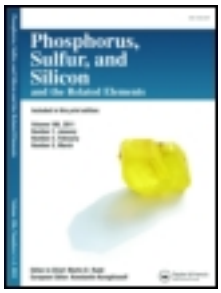

Phosphorus, Sulfur, and Silicon and the Related Elements

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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL THIOSEMICARBAZONES AS POSSIBLE ANTIBACTERIAL AND ANTIOXIDANT AGENTS

Ayşegül Karaküçük-İyidoğan [a] , Zeliha Mercan [a] , Emine Elçin Oruç-Emre [a] , Demet Taşdemir [a] , Derya İşler [b] , İbrahim Halil Kılıç [b] & Mehmet Özaslan [b]

[a] Department of Chemistry, Faculty of Arts and Sciences , Gaziantep University , Universite Bulvari, 27310 , Gaziantep , Turkey

[b] Department of Biology, Faculty of Arts and Sciences , Gaziantep University , Universite Bulvari, 27310 , Gaziantep , Turkey

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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL THIOSEMICARBAZONES AS POSSIBLE ANTIBACTERIAL AND ANTIOXIDANT AGENTS

Ayşegül Karaküçük-İyidoğan[a]*, Zeliha Mercan[a], Emine Elçin Oruç-Emre[a], Demet Taşdemir[a], Derya İşler[b], İbrahim Halil Kılıç[b], Mehmet Özaslan[b]

[a]*Department of Chemistry, Faculty of Arts and Sciences, Gaziantep University, Universite Bulvari, 27310, Gaziantep, Turkey.*

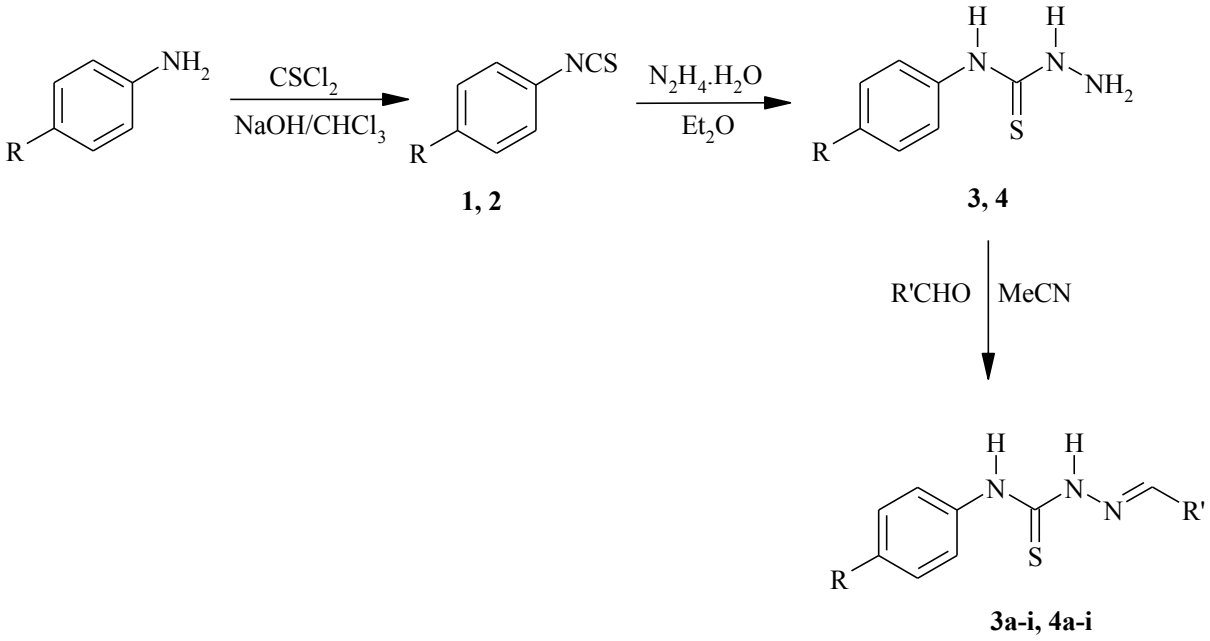
[b]*Department of Biology, Faculty of Arts and Sciences, Gaziantep University, Universite Bulvari, 27310, Gaziantep, Turkey.*

*E-mail: iyidogan@gantep.edu.tr

Phone: +90 342 317 18 30; Fax: +90 342 360 10 32.

Abstract

The synthesis and characterization of eighteen novel thiosemicarbazones, have been investigated as part of a research program on development of compounds with antibacterial and antioxidant activities. Among the tested compounds, 2-(4-hydroxybenzylidene)-*N*-[4-(trifluoromethoxy)phenyl]hydrazine carbothioamide (**3g**) and 2-(thiophen-2-ylmethylidene)-*N*-[4-(morpholin-4-yl)phenyl]hydrazine carbothioamide (**4b**) showed excellent inhibition potency at low concentration (0.5 μg/mL) against Gram-positive pathogens (*E. faecalis* and *S. aureus*). All tested compounds were also found to possess antioxidant activity against DPPH radical and ABTS radical cation.



Keywords: antibacterial, antioxidant, isothiocyanate, thiosemicarbazide, thiosemicarbazone.

INTRODUCTION

Since the discovery of the first antibiotic penicillin by Alexander Fleming in 1928, the antibiotics have been of critical importance in the fight against infectious diseases caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi.[1] However, the increasing resistance against Gram-positive pathogens and Gram-negative pathogens is a serious health problem in both developed and developing countries.[2,3] Especially, the existence of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterecoccus faecalis* (VRE) in the nosocomial (hospital) environments has increased considerably.[4] Furthermore, *Pseudomonas aeruginosa* is an opportunistic human pathogen and naturally resistant to a wide range of antibiotics.[5] Reactive oxygen species (ROS) normally exist in all aerobic cells in balance with biochemical antioxidants which may be enzymes such as superoxide dismutate, glutathione peroxidase and catalase or organic molecules such as ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids, flavonoids and thiols.[6] Oxidative stress is generated leading to oxidative damages to biomolecules when this critical balance is disrupted because of excess ROS, antioxidants depletion, or both.[7] Various antioxidant agents have been widely used in food industry and medicine to interrupt radical chain oxidation processes.[8]

Herein, to find potent more specific and effective antibacterial and antioxidant agents, we have designed a series of novel compounds containing thiosemicarbazone skeleton. Thiosemicarbazones are thiourea derivatives and analogues of semicarbazones which contains a sulfur atom in place of the oxygen atom.[9] They have attracted considerable interest since the discovery of their activity against *Mycobacterium tuberculosis*.[10] The biological properties of

thiosemicarbazone derivatives such as antibacterial,[11] antifungal,[12] antimalarial,[13] anticancer,[14,15] anti-inflammatory,[16] anticonvulsant,[17] antioxidant,[18] antiviral,[19,20] activities have been extensively studied over the last 50 years. Some thiosemicarbazone derivatives, such as triapine (anticancer), thiacetazone (antitubercular) and methisazone (antiviral) are already used in clinical treatment (*Figure 1.*).[21] Generally, thiosemicarbazone derivatives are synthesized by condensation of the corresponding thiosemicarbazides with aldehydes or ketones. Therefore, their biologicial activities depend on the parent aldehyde and ketone.[22]

On the basis of the above ideas, novel eighteen thiosemicarbazone derivatives have been synthesized by condensation of five 4-substituted benzaldeydes and four heteroaromatic aldehydes with two 4-substitutedphenyl thiosemicarbazides. The chemical structures of all compounds including intermediates were eludicated by UV-Vis, IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis. These thiosemicarbazones were tested against a variety of bio assays. Antibacterial activity was evaluated against four pathogenic bacterial strains. Antioxidant activity of the synthesized thiosemicarbazones was also screened using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method as free radical scavenging assays. The results showed that novel thiosemicarbazone analogues possess a great potential to be developed as antibacterial and antioxidant agents.

