Synthesis, Characterization and antioxidant activities of Semicarbazide and Thiosemicarbazide Derivatives

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Summary: In this research work Semicarbazide, thiosemicarbazide derivatives **3** to **25** were synthesized by conventional methods with high percentage yield and reaction rate. ^{1}H-NMR and EIMS spectroscopic techniques were used to elucidate the structure of the synthesized compounds. The effect of thiosemicarbazide and semicarbazide derivatives as an antioxidant agents were studied by DPPH free radical scavenging, ferric ion reducing, ferrous ion chelating assays. Higher DDPH radical scavenging activity exhibited by most of the compounds as compared to standard vitamin C. Excellent ferric ion reducing activity was indicated by compounds of theseriesas compared to standard vitamin C. However most of the compounds generally showed average ferrous ion chelating activity than standard EDTA.

Keywords: Semicarbazide, Thiosemicarbazide, DPPH, Ferric ion reducing activity, Ferrous ion chelating activity.

Introduction

In recent years, thiosemicarbazides and semicarbazides have attracted much attention because of their fungicidal,[1]bactericidal[2] and antioxidant activities. Aryl semicarbazides are investigated to display an outstanding anticonvulsant activity in mice and rats compared to that of phenytoin.[3] The aryl semicarbazides were believed to interact at the putative binding site designated as aryl binding, a hydrogen bonding domain and an auxiliary aryl binding site.[4] The aryl binding site can be phenyl or other hydrophobic moieties with retention of the anticonvulsant activity.

Thiosemicarbazides had central role in drug industry. The Use of thiosemicarbazides in organic synthesis has become a classical strategy for the synthesis of several heterocycles and other derivatives. Their reactions with compounds containing C=O and C=N groups is mainly involved for the synthesis of bioactive compounds, viztriazoles and thiazoles. A better understanding of their biological activity can be derived from their oxidation mechanisms. It is widely accepted that the prerequisite for thio compounds to express their physiological effects is through S-oxygenation.[5] Oxidation of sulfur containing compounds are involved in various cellular functions,[6] including the reductive degradation of polypeptide proteins , hormones and regulating the protein synthesis, maintaining of intracellular redox potential, minimize cellular oxidative damage, etc. Thiosemicarbazides and their derivatives display interesting biological activities, including anticancer,[7] anti HIV,[8], antibacterial,[9] antiviral[10] and antifungalowing to their ability to diffuse through the semipermeable membrane of cell lines.[11-13] They play vital role in the plant growth regulation. Some commercially important activities, such as antifouling effects and anti-corrosion [14] have also been observed for these compounds.

Antioxidants play an important role in several important biological processes such as protection against tissue damage, immunity, reproduction and development or growth. It is recognized that many human diseases e.g. cancer, arthritis and atherosclerosis have been correlated with oxidative damage, caused by reactive free radicals. The literature suggests that supplementation with antioxidants may be useful in the prevention and treatment of Parkinson's and Alzheimer's disease. [15], [16] Therefore, the search for a new active antioxidant has received much attention. Reactive oxygen species and free radical-mediated reactions are also involved in degenerative or pathological processes such as aging, [17], [18]cancer and coronary heart disease.[19]

Ghosh*et. al.* synthesized and evaluated a series of thiosemicarbazone, arylthiosemicarbazide derivatives and *N*-per-*O*-acetyl-glucosyl for their in vitro antioxidant and in vivo anti-dyslipidemic activities.[20]

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Experimental

To monitor the progress of reaction, thin layer chromatographic analysis technique was adopted, by using aluminum plates precoated with silica gel (Kieselgel 60, 254), Merck,Germany.The chromatograms were visualized usingUV at 254 and 365 nm. Advance Bruker AM 300 MHz was used for ^{1}HNMR experiments. A Finnigan MAT-311A, Germany was used to determined Electron impactmassspectra(EIMS).2,2-Diphenyl-1-picrylhydrazylradical(DPPH),ethylenediamine tetra-acetic acid (EDTA), *Tris*HCl buffer, ferrous sulphate, ferric chloride ($FeCl_3$), sulfuric acid, *O*-phenenthroline, potassium phosphate (mono phosphate and diphosphate), ethyl benzoate, CS_2, Hydrazine hydrate hydrogen peroxide (H_2O_2), different cyanate, thiocyanates,acetic acid and ethanol analytical grade were purchased from Sigma Aldrich.Dichloromethane (DCM), chloroform and silica gel were of Merck,Germany. Commercialgradeethyl acetate and n-hexane were purchased from local market and were distilled two times before use.

Condensation of Semicabazide and Thiosemicarbazide Derivativeswith Different Isocyanates and Thiocynates

Benzohydrzide was treated with twenty three different substituted isocyanates and thiocyanates in (1:1) ratio respectively. Ethanol was used as solvent while acetic acid ascatalyst with constant heating and reflux in a round bottom flask. The thiosemicarbazide (**1)** and semicarbazides (**2**) derivatives were precipitated quickly after the start of reaction. The reaction was given 10-60 minutes to complete.The progress of reaction was monitored with the help of TLC.

