2O). Found, %: C, 64.90; H, 4.95; N, 3.55. $C_{20}H_{19}NO_2S_2$. Calculated, %: C, 65.01; H, 5.18; N, 3.79.

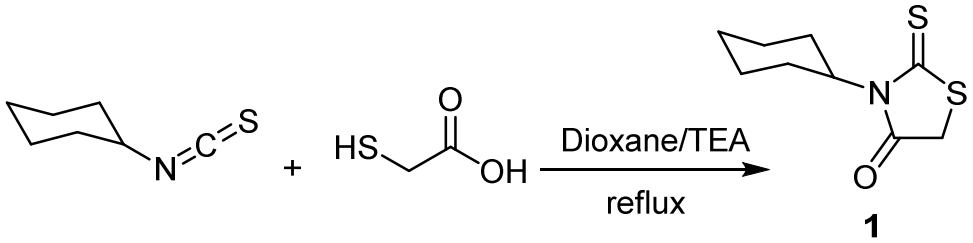
Antimicrobial evaluation

The disks of Whitman filter paper were prepared with standard size (6.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These bottles are kept into hot air oven at a temperature of 150 ºC. Then, the standard sterilized filter paper disks impregnated with a solution of the test compound in DMF (100 µL, 5 mg/mL) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard concentrations of 10^6 CFU/mL (Colony Forming Units/mL) and 10^4 CFU/mL were used for antibacterial and antifungal assay, respectively. Pyrex glass Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. The utilized test organisms were *S. aureus, S.epidermidis* and *B. subitilis* as examples of Gram-positive bacteria and *P.aeruginosa, P. vulgaris* and *K. pneumonia* as examples of Gram-negative bacteria. They were also evaluated for their in vitro antifungal potential against *A. fumigatus, A. clavatus* and *G. candidium* fungal strain. Ampicillin and gentamycin were used as standard antibacterial agents; while amphotericin B was used as standard antifungal agent. DMF alone was used as control at the same above-mentioned concentration and due this there was no visible change in bacterial growth. The plates were incubated at 37 ºC for 24 h for bacteria and for 48 h at 25 ºC for fungi. The mean zone of inhibition measured in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms.