[Insert Figure 1]

RESULTS AND DISCUSSION

Synthesis

The starting compounds, 4-substitutedphenyl isothiocyanates (**1** and **2**), were prepared by treatment of 4-substituted aniline derivatives with thiophosgene in the presence of NaOH in water-chloroform medium at 0°C for 24 h. 4-(trifluoromethoxy)phenyl isothiocyanate (**1**) and 4-morpholinophenyl isothiocyanate (**2**) are commercially avaliable. 4-substitutedphenyl thiosemicarbazides (**3** and **4**) were reported previously.[23,24] These compounds were obtained by the reaction of 4-substitutedphenyl isothiocyanates with hydrazine monohydrate in diethyl ether at room temperature. A series of thiosemicarbazones (**3a-i** and **4a-i**) were synthesized by refluxing an equimolar ratio of the appropriate aldehydes and 4-substituedphenyl thiosemicarbazides in acetonitrile medium and gave 80-86% yields. All thiosemicarbazone derivatives were firstly synthesized. The general synthetic pathway to prepare thiosemicarbazone derivatives was exhibited in *Scheme 1*. The chemical structures of the all synthesized thiosemicarbazones were supported by elemental analysis and the spectral data achieved from UV-Vis, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy which were in agreement with the proposed structures.

[Insert Scheme 1]

Antibacterial activity

Both series of thiosemicarbazone derivatives, **3a-i** and **4a-i** were evaluated for their *in vitro* antibacterial activity against two Gram-positive (*Enterococcus faecalis* ATTC 29212 and *Staphylococcus aureus* ATTC 25923) and two Gram-negative (*Escherichia coli* ATTC 25322 and *Pseudomonas aeruginosa* ATTC 27853) bacteria using conventional microdilution broth method.[25] The MIC (Minimum Inhibitory Concentration) values were determined by comparison

to ampicillin trihydrate as a reference drug. The results of antibacterial activity study were presented in *Table S 1 (Supplemental Materials).*

DPPH radical scavenging activity

The scavenging effect of the synthesized thiosemicarbazones **3a-i** and **4a-i** on the DPPH radical was evaluated by modifying the literature procedure.[26] The results of antioxidant activity study were presented in *Table S 2 (Supplemental Materials).*

ABTS radical cation scavenging activity

In ABTS radical cation scavenging method, the antioxidant activity of tested compounds was expressed also as the TEAC, the concentration of Trolox solution having an antioxidant activity equivalent to 1 mM concentration of the tested compounds.[27]

Determination of drug-like properties

In order to improve the discovery and development of new drug candidates, a broad effort is being made to assess the 'drug-like' properties of molecules in early stages of the discovery-research process.[28,29] Lipinski's rule of five also known as the Rule of Five (ROF) is a rule of thumb to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by the medical chemist Christopher A. Lipinski and his

colleagues in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules. The rule describes molecular properties of a compound that directly influence its pharmacokinetics in human body like a drug, including their absorption, distrubution, metabolism and excretion (ADME). Generally, the ROF is based on four properties of molecules: molecular weight (MW), octanol-water partition coefficient (log*P*), the number of hydrogen-bond donors (HBD: the number of –OH and –NH groups) and the number of hydrogen-bond acceptors (HBA: the number of oxygen and nitrogen atoms). These parameters allow to ascertain a poor oral absorption, or membrane permeability, that occurs when the tested compounds present values higher than five H-bond donors, 10 H-bond acceptors, molecular weight is greater than 500 Da and lipophilicity (clog*P*) is greater than 5.[29] However, it is important to note that the rule does not guarantee a molecule is drug-like and predict if a compound is pharmacologically active. Another very useful parameter for the prediction of absorption is the polar surface area (PSA) defined as the sum of surfaces of polar atoms in a molecule. This parameter is easy to understand and, most importantly, provides good correlation with experimental transport data. Topolocigal polar surface area (TPSA) is a good descriptor for prediction of drug transport properties in the human intestines, Caco-2 monolayers penetration and blood-brain barrier crossing.[30]

These parameters were determined for the thiosemicarbazone derivatives in analysis and the results are given in *Table 1*. From the obtained data, all thiosemicarbazone derivatives possess a suitable number of H-bond donor and H-bond acceptor atoms to ensure efficient interaction with receptors and their log*P* were found less than 5 except for compound **3h**.

According to predictive TPSA data of all thiosemicarbazones, they could have a good ability for penetrating cell membranes.

[Insert Table 1]

EXPERIMENTAL

All chemicals and solvents were purchased from Merck and Aldrich Chemical Company and were used without further purification. Reactions were monitored by thin layer chromatography (TLC). TLC was performed on Merck Silica Gel 60 F_{254} plates with visualization by exposure to iodine vapor and UV-light using EtOAc/hexane (v/v 1:1 and 1:3) as solvent system. Melting points were determined on a EZ-Melt MPA120 Automated Melting Point apparatus and were uncorrected. Electronic spectra were recorded in DMF on a PG Instruments T80+ UV-visible Spectrophometer. IR spectra were recorded on a Perkin Elmer 100 FT IR Spectrometer with universal ATR sampling accessory. ^{1}H NMR and ^{13}C NMR spectra were obtained at room temperature with a Bruker Avance-DPX-400 NMR spectrometer in DMSO-d_6 using TMS as a internal standard. Chemical shifts were recorded in parts per million (ppm) downfield from tetramethylsilane. The splitting patterns of ^{1}H NMR were designed as follows: s: singlet, brs: broad singlet, d: doublet, dd: double doublet, t: triplet, m: multiplet. The coupling constants (*J*) are given in Hertz. The mass spectra were recorded on LC–MS-Agilent 1100 MSD series in the electrospray mode. Elemental analyses (CHNS) were performed on a VarioMICRO elemental analyzer.

General procedure for the synthesis of thiosemicarbazones (3a-i and 4a-i)

To a hot solution of thiosemicarbazide (1.20 mmol) in CH_3CN (30 mL) was added dropwise a solution of the an appropriate aldehyde (1.20 mmol) in CH_3CN (10 mL) with continuous stirring and the reaction mixture was refluxed for a period of time specific to each synthesized compound. The progress of the reaction was monitored by TLC using appropriate thiosemicarbazide and aldehyde as the reference standard. The reaction mixture was cooled. The precipitate was filtered and the filtrate was concentrated under reduced pressure. The crude product was recrystallized from acetonitrile.