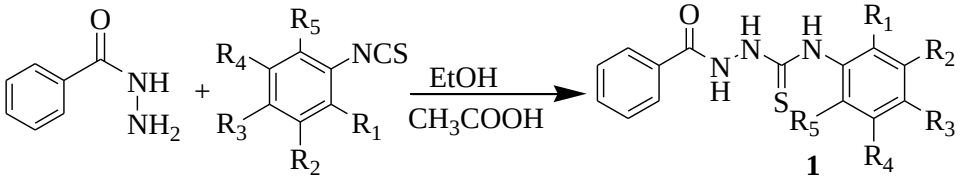
Analytical, Physical and Spectral Data of Thiosemicarbazide and Semicarbazides Derivatives.

1-[(Phenyl)carbonyl-4-(4-fluoro-2-methylphenyl)thiosemicarbazide **(3)**

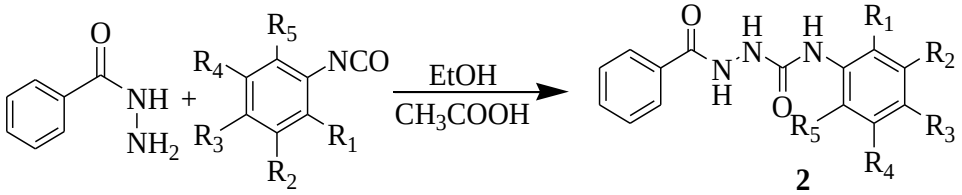
Chemical formula: $C_{15}H_{14}FN_3OS$. Molecular weight: 303.08 g/mol. Yield 87%; (cream).^{1}H-NMR (300 MHz, DMSO),δ10.54 (s,1H, NH), 9.74 (s,1H, NH), 9.57 (s, 1H, NH), 8.03 (d, 2H, *J* = 7.5 Hz, H-2/6), 7.70-7.63 (m, 3H, H-3/4/5), 6.69 (d, 1H, *J* = 7.6 Hz,H-3′) 6.48 (d, 1H, J = 7.6 Hz,H-4′), 6.45 (s, 1H, H-6′), 2.12 (s, 3H, -CH_3). EI MS *m/z* (% rel. abund.):105 (100%), 77 (81%), 167 (72%), 303.1 (18.65%), 304.1 (2.91%). Elemental Analysis: C, 59.37; H, 4.67; F, 6.28; N, 14.05; O, 5.26; S, 10.59.

1-[(Phenyl)carbonyl]-4-(4-ethoxyphenyl) thiosemicarbazide **(4).**

Chemical formula: $C_{16}H_{17}N_3O_2S$ Molecular weight: 315.10 g/mol. Yield 86%; (White). ^{1}H-NMR (300 MHz, DMSO),δ9.68 (s, 1H, NH), 9.51 (s, 1H, NH), 8.9 (s, 1H, NH), 8.04 (d, 2H, *J* = 7.2 Hz, H-2/6), 7.70-7.63 (m, 3H, H-3/4/5), 6.74 (d, 2H, *J* = 7.6 Hz,H-2′/6′), 6.33, (d, 2H, *J* = 7.6HzH-3′/5′), 4.12 (q, 2H, *J*= 7.6 Hz, -OCH_2), 1.33 (t, 3H, J = 7.6 Hz,-CH_3). EI MS *m/z* (% rel. abund.):151.0 (100.0%), 1**79**.0 (83%), 108 (54.5%), 134 (45.0%), 78 (4%) 316.10 (1.9%). Elemental Analysis: C, 61.00; H, 5.93; N, 14.32; O, 9.75; S, 10.19.



Scheme-1.1: Synthesis of Thiosemicarbazide derivatives.



Scheme-1.2: Synthesis of semicarbazide derivatives.
Where R_2, R_3, R_4 and R_5 pertains to various substituents.

1-[(Phenyl)carbonyl-4-(3-trifluoromethylphenyl)thiosemicarbazide **(5)**

Chemical formula: $C_{15}H_{12}F_3N_3OS$, Molecular weight: 339.07g/mol. Yield 78%; (White). ^{1}H NMR (300 MHz, DMSO) δ 10.17 (s, 1H, NH), 9.92 (s, 1H, NH), 8.8 (s, 1H, NH), 7.98 (d, 2H, J = 7.5 Hz, H-2/6), 7.70-7.63 (m, 3H, H-3/4/5), 7.13 (t, 1H, *J* = 7.2 Hz,H-5′), 6.98-6.94 (m, 2H, H-2′/4′), 6.43 (d, H-6′, *J* =7.1 Hz). EI MS *m/z* (% rel. abund.):145 (100.0%), 161.07 (62.56%), 235.0 (13.17%), 331.3 (2.1%). Elemental Analysis: C, 53.10; H, 3.47; F, 17.01; N, 13.02; O, 5.73; S, 8.99.