RESULTS AND DISCUSSION

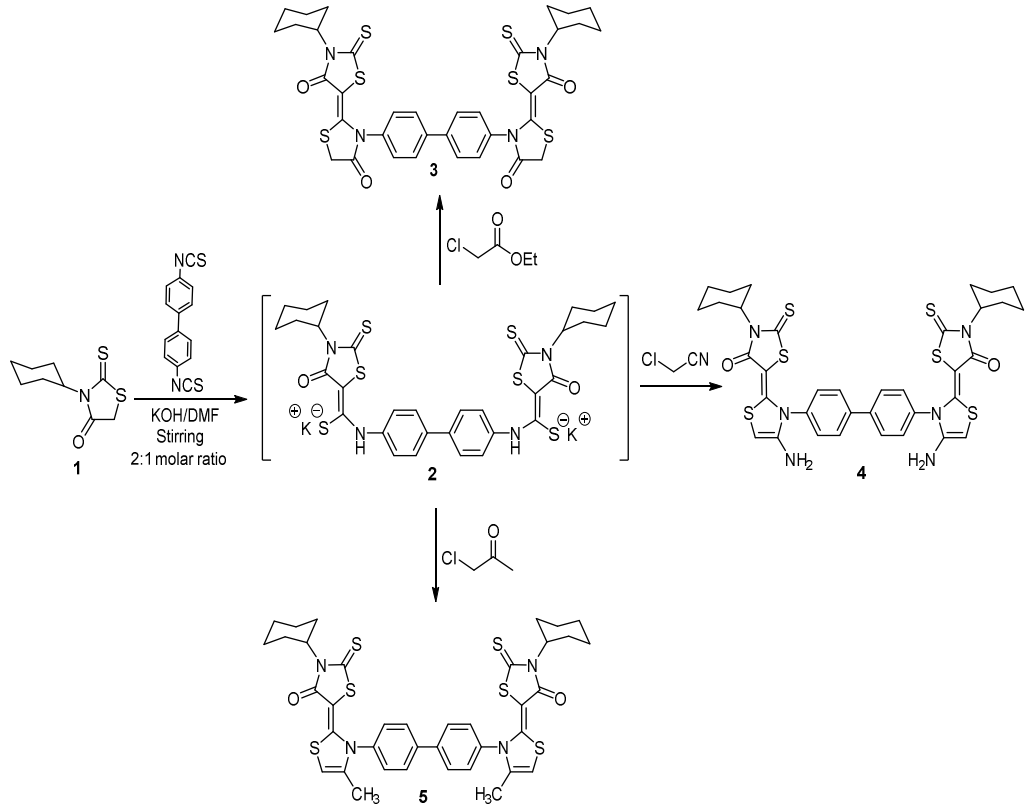
Chemistry

Isothiocyanates are useful, widely-used building blocks in the synthesis of nitrogen, sulfur and oxygen heterocycles and organometallic compounds of academic, pharmaceutical and industrial interests [30, 31]. Rhodanine **1** was synthesized by Cyclocondensation of cyclohexyl-isothiocyanate with sulfanylacetic acid in 1,4-dioxane in the presence of triethylamine under reflux conditions [32] (Scheme **1**).



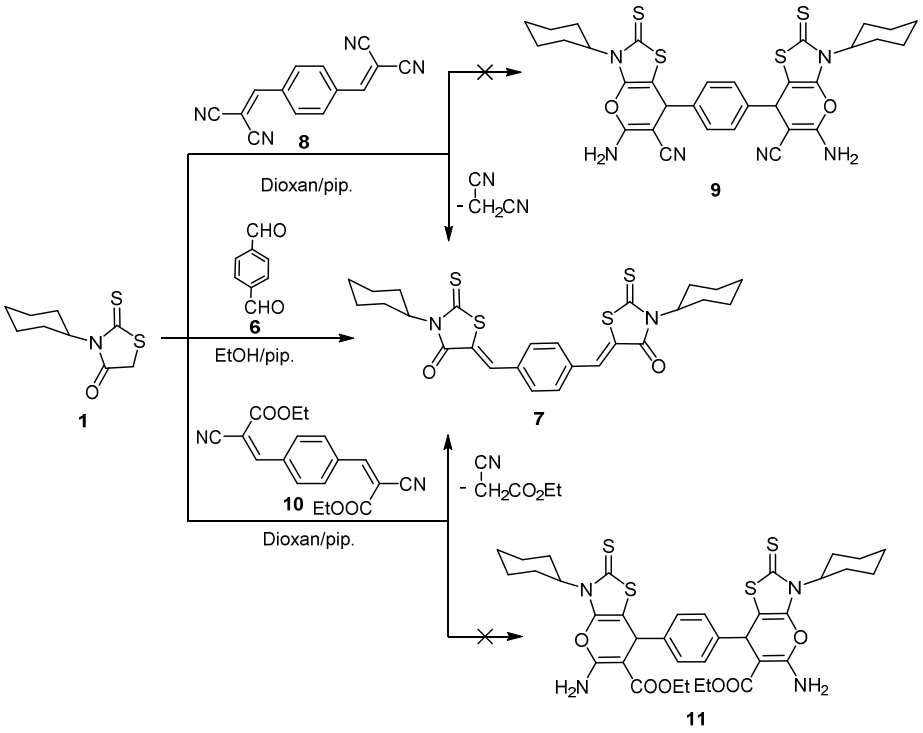
Scheme 1. synthesis of rhodanine derivative 1