2-(furan-2-ylmethylidene)-*N*-[4-trifluoromethoxy)phenyl]hydrazine carbothioamide (3a). Brown solid. Yield: 89%; m.p.: 139-140 ^{o}C. UV/Vis λ_{max} (nm): 332; 269. IR ν_{max} (cm^{-1}): 3295, 3134 (N-H); 1539 (C=N); 1199 (C-F); 1132 (C-N); 1016 (N-N); 750 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 11.91 (s, 1H, CSN*H*); 9.99 (s, 1H, PhN*H*); 8.09 (s, 1H, *H*C=N); 7.87 (d, 1H, Ar*H*, *J*=1.59 Hz, C_2 proton of furan ring); 7.72 (d, 2H, Ar*H*, *J*=8.97 Hz, *meta* protons to OCF_3); 7.35 (d, 2H, Ar*H*, *J*=8.46 Hz, *ortho* protons to OCF_3); 7.09 (d, 1H, Ar*H*, *J*=3.41 Hz, C_4 proton of furan ring); 6.67 (dd, 1H, Ar*H*, J_1=3.42 Hz, J_2=1.76 Hz, C_3 proton of furan ring). ^{13}C NMR (DMSO-d_6, δ ppm): 176.5 (*C*=S); 146.2 (Ar*C*, *ipso* carbon to OCF_3); 139.4 (H*C*=N); 136.1, 130.5, 128.9 (Ar*C*), 127.7 (O*C*F_3), 122.0, 120.3, 119.6 (Ar*C*). MS-ES: (m/z) 329 [M^+], 330 $[M+H]^+$. Analysis calculated for $C_{13}H_{10}F_3N_3O_2S$ (329.30): C, 47.42; H, 3.06; N, 12.76; S, 9.74%. Found: C, 47.48; H, 3.10; N, 12.71; S, 9.67%.

2-(thiophen-2-ylmethylidene)-*N*-[4-(trifluoromethoxy)phenyl]hydrazine carbothioamide (3b). Brown solid. Yield: 86%; m.p.: 181-182 ^{o}C. UV/Vis λ_{max} (nm): 349; 269. IR ν_{max} (cm^{-1}): 3379, 3134 (N-H); 1541 (C=N); 1203 (C-F); 1138 (C-N); 1018 (N-N); 749 (C=S);

690 (C-S-C). ^{1}H NMR (DMSO-d_6, δ ppm): 11.91 (s, 1H, CSN*H*); 9.93 (s, 1H, PhN*H*); 8.37 (s, 1H, *H*C=N); 7.71 (d, 1H, Ar*H*, *J*=3.75 Hz, C_2 proton of thiophene ring); 7.67 (d, 2H, Ar*H*, *J*=8.74 Hz, *meta* protons to OCF_3); 7.54 (d, 1H, Ar*H*, *J*=3.70 Hz, C_4 proton of thiophene ring); 7.36 (d, 2H, Ar*H*, *J*=8.71 Hz, *ortho* protons to OCF_3); 7.15 (t, 1H, Ar*H*, J_1=3.75 Hz, J_2=3.71 Hz, C_3 proton of thiophene ring). ^{13}C NMR (DMSO-d_6, δ ppm): 176.2 (*C*=S); 145.7 (Ar*C*, *ipso* carbon to OCF_3); 140.9 (H*C*=N); 138.7, 131.6, 129.9 (Ar*C*), 128.5 (O*C*F_3), 121.9, 121.2, 119.3 (Ar*C*). MS-ES (m/z): 345 [M^+], 346 [M+H]$^+$. Analysis calculated for $C_{13}H_{10}F_3N_3OS_2$ (345.37): C, 45.21; H, 2.92; N, 12.17; S, 18.57%. Found: C, 45.25; H, 2.89; N, 12.11; S, 18.61%.

2-(5-nitrothiophen-2-ylmethylidene)-*N*-[4-trifluoromethoxy)phenyl]hydrazine carbothioamide (3c). Yellow solid. Yield: 81%; m.p.: 223-224 ^{o}C. UV/Vis λ_{max} (nm): 400; 296; 270. IR ν_{max} (cm^{-1}): 3333, 3118 (N-H); 1542 (C=N); 1528 (NO_2, asymmetric); 1335 (NO_2, symmetric); 1227 (C-F); 1153 (C-N); 1024 (N-N); 812 (C=S); 739 (C-S-C). ^{1}H NMR (DMSO-d_6, δ ppm): 12.30 (s, 1H, CSN*H*); 10.25 (s, 1H PhN*H*,); 8.32 (s, 1H, *H*C=N); 8.12 (d, 1H, Ar*H*, *J*= 4.35 Hz, C_3 proton of thiophene ring); 7.65 (d, 2H, Ar*H*, *J*= 8.75 Hz, *meta* protons to OCF_3); 7.62 (d, 1H, Ar*H*, *J*=4.39 Hz, C_4 proton of thiophene ring); 7.39 (d, 2H, Ar*H*, *J*=8.77 Hz, *ortho* protons to OCF_3). ^{13}C NMR (DMSO-d_6, δ ppm): 176.9 (*C*=S); 151.6 (Ar*C*, *ipso* carbon to NO_2); 146.7 (Ar*C*, *ipso* carbon to OCF_3); 146.1 (Ar*C*); 138.5 (H*C*=N); 136.6, 130.8, 130.3 (Ar*C*), 128.4 (O*C*F_3), 121.3, 119.3 (Ar*C*). MS-ES (m/z): 390 [M^+], 391 [M+H]$^+$. Analysis calculated for $C_{13}H_9F_3N_4O_3S_2$ (390.37): C, 40.00; H, 2.32; N, 14.35; S, 16.43%. Found: C, 40.16; H, 2.43; N, 14.24; S, 16.48%.

2-(1*H*-pyrrol-2-ylmethylidene)-*N*-[4-(trifluoromethoxy)phenyl]hydrazine carbothioamide (3d). Black solid. Yield: 87%; m.p: 165-166 ^{o}C. UV/Vis λ_{max} (nm): 350; 270.

IR ν_{max} (cm^{-1}): 3299, 3248, 3146 (N-H); 1555 (C=N); 1167 (C-F); 1122 (C-N); 1016 (N-N); 733 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 11.79 (s, 1H, CSN*H*); 11.52 (s, 1H, pyrrole N*H*); 10.11 (s, 1H, PhN*H*); 7.96 (s, 1H, *H*C=N); 7.75 (d, 2H, Ar*H*, *J*=8.27 Hz, *meta* protons to OCF_3); 7.40 (d, 2H, Ar*H*, *J*=8.28 Hz, *ortho* protons to OCF_3); 7.08 (m, 1H, Ar*H*, C_2 proton of pyrrole ring); 6.50 (m, 1H, Ar*H*, C_4 proton of pyrrole ring); 6.15 (m, 1H, Ar*H*, C_3 proton of pyrrole ring). ^{13}C NMR (DMSO-d_6, δ ppm): 177.2 (*C*=S), 144.7 (Ar*C*, *ipso* carbon to OCF_3), 142.9 (*C*=N), 138.5, 130.1, 129.1 (Ar*C*), 127.4 (O*C*F_3), 125.26, 118.8, 114.7, 111.4 (Ar*C*). MS-ES (m/z): 328 [M^+], 329 $[M+H]^+$. Analysis calculated for $C_{13}H_{11}F_3N_4OS$ (328.32): C, 47.56; H, 3.38; N, 17.07; S, 9.77%. Found: C, 47.58; H, 3.28; N, 17.11; S, 9.87%.