1-[(Phenyl)carbonyl-4-(2-fluorophenyl)thiosemicarbazide **(6)**

Chemical formula: $C_{14}H_{12}FN_3OS$, Molecular weight: 289.07 g/mol. Yield 81%; (White). ^{1}H NMR (300 MHz, DMSO) δ 9.98 (s, 1H, NH), 9.60 (s, 1H, NH), 8.7 (s, 1H, NH) 8.02 (d, 2H, *J* = 7.4 Hz, H-2/6), 7.71-7.63 (m, 3H, H-3/4/5), 6.99-6.96 (2H, m, H-3′/5′), 6.78 (d, 1H, *J* = 7.1 Hz, H-6′), 6.62 (t, 1H, *J* = 7.1 Hz, H-4′). EI MS EI MS *m/z* (% rel. abund.):111.1 (100.0%), 226.9 (75.1%), 185 (43.3%), 168 (36.0%). Elemental Analysis: C, 58.12; H, 4.18; F, 6.57; N, 14.52; O, 5.53; S, 11.08.

1-[(Phenyl)carbonyl]4-(3-Chlorophenyl)-thiosemicarbazide **(7)**

Chemical formula: $C_{14}H_{12}ClN_3OS$, Molecular weight: 305.04 g/mol. Yield **79**%; (White). ^{1}H NMR (300 MHz, DMSO) δ 10.03 (s, 1H, NH), 9.87 (s, 1H, NH), 9.83 (s, 1H, NH). 7.75 (d, 2H, *J* = 7.1 Hz, H-2/6), 7.70-7.62 (m, 3H, H-3/4/5), 7.14 (t, 1H, J = 7.3 Hz, H-5′), 6.85-680 (m, 2H, H-2′/4′), 6.31 (d, 1H, *J* = 7.1 Hz, H-6′). MS *m/z* (% rel. abund.):105 (100.0%), 77.0 (80.76%), 168.9 (70.1%), 136 (52.32%), 305.03 (26.77%), 306.0 (3.46%). Elemental Analysis: C, 55.00; H, 4.00; Cl, 11.99; N, 14.78; O, 6.00; S, 10.91.

1-[(Phenyl)carbonyl]4-(2,5-dichlorophenyl)-thiosemicarbazide **(8)**

Chemical formula: $C_{14}H_{11}Cl_2N_3OS$ Molecular weight: 339.00 g/mol. Yield 84%; (Light yellow). ^{1}H-NMR (300 MHz, DMSO) δ 9.65 (s, 1H, NH), 8.60 (s, 1H, NH), 8.5 (s, 1H, NH), 7.50 (d, 2H, J = 7.1 Hz, H-2/6), 7.70-7.61 (m, 3H, H-3/4/5), 7.31 (d, 1H, *J* = 7.1 Hz, H-3′), 7.08 (d, 1H, *J* = 7.4Hz, H-4′), 6.75 (s, 1H, H-6′). MS *m/z* (% rel. abund.):183.0 (39.2%), 172.0 (27.3%), 105 (21.1%), 77 (11.9%), 339.00 (11.0%). Elemental Analysis: C, 48.99; H, 3.83; Cl, 21.04; N, 12.34; O, 5.11; S, 8.55.

1-[(Phenyl)carbonyl-4-(2,3dichlorophenyl)thiosemicarbazide **(9)**

Chemical formula: $C_{14}H_{11}Cl_2N_3OS$, Molecular weight: 339.00 g/mol. Yield 78%; (White). ^{1}H NMR (300 MHz, DMSO) δ 9.57 (s, 1H, NH), 8.35 (s, 1H, NH), 8.32 (s, 1H, NH), 7.91 (d, 2H, *J* = 7.1 Hz, H-2/6), 7.70-7.61 (m, 3H, H-3/4/5), 7.15 (d, 1H, *J* = 7.3 Hz, H-4′), 7.02 (t, 1H, *J* = 7.7 Hz, H-5′), 6.74 (d, 1H, *J* = 7.1 Hz, H-6′). MS *m/z* (% rel. abund.):133.0 (100.0%), 182.0 (97.2%), 109.00 (74.0%), 172.99 (59.1%), 218.00 (13.0%), 334.00 (2.0%). Elemental Analysis: C, 48.99; H, 3.38; Cl, 21.81; N, 11.98; O, 4.74; S, 10.01.

1-[(Phenyl)carbonyl-4-(2,4-difluorophenyl)thiosemicarbazide **(10)**

Chemical formula: $C_{14}H_{11}F_2N_3OS$ Molecular weight: 307.06g/mol. Yield 68%; (White). ^{1}H-NMR (300 MHz, DMSO) δ 10.00 (s, 1H, NH), 9.70 (s, 1H, NH), 8.8 (s, 1H, NH),7.81 (d, 2H, *J* = 7.1 Hz, H-2/6), 7.73-7.62 (m, 3H, H-3/4/5), 6.76 (d, 1H, *J* = 7.4Hz, H-5′), 6.59 (d, 1H, *J* = 7.4Hz, H-6′), 6.43 (s, 1H, H-3′). MS *m/z* (% rel. abund.):129 (100.0%), 171.0 (45.42%), 187.05 (7.74%), 154.1 (6.04%). Elemental Analysis: C, 55.00; H, 3.69; F, 13.01; N, 13.65; O, 5.41; S, 10.43.