The active methylene moiety of rhodanine **1** was allowed to react with 4,4'-diisothiocyanato-1,1'-biphenyl in *N,N*-dimethylformamide in the presence of an equimolar amount of potassium hydroxide yielded the non-isolable intermediate potassium sulphide salt **2**, which was allowed to react with ethyl chloroacetate to afford the novel 3,3''-([1,1'-biphenyl]-4,4'-diyl)bis(3'-cyclohexyl-2'-thioxo-2',3'-dihydro-3H,4'H-[2,5'-bithiazolylidene]-4,4'(5H)-dione) **3** (Scheme **2**). The structure of compound **3** was readily established by analytical and spectral data. Its infrared spectrum exhibited absorption bands at 3034, 2977 and 1701 cm^{-1} due to stretching vibrations of CH- arom, CH-aliph and C=O groups . The ^{1}H NMR spectrum (DMSO-d_6) displayed signals at δ 1.23- 1.28, 1.71- 1.76, 1.85- 1.89, 2.29- 2.33, 4.11, 4.48-4.57 and 7.44- 7.61 ppm which were readily assigned to the cyclohexyl, methylene (4H,thiazolidinone) and aromatic protons, respectively. The mass spectrum of compound **3** showed a molecular ion peak at m/z = 779 (M^+, 30.6%) corresponding to the molecular formula $C_{36}H_{34}N_4O_4S_6$. The base peak was found in the spectrum at m/z = 307. The formation **3** is assumed to proceed *via* initial alkylation followed by cycloalkylation by ethanol elimination. Also, it has been found that, the in situ reaction of **2** with chloroacetonitrile afforded aminothiazole derivative **4.** Cycloalkylation of non-isolable salt **2** with chloroacetone produced 4-methylthiazole derivative **5** (Scheme 2). The IR spectrum of compound **5** showed the appearance of absorption band at 2977 and 1704 cm^{-1} corresponding to the CH-aliph and C=O groups, respectively. The ^{1}H NMR (DMSO-d_6) spectrum of compound **5** exhibited signals at δ 1.18- 1.23, 1.67- 1.75, 1.81- 1.89, 2.18, 2.28- 2.33, 4.30-4.41, 6.62, 7.28- 7.72. corresponding to cyclohexyl, methyl, thiazole and aromatic protons, respectively. The formation of **5** was assumed to proceed via an initial alkylation followed by intramolecular cyclization by elimination of water**.**



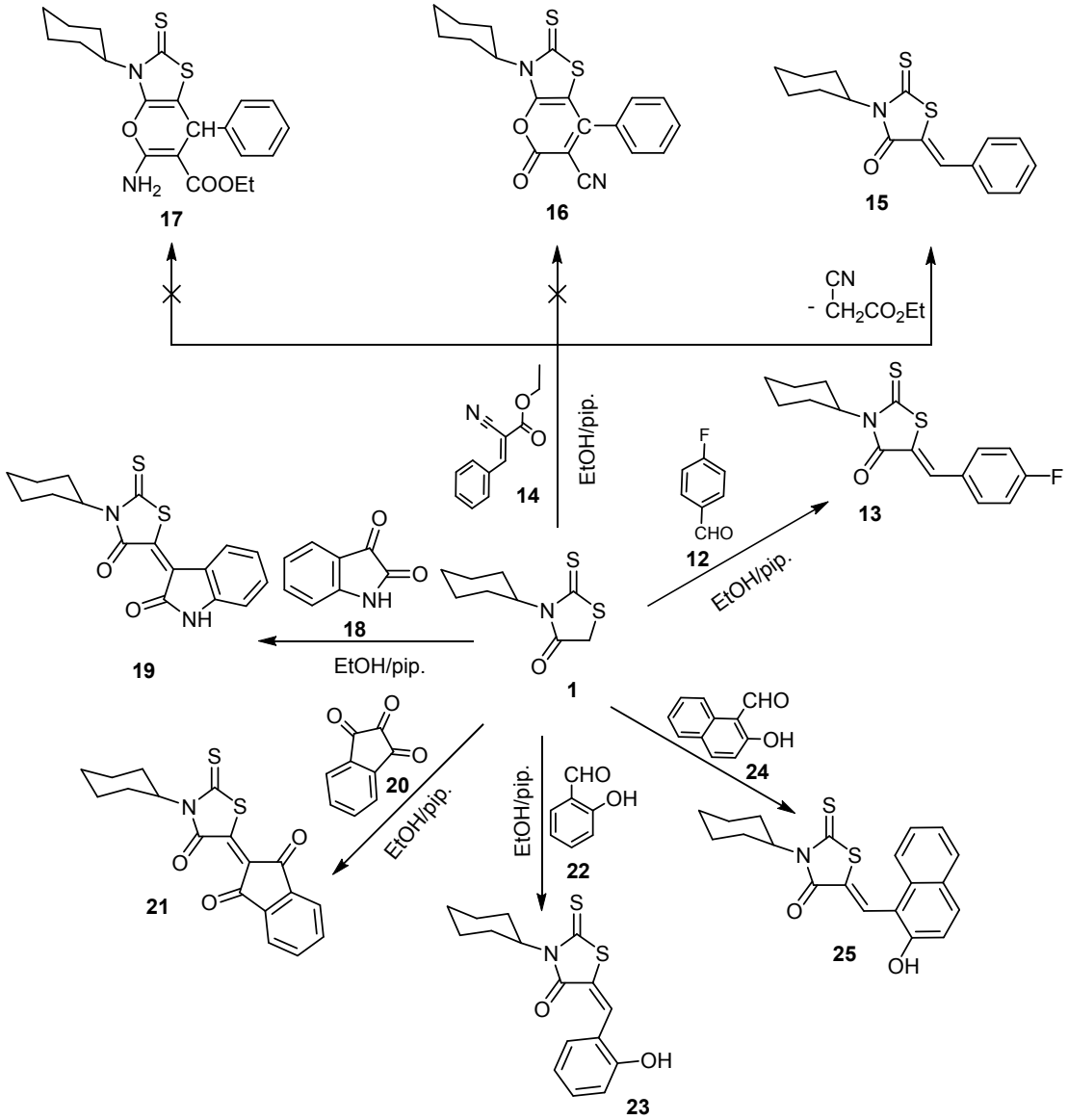
Scheme 2. Synthetic pathways for bisrhodanine derivatives 3-5