2-(4-fluorobenzylidene)-*N*-[4-(trifluoromethoxy)phenyl]hydrazinecarbothioamide (3e). Brown solid. Yield: 85%; m.p.: 172-173 °C. UV/Vis λ_{max} (nm): 332; 270. IR ν_{max} (cm^{-1}): 3291, 3152 (N-H); 1559 (C=N); 1150 (C-F); 1071 (C-N); 1014 (N-N); 830 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 11.94 (s, 1H, CSN*H*); 10.21 (s, 1H, PhN*H*); 8.17 (s, 1H, *H*C=N); 8.00 (dd, 2H, Ar*H*, *J*=8.52 Hz, *ortho* protons to F); 7.70 (d, 2H, Ar*H*, *J*=8.64 Hz, *meta* protons to OCF_3); 7.36 (d, 2H, Ar*H*, *J*=8.60 Hz, *ortho* protons to OCF_3); 7.28 (t, 2H, Ar*H*, *J*=8.51 Hz, *meta* protons to F). ^{13}C NMR (DMSO-d_6, δ ppm): 176.9 (*C*=S), 164.2 (Ar*C*, *ipso* carbon to F), 146.7 (Ar*C*, *ipso* carbon to OCF_3), 141.7 (*C*=N), 133.4, 131.8, 129.9 (Ar*C*), 127.5 (O*C*F_3), 125.4, 117.7, 114.2 (Ar*C*). MS-ES (m/z): 357 [M^+], 358 $[M+H]^+$. Analysis calculated for $C_{15}H_{11}F_4N_3OS$ (357.33): C, 50.42; H, 3.10; N, 11.76; S, 8.97%. Found: C, 50.58; H, 3.16; N, 11.67; S, 8.99%.

2-(4-nitrobenzylidene)-*N*-[4-(trifluoromethoxy)phenyl]hydrazinecarbothioamide (3f). Yellow solid. Yield: 82%; m.p.: 227-228 °C. UV/Vis λ_{max} (nm): 383; 275; 270. IR ν_{max} (cm^{-1}): 3345, 3133 (N-H); 1539 (C=N); 1512 (NO_2, asymmetric); 1337 (NO_2, symmetric); 1161 (C-

F); 1088 (C-N); 1019 (N-N); 749 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 12.19 (s, 1H, CSN*H*); 10.38 (s, 1H, PhN*H*); 8.27 (s, 1H, *H*C=N); 8.26 (d, 2H, Ar*H*, *J*=8.91 Hz, *ortho* protons to NO_2); 8.19 (d, 2H, Ar*H*, *J*=8.95 Hz, *meta* protons to NO_2); 7.68 (d, 2H, Ar*H*, *J*=8.96 Hz, *meta* protons to OCF_3); 7.40 (d, 2H, Ar*H*, *J*=8.25 Hz, *ortho* protons to OCF_3). ^{13}C NMR (DMSO-d_6, δ ppm): 176.5 (*C*=S), 151.3 (Ar*C*, *ipso* carbon to NO_2), 145.4 (Ar*C*, *ipso* carbon to OCF_3), 141.9 (*C*=N), 139.0, 130.5, 129.7 (Ar*C*), 127.5 (O*C*F_3), 125.9, 124.8, 114.7 (Ar*C*). MS-ES (m/z): 384 [M^+], 385 [M+H]$^+$. Analysis calculated for $C_{15}H_{11}F_3N_4O_3S$ (384.34): C, 46.88; H, 2.88; N, 14.58; S, 8.34%. Found: C, 46.65; H, 2.82; N, 14.29; S, 8.15%.

2-(4-hydroxybenzylidene)-*N*-[4-(trifluoromethoxy)phenyl]hydrazine carbothioamide (3g). Yellow solid. Yield: 89%; m.p.: 193-194 ^{o}C. UV/Vis λ_{max} (nm): 336; 270. IR ν_{max} (cm^{-1}): 3305, 3290 (N-H); 1541 (C=N); 1160 (C-F); 1092 (C-N); 1020 (N-N); 831 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 11.76 (s, 1H, CSN*H*); 10.07 (s, 1H, PhN*H*); 9.94 (s, 1H, O*H*); 8.08 (s, 1H, *H*C=N); 7.73 (d, 2H, Ar*H*, *J*=8.38 Hz, *meta* protons to OH); 7.71 (d, 2H, Ar*H*, *J*=8.87 Hz, *meta* protons to OCF_3); 7.36 (d, 2H, Ar*H*, *J*=8.63 Hz, *ortho* protons to OCF_3); 6.83 (d, 2H, Ar*H*, *J*=8.60 Hz, *ortho* protons to OH). ^{13}C NMR (DMSO-d_6, δ ppm): 177.8 (*C*=S), 153.5 (Ar*C*, *ipso* carbon to OH), 147.2 (Ar*C*, *ipso* carbon to OCF_3), 142.0 (*C*=N), 132.5, 131.9, 130.2 (Ar*C*), 127.2 (O*C*F_3), 126.1, 117.5, 115.5 (Ar*C*). MS-ES (m/z): 355 [M^+], 356 [M+H]$^+$. Analysis calculated for $C_{15}H_{12}F_3N_3O_2S$ (355.34): C, 50.70; H, 3.40; N, 11.83; S, 9.02%. Found: C, 50.57; H, 3.55; N, 11.78; S, 9.05%.

2-{4-[bis(2-chloroethyl)amino]benzylidene}-*N*-[4-(trifluoromethoxy)phenyl] hydrazine carbothioamide (3h). Brown solid. Yield: 88%; m.p.: 191-192 ^{o}C. UV/Vis λ_{max} (nm): 371; 270. IR ν_{max} (cm^{-1}): 3310, 3143 (N-H); 1544 (C=N); 1189 (C-F); 1056 (C-N); 1017 (N-N);

815 (C=S); 717 (C-Cl). ^{1}H NMR (DMSO-d_6, δ ppm): 11.78 (s, 1H, CSN*H*); 10.18 (s, 1H, PhN*H*); 8.12 (s, 1H, *H*C=N); 7.79 (d, 4H, Ar*H*, *J*=8.86 Hz, *ortho* and *meta* protons to $N(CH_2CH_2Cl)_2$ group); 7.42 (d, 2H, Ar*H*, *J*=8.50 Hz, *meta* protons to OCF_3); 6.86 (d, 2H, Ar*H*, *J*=8.91 Hz, *ortho* protons to OCF_3); 3.89-3.79 (m, 8H, $N(CH_2CH_2Cl)_2$). ^{13}C NMR (DMSO-d_6, δ ppm): 175.9 (*C*=S), 148.4 (Ar*C*, *ipso* carbon to $N(CH_2CH_2Cl)_2$ group), 146.8 (Ar*C*, *ipso* carbon to OCF_3), 1439 (*C*=N), 136.7, 134.56 129.7 (Ar*C*), 127.4 (O*C*F_3), 126.1, 122.6, 112.0 (Ar*C*), 52.3 ($N(CH_2CH_2Cl)_2$), 41.9 ($N(CH_2CH_2Cl)_2$). MS-ES: (m/z) 478 [M^+]; 479 [M+H]$^+$. Analysis calculated for $C_{19}H_{19}Cl_2F_3N_4OS$ (478.35): C, 47.61; H, 4.00; N, 11.69; S, 6.69%. Found: C, 47.48; H, 4.07; N, 11.62; S, 6.74%.