1-[(Phenyl)carbonyl-4-(2-trifluoromethylphenyl)thiosemicarbazide **(11)**

Chemical formula: $C_{15}H_{12}F_3N_3OS$, Molecular weight: 339.07 g/mol. Yield 76%; (Light brown). ^{1}H NMR (300 MHz, DMSO) δ 10.56 (s, 1H, NH), 10.03 (s, 1H, NH), 9.82 (s, 1H, NH), 8.03 (d, 2H, *J* = 7.1Hz H-2/6), 7.73-7.64 (m, 3H, H-3/4/5), 7.48 (d, 1H, *J*= 7.3Hz, H-3′), 7.20 (t, 1H, *J* = 7.4Hz, H-5′), 7.08 (t,1H *J* = 7.4 Hz, H-4′), 6.17 (d, 1H, J = 7.1, H-6′). MS *m/z* (% rel. abund.):105 (100.0%), 145.0 (90.4%), 77.0 (85.8%), 203.0 (80.8%), 339.2 (2.8%). Elemental Analysis: C, 53.10; H, 4.01; F, 17.11; N, 12.40; O, 5.01; S, 9.58.

1-[(Phenyl)carbonyl-4-(2,6-ditrifluorophenyl)thiosemicarbazide **(12)**

Chemical formula: $C_{14}H_{11}F_2N_3OS$, Molecular weight: 307.06 g/mol. Yield 76%; (Cream color). ^{1}H NMR (300 MHz, DMSO) δ 10.65 (s, 1H, NH), 10.03 (s, 1H, NH), 9.432 (s, 1H, NH), 7.97 (d, 2H, *J* = 7.1 Hz, H-2/6), 7.57-7.38 (m, 3H, H-3/4/5), 7.34 (m, 2H, H-3′/5′), 7.24 (t, 1H, *J* = 7.4 Hz, H-4′). MS *m/z* (% rel. abund.):129.0 (100.0%), 171.0 (78.3%), 154.05 (57.9%), 178.07 (24%), 203

(11.4%). Elemental Analysis: C, 54.00; H, 4.61; F, 12.36; N, 13.78; O, 5.21; S, 9.99.

1-[(Phenyl)carbonyl-4-(methyl)thiosemicarbazide **(13)**

Chemical formula: $C_9H_{11}N_3OS$ Molecular weight: 209.6, Yield 75%; (White).^{1}H NMR (300 MHz, DMSO) δ 10.05 (s, 1H, NH), 10.03 (s, 1H, NH), 8.6 (s, 1H, NH), 7.87 (d, 2H, J = 7.1 Hz H-2/6), 7.75-7.59 (m, 3H, H-3/4/5), 2.81 (s, 3H, -CH_3). MS *m/z* (% rel. abund.):120 (100.0%), 105.0 (9.9%), 74.9 (4.6%). Elemental Analysis: C, 50.65; H, 5.80; N, 21.08; O, 8.65; S, 14.32.

1-[(Phenyl)carbonyl]-4-(2,6-dimethylphenyl)-thiosemicarbazide **(14)**

Chemical formula: $C_{16}H_{17}N_3OS$, Molecular weight: 299.11 g/mol. Yield 83%, (White).^{1}H NMR (300 MHz, DMSO) δ 10.04 (s, 1H, NH), 10.02 (s, 1H, NH), 8.7 (s, 1H, NH), 7.97 (d, 2H, J = 7.1 Hz, H-2/6), 7.72-7.63 (m, 3H, H-3/4/5), 6.96-6.94 (m, 2H, H-3′/5′), 6.79 (t, 1H, J= 7.5 Hz, H-4′), 2.6 (s, 6H, -CH_3). MS *m/z* (% rel. abund.):121.0 (100.0%), 163.11 (91.8%), 106.11 (60.7%), 77.0 (13.5%) 195.12 (11.2%). Elemental Analysis: C, 63.99; H, 5.79; N, 14.07; O, 5.42; S, 11.81.

1-[(Phenyl)carbonyl-4-(phenyl)thiosemicarbazide **(15)**

Chemical formula: $C_{14}H_{13}N_3OS$, Molecular weight: 271.08 g/mol. Yield 78%; (White). ^{1}H NMR (300 MHz, DMSO) δ 9.86 (s, 1H, NH), 9.68 (s, 1H, NH), 9.66 (s, 1H, NH), 7.46 (d, 2H, J = 7.4Hz, H-2/6), 7.74-7.65 (m, 5H, H-3/4/5/2′/6′), 7.20-7.24 (m, 2H, H-3′/5′), 6.81 (t, 1H, J =7.1Hz,H-4′). MS *m/z* (% rel. abund.):105.0 (100.0%), 77.0 (91.7%), 135.0 (41.34%), 178.0 (178.16%), 271(21.1%). Elemental Analysis: C, 62.0; H, 4.85; N, 15.51; O, 6.02; S, 12.01.

1-(2-Bromophenyl)-1-[(2-chlorophenyl)carbonyl]thiosemicarbazide **(16)**

Chemical formula: $C_{14}H_{12}ClN_3OS$, Molecular weight: 305.04 g/mol. Yield 81%; (White). ^{1}H NMR (300 MHz, DMSO) δ 9.33 (s, 1H, NH), 8.32 (s, 1H, NH), 8.28 (s, 1H, NH), 7.45 (d, 2H, J = 7.1 Hz, H-2/6), 7.73-7.62 (m, 3H, H-3/4/5), 7.41 (d, 1H, J=7.2Hz, H-3′), 7.08 (t, 1H, J = 7.3Hz, H-5′) 6.86 (d, 1H, J=7.6Hz, H-6′) 6.75 (t, 1H, J = 7.1Hz, H-4′). MS *m/z* (% rel. abund.):184.0 (28.2%), 138.0 (28.5%), 167 (16.5%), 111 (15.6%), 305.1 (1.1%). Elemental Analysis: C, 54.98; H, 4.11; Cl, 11.61; N, 13.76; O, 5.23; S, 10.59.