Reaction of rhodanine **1** with 1,4-bis-(benzylidene)derivatives **8** and / or **10** (2:1 molar ratio) by refluxing in 1,4-dioxane in the presence of piperidine afforded bisbenzylidene derivative **7** (Scheme 3). The structure formula of compound **7** resulting from this reaction has been confirmed using elemental analyses and spectral methods. Mass spectrum of compound **7** showed a molecular ion peak at m/z = 528 corresponding to a molecular formula $C_{26}H_{28}N_2O_2S_4$. Additionally, the structure of **7** was established chemically through the reaction of compound **1** with terephthalaldehyde **6** in ethanol in the presence of a catalytic amount of piperidine.



Scheme 3. Synthesis of Bisrhodanine derivative

The methylene moiety in compound **1** was exploited to synthesize hitherto unknown rhodanine derivatives through its reaction with some electrophiles. Treatment of compound **1** with 4-flourobenzaldehyde **12** led to the formation of 5-(4-fluorobenzylidene)-2-thioxothiazolidin-4-one derivative **13** (Scheme 4). The ^{1}H NMR spectrum of compound **13** in DMSO-d_6 revealed the absence of methylene moiety which present in the parent compound in addition to the presence of cyclohexyl, methylidene and aromatic protons. Its mass spectrum revealed a molecular ion peak at m/z = 321 which is the base peak in the spectrum. Treatment of compound **1** with ethyl-2-cyano-3-phenylacrylate **14** led to the formation of 5-benzylidene-2-thioxothiazolidin-4-one derivative **15** and the other possible structures pyranothiazole **16 & 17** was eliminated on the basis of analytical and spectral data. Compound **15** was assumed to be formed via Michael addition of the active methylene group in **1** to the activated double bond in arylidine **14** followed by the elimination of ethyl cyanoacetate [33]. This work was extended to cover the reactivity of compound **1** towards carbonyl compounds to synthesized rhodanine derivatives. Thus, condensation of compound **1** with isatine **18** in refluxing ethanol gave the 5-(2-oxoindolin-3-ylidene)-2-thioxothiazol-idin-4-one derivative **19**. The structure of isolated product **19** was established on the basis of its elemental analysis and spectral data. In a similar manner, the reaction of rhodanine **1** with ninhydrine **20** afforded 2-(3-cyclohexyl-4-oxo-2-thioxothiazolidin-5-ylidene)-1*H*-indene-1,3 (2*H*)-dione **21**. In addition, Condensation of compound **1** with salicylaldehyde **22** and /or 2-hydroxy-1-naphthaldehyde **24** in refluxing ethanol in the presence of piperdine furnished 5-(2-hydroxybenzylidene)-2-thioxothia-zolidin-4-one **23** and 5-((2-hydroxynaphthalen-1-yl) methylene)-2-thioxothiazolidin-4-one **25,** respectively. ^{1}HNMR spectrum of compound **23** in DMSO- d_6 revealed the absence of methylene moiety and appears OH group at 10.33 ppm. Mass spectrum showed a molecular ion peak at m/z = 319 corresponding to a molecular formula $C_{16}H_{17}NO_2S_2$.