2-(4-methoxybenzylidene)-*N*-[4-(trifluoromethoxy)phenyl]hydrazine carbothioamide (3i). Beige solid. Yield: 79%; m.p.: 165-166 ^{o}C. UV/Vis λ_{max} (nm): 346; 270. IR ν_{max} (cm^{-1}): 3343, 3143 (N-H); 1543 (C=N); 1166 (C-F); 1132 (C-N); 1024 (N-N); 829 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 11.82 (s, 1H, CSN*H*); 10.12 (s, 1H, PhN*H*); 8.12 (s, 1H, *H*C=N); 7.85 (d, 2H, Ar*H*, *J*=8.85 Hz, *meta* protons to OCH_3); 7.71 (d, 2H, Ar*H*, *J*=8.97 Hz, *meta* protons to OCF_3); 7.37 (d, 2H, Ar*H*, *J*=8.90 Hz, *ortho* protons to OCF_3); 6.90 (d, 2H, Ar*H*, *J*=8.86 Hz, *ortho* protons to OCH_3); 3.81 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, δ ppm): 176.3 (*C*=S), 161.3 (Ar*C*, *ipso* carbon to OCH_3), 147.3 (Ar*C*, *ipso* carbon to OCF_3), 143.4 (*C*=N), 136.7, 134.8, 129.7 (Ar*C*), 127.8 (O*C*F_3), 126.7, 126.1, 114.5 (Ar*C*), 55.3 (O*C*H_3). MS-ES (m/z): 369 [M^+], 370 [M+H]$^+$. Analysis calculated for $C_{16}H_{14}F_3N_3O_2S$ (369.37): C, 52.03; H, 3.82; N, 11.38; S, 8.68%. Found: C, 52.06; H, 3.76; N, 11.44; S, 8.80%.

2-(furan-2-ylmethylidene)-*N*-[4-(morpholin-4-yl)phenyl]hydrazine carbothioamide (4a). Brown solid. Yield: 72%; m.p.: 219-220 ^{o}C. UV/Vis λ_{max} (nm): 331; 271. IR ν_{max} (cm^{-1}):

3286, 3193 (N-H); 1564 (C=N); 1069 (C-N), 1018 (N-N); 811 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 11.69 (s, 1H, CSN*H*); 9.67 (s, 1H, PhN*H*); 8.07 (s, 1H, *H*C=N); 7.85 (d, 1H, Ar*H*, *J*=1.60 Hz, C_2 proton of furan ring); 7.38 (d, 2H, Ar*H*, *J*=8.96 Hz, *meta* protons to morpholino group); 7.07 (d, 1H, Ar*H*, *J*=3.60 Hz, C_4 proton of furan ring); 6.92 (d, 2H, Ar*H*, *J*=8.65 Hz, *ortho* protons to morpholino group); 6.65 (dd, 1H, Ar*H*, J_1=3.60 Hz, J_2=1.60 Hz, C_3 proton of furan ring); 3.74 (t, 4H, NCH_2CH_2O); 3.11 (t, 4H, NCH_2CH_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 176.4 (*C*=S), 151.9, 146.1 (Ar*C*), 143.4 (*C*=N), 130.8, 129.7, 127.9, 116.4, 113.7, 113.3 (Ar*C*), 66.4 (NCH_2CH_2O), 47.2 (NCH_2CH_2O). MS-ES (m/z): 330 [M^+], 331 [M+H]$^+$. Analysis calculated for $C_{16}H_{18}N_4O_2S$ (330.41): C, 58.16; H, 5.49; N, 16.96; S, 9.70%. Found: C, 58.29; H, 5.47; N, 16.81; S, 9.58%.

2-(thiophen-2-ylmethylidene)-*N*-[4-(morpholin-4-yl)phenyl]hydrazine carbothioamide (4b). Dark green solid. Yield: 72%; m.p.: 195-196 ^{o}C. UV/Vis λ_{max} (nm): 355; 268. IR ν_{max} (cm^{-1}): 3220, 3100 (N-H); 1542 (C=N); 1135 (C-N); 1041 (N-N); 819 (C=S); 714 (C-S-C). ^{1}H NMR (DMSO-d_6, δ ppm): 11.75 (s, 1H, CSN*H*); 9.70 (s, 1H PhN*H*); 8.32 (s, 1H, *H*C=N); 7.69 (d, 1H, Ar*H*, *J*=3.81 Hz C_2 proton of thiophene ring); 7.52 (d, 1H, Ar*H*, *J*=3.64 Hz C_4 proton of thiophene ring); 7.36 (d, 2H, Ar*H*, *J*=8.82 Hz, *meta* protons to morpholino group); 7.14 (dd, 1H, Ar*H*, J_1=3.80 Hz, J_2=3.62 Hz, C_3 proton of thiophene ring); 6.93 (d, 2H, Ar*H*, *J*=8.81 Hz, *ortho* protons to morpholino group); 3.75 (t, 4H, NCH_2CH_2O); 3.11 (t, 4H, NCH_2CH_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 176.5 (*C*=S), 154.5 (Ar*C*), 141.7 (*C*=N), 135.6, 132.6, 130.8, 129.1, 121.8, 116.5, 113.7 (Ar*C*), 66.4 (NCH_2CH_2O), 47.3 (NCH_2CH_2O). MS-ES: (m/z) 346 [M^+], 347 [M+H]$^+$. Analysis calculated for $C_{16}H_{18}N_4OS_2$ (346.48): C, 55.47; H, 5.24; N, 16.17; S, 18.51%. Found: C, 55.17; H, 5.12; N, 16.09; S, 18.63%.