1-[(Phenyl)carbonyl]-4-(phenyl) semicarbazide **(17)**

Chemical formula: $C_{14}H_{13}N_3O_2$, Molecular weight: 255.10 g/mol. Yield 89%; (White).

^{1}H NMR (300 MHz, DMSO) δ 10.27 (s, 1H, NH), 8.84 (s, 1H, NH), 8.74 (s, 1H, NH), 8.15 (d, 2H, J = 7.1 Hz, H-2/6), 7.94-7.67 (m, 5H, H-3/4/5/2′/6′), 7.43 (m, 2H, H-3′/5′), 7.19 (t, 1H, J = 7.1Hz, H-4′), MS *m/z* (% rel. abund.):105.0 (100.0%), 77.1 (65.0%). 93 (51.1%). 119 (38.6%), 255 (1.8%). Elemental Analysis: C, 66.01; H, 5.14; N, 15.99; O, 11.98.

1-[(Phenyl)carbonyl-4-(3-chlorophenyl)semicarbazide **(18)**

Chemical formula: $C_{14}H_{12}ClN_3O_2$, Molecular weight: 269.12 g/mol. Yield 69%; (White)**,** ^{1}H NMR (300 MHz, DMSO) δ 10.06 (s, 1H, NH), 9.1 (s, 1H, NH), 8.1 (s, 1H, NH), 8.03-7.93 (m, 3H, H-2/6/2′), 7.76-7.60 (m, 3H, H-3/4/5), 7.49 (d, 1H, J = 7.1Hz, H-6′), 7.37 (t, 1H, J =7.3Hz, H-5′), 7.23 (d, 1H, J = 7.2 Hz, H-4′). MS *m/z* (% rel. abund.):127.0 (100.0%), 153 (91.73%), 185.0 (76%), 90.0 (88.0%). Elemental Analysis: C, 57.98; H, 4.29; Cl, 12.38; N, 14.49; O, 11.17.

1-[(Phenyl)carbonyl-4-(4-trifluoromethylphenyl)semicarbazide **(19)**

Chemical formula: $C_{15}H_{12}F_3N_3O_2$, Molecular weight: 323.09 g/mol. Yield 87%; (White). ^{1}H NMR (300 MHz, DMSO) δ 10.31 (s, 1H, NH), 9.28 (s, 1H, NH), 8.39 (s, 1H, NH), 8.03 (d, 2H, J = 7.1Hz H-2/6), 7.73-7.64 (m, 3H, H-3/4/5), 7.48 (d, 1H, J =7.3Hz, H-2′), 7.20 (t, 1H, J = 7.4Hz, H-3′), 7.08 (t,1H,J = 7.4 Hz, H-5′), 6.17 (d, 1H, J = 7.1, H-6′). MS *m/z* (% rel. abund.):105.1 (100.0%), 77.1 (89.85%), 136 (58.18%), 187.0 (31.21%). Elemental Analysis: C, 56.03; H, 4.22; F, 18.12; N, 13.12; O, 10.10.

1-[(Phenyl)carbonyl]-4-(3-trifluoromethylphenyl)semicarbazide **(20)**

Chemical formula: $C_{15}H_{12}F_3N_3O_2$, Molecular weight: 323.09 g/mol. Yield 63%; (White)·NMR (300 MHz, DMSO) δ 10.30 (s, 1H, NH), 9.97 (s, 1H, NH), 9.19 (s, 1H, NH),8.03 (d, 2H, J= 7.1Hz H-2/6), 7.76-7.63 (m, 3H, H-3/4/5),, 7.36 (d, 1H, J = 7.4Hz, H-4′/5′), 7.23 (d, 1H, J = 7.5Hz,h-6′), 3.85 (s, 1H, H-2′). MS *m/z* (% rel. abund.):105.1 (100.0%), 77.0 (65.6%), 136.0 (59.9%), 187 (56.4%),

323.2 (6%). Elemental Analysis: C, 56.01; H, 4.01; F, 18.01; N, 13.04; O, 10.01.