Scheme 4. Synthetic pathways for compounds 13-25

Antimicrobial evaluation

Applying the agar plate diffusion technique [34], all the synthesized compounds **3-25** were screened for their in vitro antibacterial activity against Gram positive bacteria and Gram negative bacteria. The antibacterial activity was evaluated against Staphylococcus aureus (RCMB 010010), Bacillus subtilus (RCMB 010067) and Salmonella sp. (RCMB 010043), Escherichia coli (RCMB 010052). Ampicillin and Gentamycin are used as a standard drug for the comparison of antibacterial activity. Also, the antifungal activity was evaluated against Aspergillus fumigatus (RCMB 02568) and Candida albicans (RCMB 05036). Amphotericin B is used as standard drugs for the comparison of antifungal activity. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The results depicted in table 1 revealed that most of tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains and also against antifungal strains.

Table 1. The results of antimicrobial screening. Mean zone of inhibition (mm) beyond the diameter produced on a range of pathogenic microorganisms

Compounds no.	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	S. aureus	*B. subitilis*	*Salmonella sp.*	*E. coli*	*A. fumigatus*	*C. albicans*
3	22	27	15	17	23	25
4	21	26	17	18	20	21
5	20	27	15	17	20	21
7	14	16	17	16	20	25
13	13	16	17	17	19	28
15	14	13	15	17	18	22
19	16	12	NA	NA	16	25
21	14	16	14	13	20	21
23	14	16	15	15	17	23
25	15	*NA	NA	NA	17	20
Ampicillin	23	32	*NT	NT	NT	NT
Gentamycin	NT	NT	17	19	NT	NT
Amphotericin B	NT	NT	NT	NT	23	25

**NA: no activity, *NT: not tested*

The data revealed that bis-rhodanines **3**-**5**, benzylidene derivatives **13**, **15**, **23** and bis-benzylidene **7** were the most active among the synthesized compounds exhibited against the tested organisms (fig.1 & 2). In this view, bis-rhodanines **3**-**5** have comparable activity to Ampicillin and Gentamycin against *Staphylococcus aureus*, *Bacillus subtilus*, *Salmonella sp*., and *Escherichia coli*. benzylidene derivatives **13** have similar activity to Gentamycin against *Salmonella sp*. and *Escherichia coli*. Aso, benzylidene derivatives **15**, **23** and bis-benzylidene **7** have comparable activity to Gentamycin against *Salmonella sp*. and *Escherichia coli*. While the activity of benzylidene derivatives **13**, **15**, **23** and bis-benzylidene **7** were 50% lower than Ampicillin in inhibiting the growth of *Staphylococcus aureus* and *Bacillus subtilus*. Concerning the antibacterial activity of the compounds **19**, **21** and **25** showed weak activities against the tested Gram-positive bacteria and have completely inactive toward Salmonella sp. and Escherichia coli compared to gentamycin.

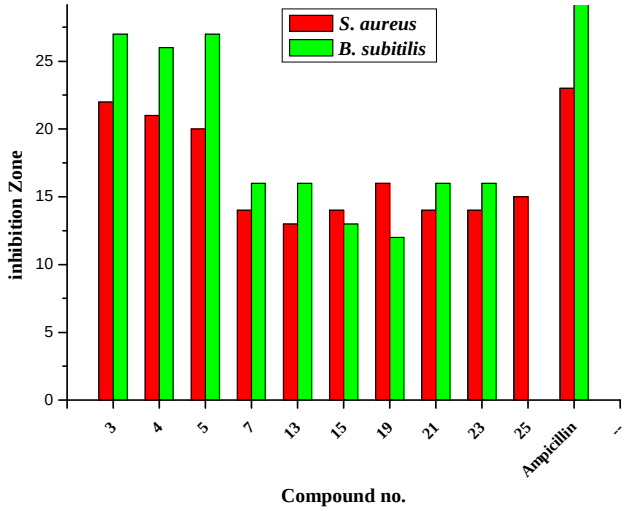


Fig. 1: Antibacterial activity of the synthesized compounds against Gram-positive bacteria