2-(5-nitrothiophene-2-ylmethylidene)-*N*-[4-(morpholin-4-yl)phenyl]hydrazine carbothioamide (4c). Brown solid. Yield: 74%; m.p.: 230-231 ^{o}C. UV/Vis λ_{max} (nm): 398; 291; 270. IR ν_{max} (cm^{-1}): 3344, 3107 (N-H); 1543 (C=N); 1518 (NO_2, asymmetric); 1334 (NO_2, symmetric); 1119 (C-N); 1032 (N-N); 813 (C=S); 730 (C-S-C). ^{1}H NMR (DMSO-d_6, δ ppm): 12.11 (s, 1H, CSN*H*); 10.07 (s, 1H PhN*H*,); 8.28 (s, 1H, *H*C=N); 8.11 (d, 1H, Ar*H*, *J*= 4.40 Hz, C_3 proton of thiophene ring); 7.60 (d, 1H, Ar*H*, *J*= 4.40 Hz, C_4 proton of thiophene ring,); 7.32 (d, 2H, Ar*H*, *J*=8.80 Hz, *meta* protons to morpholino group); 6.95 (d, 2H, Ar*H*, *J*=8.80 Hz, *ortho* protons to morpholino group); 3.75 (t, 4H, $NCH_2C\underline{H}_2O$); 3.12 (t, 4H, $NC\underline{H}_2CH_2O$). ^{13}C NMR (DMSO-d_6, δ ppm): 176.8 (*C*=S); 151.33 (Ar*C*, *ipso* carbon to NO_2); 149.5, 147.1, (Ar*C*); 135.8 (H*C*=N); 131.0, 130.8, 129.9, 127.5, 115.0 (Ar*C*), 66.6 ($NCH_2\underline{C}H_2O$), 49.0 ($N\underline{C}H_2CH_2O$). MS-ES (m/z): 391 [M^+], 392 $[M+H]^+$. Analysis calculated for $C_{16}H_{17}N_5O_3S_2$ (391.48): C, 49.09; H, 4.38; N, 17.89; S, 16.38%. Found: C, 49.21; H, 4.49; N, 17.65; S, 16.42%.

2-(1*H*-pyrrol-2-ylmethylidene)-*N*-[4-(morpholin-4-yl)phenyl]hydrazine carbothioamide (4d). Orange solid. Yield: 65%; m.p: 175-176 ^{o}C. UV/Vis λ_{max} (nm): 348; 269. IR ν_{max} (cm^{-1}): 3280, 3254, 3123 (N-H); 1579 (C=N); 1123 (C-N); 1027 (N-N); 813 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 11.54 (s, 1H, CSN*H*); 11.52 (s, 1H, pyrrole N*H*); 9.90 (s, 1H, PhN*H*); 7.94 (s, 1H, *H*C=N); 7.40 (d, 2H, Ar*H*, *J*=8.40 Hz, *meta* protons to morpholino group); 7.04 (brs, 1H, Ar*H*, C_2 proton of pyrrole ring); 6.97 (d, 2H, Ar*H*, *J*= 8.40 Hz, *ortho* protons to morpholino group); 6.46 (brs, 1H, Ar*H*, C_4 proton of pyrrole ring); 6.15 (t, 1H, Ar*H*, C_3 proton of pyrrole ring); 3.76 (t, 4H, $NCH_2C\underline{H}_2O$); 3.12 (t, 4H, $NC\underline{H}_2CH_2O$). ^{13}C NMR (DMSO-d_6, δ ppm): 176.9 (*C*=S), 145.7 (Ar*C*), 142.3 (*C*=N), 132.7, 128.4, 127.1, 124.8, 119.2, 113.2, 111.0 (Ar*C*), 66.4 ($NCH_2\underline{C}H_2O$), 48.1 ($N\underline{C}H_2CH_2O$). MS-ES (m/z): 329 [M^+], 330 $[M+H]^+$. Analysis

calculated for $C_{16}H_{19}N_5OS$ (329.43): C, 58.34; H, 5.81; N, 21.26; S, 9.73%. Found: C, 58.26; H, 5.77; N, 21.12; S, 9.61%.

2-(4-fluorobenzylidene)-*N*-[4-(morpholin-4-yl)phenyl]hydrazinecarbothioamide (4e). Gray solid. Yield: 88%; m.p.: 217-218 ^{o}C. UV/Vis λ_{max} (nm): 332; 269. IR ν_{max} (cm^{-1}): 3257, 3118 (N-H); 1541 (C=N); 1230 (C-F); 1118 (C-N); 1071 (N-N); 824 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 11.52 (s, 1H, CSN*H*); 10.01 (s, 1H PhN*H*); 8.13 (s, 1H, *H*C=N); 7.99 (dd, 2H, Ar*H*, *J*=8.65 Hz *ortho* protons to F); 7.35 (d, 2H, Ar*H*, *J*=8.65 Hz, *meta* protons to morpholino group); 7.27 (t, 2H, Ar*H*, *J*=8.55 Hz, *meta* proton to F); 6.94 (d, 2H, Ar*H*, *J*=8.67 Hz, *ortho* protons to morpholino group); 3.74 (t, 4H, $NCH_2C\underline{H_2}O$); 3.10 (t, 4H, $NC\underline{H_2}CH_2O$). ^{13}C NMR (DMSO-d_6, δ ppm): 176.9 (*C*=S), 162.4, 154.6 (Ar*C*), 142.8 (*C*=N), 132.2, 131.4, 130.8, 128.0, 116.5, 113.5 (Ar*C*), 66.6 ($NCH_2\underline{C}H_2O$), 47.6 ($N\underline{C}H_2CH_2O$). MS-ES (m/z): 358 [M^+], 359 $[M+H]^+$. Analysis calculated for $C_{18}H_{19}FN_4OS$ (358.44): C, 60.32; H, 5.34; N, 15.63; S, 8.95%. Found: C, 60.24; H, 5.32; N, 15.31; S, 8.83%.

2-(4-nitrobenzylidene)-*N*-[4-(morpholin-4-yl)phenyl]hydrazinecarbothioamide (4f). Yellow solid. Yield: 75%; m.p.: 180-181 ^{o}C. UV/Vis λ_{max} (nm): 392; 276; 268. IR ν_{max} (cm^{-1}): 3279, 3254 (N-H); 1579 (C=N); 1518 (NO_2, asymmetric); 1345 (NO_2, symmetric); 1122 (C-N); 1027 (N-N); 814 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 12.01 (s, 1H, CSN*H*); 10.22 (s, 1H, PhN*H*); 8.25 (d, 2H, Ar*H*, *J*=8.91 Hz, *ortho* protons to NO_2); 8.21 (s, 1H, *H*C=N); 8.19 (d, 2H, Ar*H*, *J*=8.95 Hz, *meta* protons to NO_2); 7.34 (d, 2H, Ar*H*, *J*=8.40 Hz, *meta* protons to morpholino group); 6.96 (d, 2H, Ar*H*, *J*=8.40 Hz, *ortho* protons to morpholino group); 3.76 (t, 4H, $NCH_2C\underline{H_2}O$); 3.12 (t, 4H, $NC\underline{H_2}CH_2O$). ^{13}C NMR (DMSO-d_6, δ ppm): 177.3 ($\underline{C}$=S), 151.9 (Ar*C*, *ipso* carbon to NO_2), 147.5 (Ar*C*). 142.2 (*C*=N), 140.2, 128.6, 127.5, 125.1, 124.9, 113.3

(Ar<u>C</u>), 67.1 ($NCH_2\underline{C}H_2O$), 48.2 ($N\underline{C}H_2CH_2O$). MS-ES (m/z): 385 [M^+], 386 $[M+H]^+$. Analysis calculated for $C_{18}H_{19}N_5O_3S$ (385.45): C, 56.09; H, 4.97; N, 18.17; S, 8.32%. Found: C, 56.15; H, 4.91; N, 18.28; S, 8.44%.