1-[(Phenyl)carbonyl-4-(2-trifluoromethylphenyl)semicarbazide **(21)**

Chemical formula: $C_{15}H_{12}F_3N_3O_2$, Molecular weight: 323.09, Yield 84%; (White). ^{1}H NMR (300 MHz, DMSO) δ 10.20 (s, 1H, NH), 10.03 (s, 1H, NH), 8.32 (s, 1H, NH), 7.41 (d, 2H, *J* = 7.1 Hz, H-2/6), 7.75-7.64 (m, 3H, H-3/4/5), 7.41 (d, 1H, *J*=7.2Hz, H-3′), 7.06 (t, 1H, *J* = 7.2Hz, H-5′) 6.85 (d, 1H, *J*=7.2Hz, H-6′) 6.73 (t, 1H, *J* = 7.5Hz, H-4′).MS *m/z* (% rel. abund.):105.0 (100.0%), 77.0 (63.6%), 135.0 (58.9%), 186 (57.4%), 323.2 (6%). Elemental Analysis: C, 55.73; H, 3.74; F, 17.63; N, 13.00; O, 9.90

1-[(Phenyl)carbonyl-4-(4-nitrophenyl)semicarbazide **(22)**

Chemical formula: $C_{14}H_{12}N_4O_4$, Molecular weight: 300.09 g/mol. Yield 74%; (Yellow). ^{1}H NMR (300 MHz, DMSO) δ 10.00 (s, 1H, NH), 9.63 (s, 2H, NH),9.53 (s, 2H, NH),8.24 (m, 2H, H-3′/5′), 8.03 (d, 2H, *J* = 7.1Hz, H-2/6), 7.82 (m, 2H, H-2′/6′), 7.73-7.62 (m, 3H, H-3/4/5). MS *m/z* (% rel. abund.):105 (100.0%), 135 (60.0%), 178 (58.1%), 163 (40.4%). Elemental Analysis: C, 55.00; H, 5.03; N, 17.99; O, 20.98.

1-[(Phenyl)carbonyl-4-(2-nitrophenyl)semicarbazide **(23)**

Chemical formula: $C_{14}H_{12}N_4O_4$, Molecular weight: 300.09 g/mol. Yield 82%; (Yellow). ^{1}H NMR (300 MHz, DMSO) δ 10.03 (s, 1H, NH), 9.53 (s, 2H, NH), 9.50 (s, 2H, NH),8.53 (d, 1H, *J* = 7.1Hz, H-6′), 8.24 (1H, d, *J* = 7.1Hz, H-3′), 8.03 (d, 2H, *J* = 7.1Hz, H-2/6), 7.82-7.63 (m, 5H, H-3/4/5/4′/5′). MS *m/z* (% rel. abund.):138.0 (100.0%), 302.0 (39.22%), 164 (11.81%), 105.0 (4.45%). Elemental Analysis: C, 55.98; H, 4.12; N, 17.96; O, 20.97

1-[(Phenyl)carbonyl-4-(methyl)semicarbazide **(24)**

Chemical formula: $C_9H_{11}N_3O_2$, Molecular weight: 193.09 g/mol. Yield 57 %; (Brown).^{1}H NMR (300 MHz, DMSO) δ9.74 (s, 1H, NH), 7.81 (d, 2H, *J* = 6.9Hz, H-2/6), 7.50-7.40 (m, 3H, H-3/4/5), 4.47 (s, 2H, NH), 3.15 (s, 3H, -CH_3). MS *m/z* (% rel. abund.):85.1 (100.0%), 77.1 (96.78%), 105 (84.08%), 58.1 (73.70%), 136.1 (11.25%). Elemental Analysis: C, 56.01; H, 6.00; N, 20.99; O, 16.69.

1-[(Phenyl) carbonyl] 4-(naphthyl)semicarbazide **(25)**

Chemical formula: $C_{18}H_{15}N_3O_2$, Molecular weight: 305.12g/mol. Yield 60%; (White). ^{1}H NMR (300 MHz, DMSO) δ 9.16 (s, 1H, NH), 9.12 (d, 2H, NH), 8.29 (s, 1H, H-2′), 8.03 (d, 2H, *J* = 7.1Hz, H-2/6), 7.88 (d, 1H, *J* =7.2Hz, H-5′), 7.86 (d, 1H, *J* =7.5Hz, H-6′), 7.84 (d, *J* =7.5Hz, H-10′′),7.77 (d, *J* =7.5Hz, H-7′′).7.73 (t, 1H, *J* = 7.2Hz, H-4), 7.62 (m, 2H, H-3/5).7.50 (t, 1H, *J* =7.6Hz,H-8′′), 7.36 (t, 1H, *J* = 7.2Hz, H-9′′). MS *m/z* (% rel. abund.):143.1 100.0%), 164 (74.3%), 115 (73.0%), 127 (36.0%). Elemental Analysis: C, 71.01; H, 5.01; N, 14.02; O, 10.59.

Results and Discussion

DPPH radical scavenging activity

All synthesized Semicabazide and thiosemicarbazide derivatives were screened for *in vitro* free radical scavenging activeness. By the help of 1,1-diphenyl-2-picrylhydrazyl (DPPH) test the free radical scavenging activity of the compounds were calculated *in vitro*. A solution of 85 μM was prepared by dissolving DPPH in 100% ethanol and stored in test tubes by covering with a foil of aluminum. To the 3 ml of ethanol 510 μM DPPH solution was added and for control reading absorbance was recorded quickly at 518 nm. Then 510 μM of the prepared solution was added to 3ml of the testing compounds taken in ethanol at different concentrations (10, 50 80, 150μM) of drugs as well as standard compound vitamin-C. After shaking the mixture was allowed to stand for 30 minutes. With the help of spectrophotometer the absorbance (A) of the mixture was measured at 518 μM. For each synthetic compound and standard compound IC_{50}values in term of % inhibition were determined by applying the following formula.