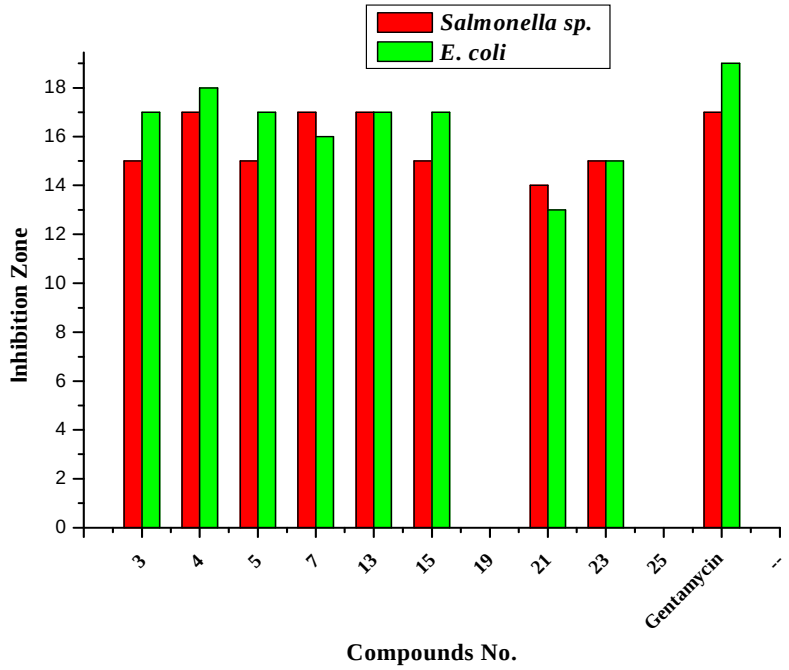


Fig. 2: Antibacterial activity of the synthesized compounds against Gram-negative bacteria

Regarding the activity of rhodanine derivatives versus antifungal strains, bis-rhodanine **3** was equipotent to Amphotericin B against *Aspergillus fumigatus* and *Candida albicans*. While, bis-rhodanines **4**, **5** were comparable to Amphotericin B against antifungal strains. Benzylidene derivative **13** displayed activities more potent than Amphotericin B against *Candida albicans*. Compounds **7** and **19** displayed equipotent activity to Amphotericin B against *Candida albicans*. Other synthesized compounds **15**, **21**, **23** and **25** showed relatively good growth inhibitory profiles versus *Aspergillus fumigatus* and *Candida albicans* when compared to Amphotericin B (fig. 3).

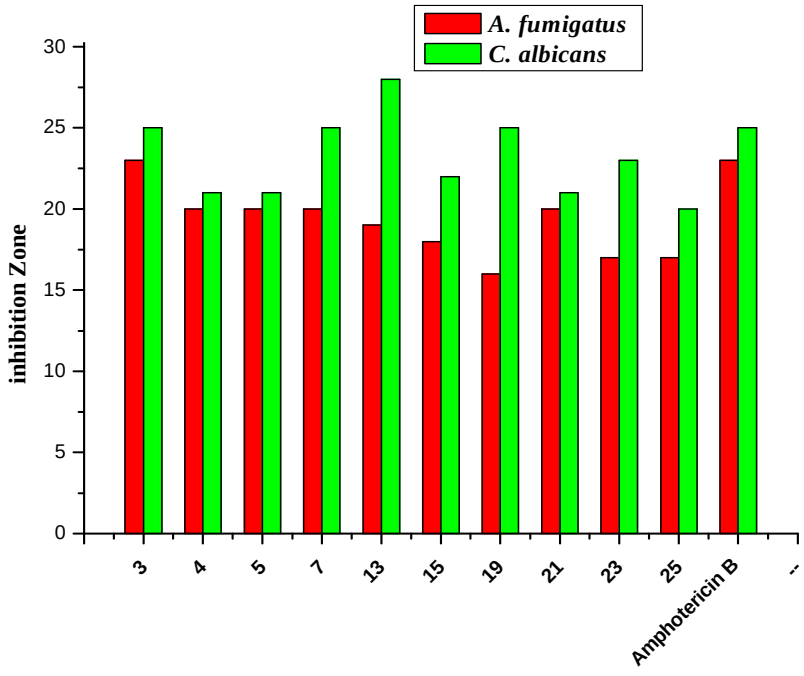


Fig. 3: Antifungal activity of the synthesized compounds

CONCLUSION

The objective of the present study was to synthesize and evaluate the antimicrobial activity of some novel rhodanine and bisrhodanine with the hope of discovering new structure serving as antimicrobial agent. The data showed clearly that most of compounds displayed good to moderate antimicrobial activity compared with Ampicillin, Gentamycin and Amphotericin B.

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