2-(4-hydroxybenzylidene)-*N*-[4-(morpholin-4-yl)phenyl]hydrazinecarbothioamide (4g). Brown solid. Yield: 86%; m.p.: 230-231 ^oC. UV/Vis λ_{max} (nm): 329; 270. IR ν_{max} (cm^{-1}): 3297, 3192 (N-H); 1577 (C=N); 1065 (C-N); 1024 (N-N); 812 (C=S). ^1H NMR (DMSO-d_6, δ ppm): 11.54 (s, 1H,CSN<u>*H*</u>); 9.91 (s, 1H, PhN<u>*H*</u>); 9.83 (s, 1H, O<u>*H*</u>); 8.04 (s, 1H, <u>*H*</u>C=N); 7.71 (d, 2H, Ar<u>*H*</u>, *J*=8.89 Hz, *meta* protons to OH); 7.36 (d, 2H, Ar<u>*H*</u>, *J*=8.58 Hz, *meta* protons to morpholino group); 6.92 (d, 2H, Ar<u>*H*</u>, *J*=8.56 Hz, *ortho* protons to morpholino group); 6.85 (d, 2H, Ar<u>*H*</u>, *J*=8.87 Hz, *ortho* protons to OH); 3.74 (t, 4H, $NCH_2C\underline{H}_2O$); 3.10 (t, 4H, $NC\underline{H}_2CH_2O$). ^{13}C NMR (DMSO-d_6, δ ppm): 178.2 (*C*=S), 156.6, 149.3 (Ar<u>C</u>), 141.5 (*C*=N), 127.9, 126.4, 125.2, 124.4, 122.7, 112.7 (Ar<u>C</u>), 66.3 ($NCH_2\underline{C}H_2O$), 47.3 ($N\underline{C}H_2CH_2O$). MS-ES (m/z): 356 [M^+]; 357 $[M+H]^+$. Analysis calculated for $C_{18}H_{20}N_4O_2S$ (356.45): C, 60.65; H, 5.66; N, 15.72; S, 9.00%. Found: C, 60.55; H, 5.57; N, 15.60; S, 9.16%.

2-{4-[bis(2-chloroethyl)amino]benzylidene}-*N*-[4-(morpholin-4-yl)phenyl] hydrazine carbothioamide (4h). Red solid. Yield: 85%; m.p.: 184-185 ^oC. UV/Vis λ_{max} (nm): 377; 271. IR ν_{max} (cm^{-1}): 3280, 3255 (N-H); 1595 (C=N); 1122 (C-N); 1027 (N-N); 814 (C=S); 698 (C-Cl). ^1H NMR (DMSO-d_6, δ ppm): 11.53 (s, 1H, CSN<u>*H*</u>); 9.81 (s, 1H, PhN<u>*H*</u>); 8.03 (s, 1H, <u>*H*</u>C=N); 7.72 (d, 4H, Ar<u>*H*</u>, *J*=8.86 Hz, *meta* protons to $N(CH_2CH_2Cl)_2$ group); 7.38 (d, 2H, Ar<u>*H*</u>, *J*=8.50 Hz, *meta* protons to morpholino group); 6.93 (d, 2H, Ar<u>*H*</u>, *J*=8.91 Hz, *ortho* protons to

morpholino group); 6.79 (d, 2H, Ar*H*, *J*=8.91, *ortho* protons to $N(CH_2CH_2Cl)_2$ group); 3.80-3.74 (m, 12H, $N(CH_2CH_2Cl)_2$ and NCH_2CH_2O); 3.11 (t, 4H, NCH_2CH_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 176.7 (*C*=S), 150.4 (Ar*C*, *ipso* carbon to $N(CH_2CH_2Cl)_2$ group), 145.5 (Ar*C*), 142.4 (*C*=N), 130.8, 128.7, 127.3, 124.8, 114.4, 111.5 (Ar*C*), 65.96 (NCH_2CH_2O), 53.3 ($N(CH_2CH_2Cl)_2$), 48.1 (NCH_2CH_2O), 42.1 ($N(CH_2CH_2Cl)_2$). MS-ES: (m/z) 479 [M^+]; 480 $[M+H]^+$. Analysis calculated for $C_{22}H_{27}Cl_2N_5OS$ (479.47): C, 55.00; H, 5.66; N, 14.58; S, 6.67%. Found: C, 55.07; H, 5.71; N, 14.46; S, 6.69%.

2-(4-methoxybenzylidene)-*N*-[4-(morpholin-4-yl)phenyl]hydrazine carbothioamide (4i). Beige solid. Yield: 80%; m.p.: 195-196 ^{o}C. UV/Vis λ_{max} (nm): 352; 269. IR ν_{max} (cm^{-1}): 3280, 3254 (N-H); 1579 (C=N); 1123 (C-N); 1026 (N-N); 814 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 11.62 (s, 1H, CSN*H*); 9.91 (s, 1H, PhN*H*); 8.09 (s, 1H, *H*C=N); 7.88 (d, 2H, Ar*H*, *J*=8.85 Hz, *meta* protons to OCH_3);.7.38 (d, 2H, Ar*H*, *J*=8.90 Hz, *meta* protons to morpholino group); 7.02 (d, 2H, Ar*H*, *J*=8.90 Hz, *ortho* protons to morpholino group); 6.98 (d, 2H, Ar*H*, *J*=8.86 Hz, *ortho* protons to OCH_3); 3.80 (s, 3H, OCH_3,); 3.73 (t, 4H, NCH_2CH_2O); 3.11 (t, 4H, NCH_2CH_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 175.5 (*C*=S), 162.9 (Ar*C*, *ipso* carbon to OCH_3), 144.2 141.2 (*C*=N), 131.5, 128.4, 127.0, 126.9, 115.1, 113.7 (Ar*C*), 67.6 (NCH_2CH_2O), 56.2 (OCH_3), 48.8 (NCH_2CH_2O). MS-ES (m/z): 370 [M^+], 371 $[M+H]^+$. Analysis calculated for $C_{19}H_{22}N_4O_2S$ (370.48): C, 61.60; H, 5.99; N, 15.12; S, 8.66%. Found: C, 61.76; H, 5.82; N, 15.36; S, 8.81%.

Biological studies

All the synthesized thiosemicarbazones (**3a-i** and **4a-i**) were evaluated for their antibacterial and antioxidant activities.

Antibacterial activity assay

Antibacterial activity of all synthesized thiosemicarbazones were sreened against two Gram-positive (*Enterococcus faecalis* ATTC 29212 and *Staphylococcus aureus* ATTC 25923) and two Gram-negative (*Escherichia coli* ATTC 25322 and *Pseudomonas aeruginosa* ATTC 27853) bacteria. The minimum inhibitory concentration (MIC) values for thiosemicarbazone derivatives (**3a-i**, **4a-i**) defined as the lowest concentration of the compounds preventing the visible growth were determined by using the microdilution broth procedure.[31]

Antioxidant activity assay

DPPH radical scavenging capacity assay

The 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$).is a stable radical and commercially available. It is characterized by an absortion band at 518 nm and its reduction by an antioxidant compound leads to a decolorisation which can be determined by UV-Vis spectrofotometer. The scavenging effect of the synthesized thiosemicarbazones **3a-i** and **4a-i** on the DPPH radical was evaluated by modifying the literature procedure.[32]

ABTS radical cation scavenging capacity assay

A common method for testing the antioxidative potential of antioxidants as hydrogen-donating agents is to measure their ability to scavenge $ABTS^{\cdot+}$radical cation.[32,33]