$$\text{DPPH Inhibition \%} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

All Compounds exhibited a diverse range of DPPH radical scavenging activity. Where IC_{50} values of compounds come out to be between 280.07±4.62μM and 482.42±2.80μM compared with standard ascorbic acid (Vitamin C) (IC_{50}= 320.51± 3.68μM) (Table 1). Compounds**4**(IC_{50}=365.15±4.05μM),**5**(IC_{50}=339.36± 3.75μM), **6**(IC_{50}=395.10±3.53μM), **11 (**IC_{50}=320.39±4.35μM), **12** (IC_{50}=328.66±4.14μM), **13**(IC_{50}=364.85±3.77μM), **14**(IC_{50}=348.80±3.92μM), **17**(IC_{50}=348.80±3.92μM), **18** (IC_{50}=369.50±3.81μM), **19**(IC_{50}=346.27±3.68μM), **20** (IC_{50}=324.53±3.98μM), **21**(IC_{50}=353.08±3.84μM), **23** (IC_{50}=354.71±3.49μM) and **24** (IC_{50}=321.83±4.58μM) are closed in activities

to the standard vitamin C. Among the series **5**(IC_{50}=μM), 69 (IC_{50}=μM), **9** (IC_{50}=μM) and **16** (IC_{50}=μM) show low to moderate activities as it is clear from their IC_{50}values.

Compound like **10** (IC_{50}=482.42±2.80μM), **15**(IC_{50}=416.66±3.37μM), **16** (IC_{50}=434.58±3.02μM) and (IC_{50}=419.36±3.04μM), are least active than the standard Vitamin C (IC_{50} = 320.51± 3.68μM). While compound 65 (IC_{50} =280.07±4.62 μM), was found to be more active than the standard ascorbic acid (Vitamin C), which is an exception.

Ferric Ion Reducing Activity

The ferric reducing power of all compounds was determined by making different concentrations (10, 50, 80, 150 μM). 0.3 ml of 100 mM*tris* buffer of PH = 7.4, 0.1 ml of 9 Mm *O*-phenanthroline and 0.2 ml of 3.6 Mm ferric chloride. The mixture was diluted up to 3 ml by addition of ultra-pure distilled water and was allowed to stand for 10 minutes at room temperature. With the help of UV spectrophotometer the increase in absorbance was measured at 510 nm. At the same concentrations vitamin C was used as reference standard and utilized as control without compound sample mixture. By comparing with vitamin C the reducing power was calculated by the following equation.

$$\text{Reducing Power (\%)} = \frac{A_{sample} - A_{control}}{A_{sample}} \times 100$$

Table-1: DPPH radical scavenging activity of compounds.

COMPOUNDS	% INHIBITION MEANS (N=3)				
	10μM	50 μM	80 μM	150μM	IC_{50}(μM) ± SEM
3	7.91	14.21	18.23	29.86	280.07±4.62
4	3.58	9.02	17.50	21.49	365.15±4.05
5	9.13	12.08	18.01	26.17	339.36±3.75
6	4.01	11.13	16.16	20.43	395.10±3.53
7	4.18	12.11	16.01	25.89	382.68±3.60
8	3.28	12.33	14.96	21.28	375.83±3.73
9	03.33	12.29	16.28	20.01	398.99±3.58
10	4.78	10.21	13.10	18.22	482.42±2.80
11	4.21	10.10	17.20	24.32	320.39 ±4.35
12	4.83	11.43	17.21	24.31	328.66±4.14
13	3.99	12.34	15.72	22.14	364.85±3.77
14	4.30	9.40	16.15	19.08	348.80±3.92
15	4.79	13.21	17.34	20.30	416.66±3.37
16	8.29	15.7	18.11	24.19	379.70±3.28
17	3.33	11.29	17.28	23.01	319.39±4.39
18	3.01	11.17	15.02	21.24	369.50±3.81
19	13.51	14.13	17.99	28.45	346.27±3.68
20	6.34	13.02	17.77	26.17	324.53±3.98
21	7.71	13.72	16.99	23.17	353.08±3.84
22	11.44	11.36	14.21	24.11	434.58±3.02
23	8.84	14.56	18.31	25.51	354.71±3.49
24	3.01	7.23	17.01	23.16	321.83±4.58
25	7.91	11.64	15.86	22.11	419.36±3.04
Vitamin C	14.11	15.43	19.66	30.36	320.51± 3.68