CONCLUSIONS

The synthesis, structural characterization, antibacterial and antioxidant evaluation of eighteen novel thiosemicarbazone derivatives have been reported. All synthesized compounds were purified by recrystallization and obtained in good yields. The spectroscopic data of thiosemicarbazones **3a-i** and **4a-i** clearly identified the proposed chemical structures. In conclusion, antibacterial screening data indicated that among all tested thiosemicarbazones compounds **3c**, **3e**, **3g**, **3h**, **3i**, **4b** and **4d** showed excellent antibacterial activity, while compounds **3g, 3i** and **4c** exhibited good antibacterial activity against *P. aeruginosa* when compared with ampicillin used as a standard substance. Especially, compound **3g** which is bearing the hydroxy and trifluoromethoxy substituents at the *para* position of phenyl ring endowed with the most potent antibacterial activity. However, compound **4b** which is bearing morpholino and thiophene groups at the *para* position of phenyl ring exhibited more potent antibacterial activity than other morpholino derivatives against tested Gram-positive bacteria. Other electron donating and electron with-drawing substituents did not significantly increase the antibacterial activity. Antioxidant screening data showed that all tested thiosemicarbazone derivatives exhibited excellent scavenging activity on ABTS radical cation. However, compounds **3e, 3i, 4e, 4g, 4h** and **4i** showed very good scavenging activity on DPPH radical. All these results can be useful for future efforts to synthesize and evaluate thiosemicarbazone derivatives in order to enhance their antibacterial and antioxidant properties.

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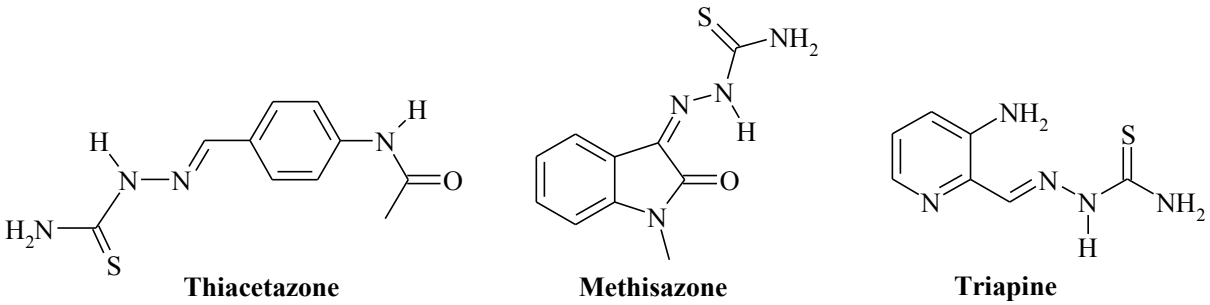
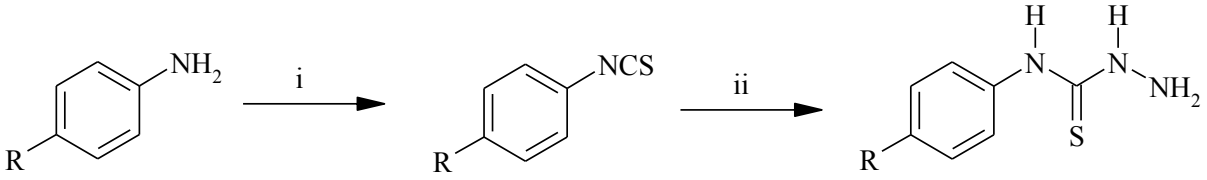
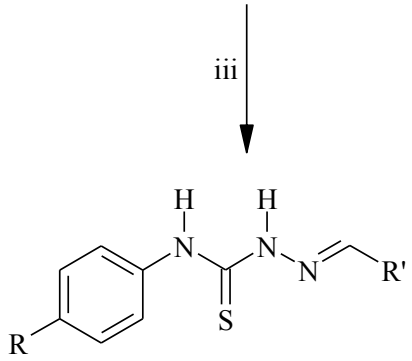


Figure 1. Biologically active thiosemicarbazones



1, 2 **3, 4**

3a: R = trifluoromethoxy, R' = furyl
3b: R = trifluoromethoxy, R' = thienyl
3c: R = trifluoromethoxy, R' = 5-nitrothienyl
3d: R = trifluoromethoxy, R' = pyrolyl
3e: R = trifluoromethoxy, R' = 4-fluorophenyl
3f: R = trifluoromethoxy, R' = 4-nitrophenyl
3g: R = trifluoromethoxy, R' = 4-hydroxyphenyl
3h: R = trifluoromethoxy, R' = 4-bis(2-chloroethylamino)phenyl
3i: R = trifluoromethoxy, R' = 4-methoxyphenyl
4a: R = morpholinyl, R' = furyl
4b: R = morpholinyl, R' = thienyl
4c: R = morpholinyl, R' = 5-nitrothienyl
4d: R = morpholinyl, R' = pyrolyl
4e: R = morpholinyl, R' = 4-fluorophenyl
4f: R = morpholinyl, R' = 4-nitrophenyl
4g: R = morpholinyl, R' = 4-hydroxyphenyl
4h: R = morpholinyl, R' = 4-bis(2-chloroethylamino)phenyl
4i: R = morpholinyl, R' = 4-methoxyphenyl



3a-i, 4a-i

Scheme 1. Synthetic patways of thiosemicarbazones **3a-i** and **4a-i.** Reagents and conditions: (i) $CSCl_2$, NaOH in water, $CHCl_3$, 0°C, 24 h; (ii) $NH_2NH_2.H_2O$ (98%), Et_2O, rt, 24 h; (iii) appropriate aldeyhde in MeCN, reflux.

Table 1: Drug-likeness properties of the synthesized thiosemicarbazone[a].

Compound	Log *P*	Volume	TPSA	Rotational bond	H-bond acceptors	H-bond donors	Molecular Weight
3a	3.54	253.229	58.79	7	5	2	329.303
3b	4.18	262.373	45.65	7	4	2	345.371
3c	4.29	285.707	91.48	8	7	2	390.368
3d	3.44	256.647	61.44	7	5	3	328.319
3e	4.45	276.592	45.65	7	4	2	357.332
3f	4.25	294.995	91.47	8	7	2	384.339
3g	3.81	279.679	65.88	7	5	3	355.341
3h	5.60	378.724	48.89	12	5	2	478.355
3i	4.34	297.207	54.88	8	5	2	369.368
4a	2.52	291.081	62.03	6	6	2	330.413
4b	3.16	300.224	48.89	6	5	2	346.481
4c	3.25	323.559	94.71	8	7	2	391.478
4d	2.42	294.498	64.68	6	6	3	329.429
4e	3.43	314.444	48.89	6	5	2	358.442
4f	3.22	332.847	94.71	8	7	2	385.449
4g	2.79	317.531	69.12	6	6	3	356.451
4h	4.58	416.575	52.13	11	6	2	479.465
4i	3.32	335.059	58.12	7	6	2	370.478

[a]TPSA, topological polar surface area. These parameters were determined with Molinspiration Calculation software.