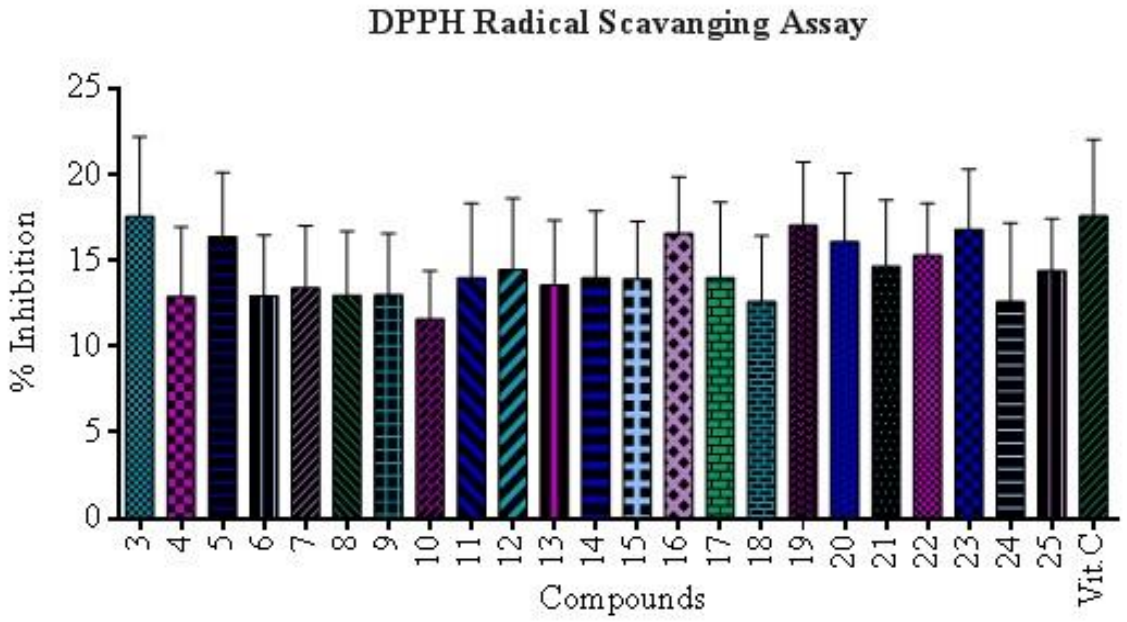


Fig1: DPPH radical scavenging activity of compounds **3-25**

The Synthesized compounds 3 to 25were examined for their ferric ion reducing activity. All Compounds were found toexhibit a varying degree of ferric ion reducing activity. Where IC_{50} values of these compounds were in the range of 52.56±13.35μM and 138.71±5.17μM compared with standard ascorbic acid (Vitamin C) (IC_{50} = 213.76 ± 9.89μM). The compounds**3** (IC_{50}=61.91±10.41μM),**5** (IC_{50}=66.28±10.04μM),**6** (IC_{50}=66.53±9.45μM),**7**(IC_{50}=65.21±10.57μM), **8**(IC_{50}=56.70±10.61μM), **10** (IC_{50}=64.13±10.62μM),**11** (IC_{50}=55.09±12.01μM), **12** (IC_{50}=52.56±13.35μM), **13** (IC_{50}=65.02±10.24μM), **16** (IC_{50}=68.25±8.84μM),**17** (IC_{50}=60.53±11.12μM),**19** (IC_{50}=53.84±11.64μM),**20** (IC_{50}=63.95±11.15μM),**83** (IC_{50}=56.47±10.62μM), **84** (IC_{50}=71.22±9.91μM) and **85** (IC_{50}=55.3±7.47μM), has comparable activities to the standard Vitamin C. While compounds **14** (IC_{50}=81.95±8.06μM), **15** (IC_{50}=89.04±7.33μM), **18** (IC_{50}=82.16±8.470μM), **24** (IC_{50}=89.16±7.93μM) and **25** (IC_{50}=85.24±8.61μM), were found to be moderately active as compared to the standard ascorbic acid (Vitamin C). The two compounds i.e.**3** (IC_{50}=138.71±5.17μM) and **9** (IC_{50}=129.91±5.63μM) have exceptionally low activities as in comparison to other members of the series.

Ferrous Ion Chelation Activity

The ferrous ion chelating activity of series 1 compounds were evaluated by making different concentrations (10, 50, 80 and 150 μM) of these compounds by mixing with 0.3 ml of 100 mM*tris*-HCl (PH = 7.4), 0.1 ml of 9 Mm *O*-phenanthroline and 0.2 ml of 3.6 mM ferrous sulphate. Then by adding ultra-pure distilled water, diluted up to 3 ml. After dilution the reaction mixture was shaken and incubated for 10 minutes. The decrease in absorbance was measured at 510 nm. At the same concentrations EDTA was taken as reference standard and without compound sample mixture was utilized as control. By applying the following formula the Fe^{+2} chelating capacity was calculated.

Table-2: Ferric Ion Reducing Activity of Compounds.

Compounds	% Reduction Mean (n=3)					$IC_{50}(\mu M)$± SEM
	20μM	30μM	50μM	70μM	100μM	
3	5.70	13.71	22.32	41.21	63.63	61.91±10.41
4	4.84	4.46	12.83	17.12	32.74	138.71±5.17
5	6.31	11.01	24.28	45.14	58.9	66.28±10.04
6	4.80	8.67	16.24	35.85	55.35	66.53±9.45
7	8.11	14.63	28.64	48.73	64.83	65.21±10.57
8	4.09	8.95	21.32	39.91	61.68	56.70±10.61
9	4.83	8.92	14.28	26.98	34.84	129.91±5.63
10	8.19	12.98	25.10	49.28	63.29	64.13±10.62
11	7.23	16.24	28.37	54.98	71.37	55.09±12.01
12	10.01	16.98	38.47	67.01	77.73	52.56±13.35
13	6.87	12.81	23.64	44.93	61.68	65.02±10.24
14	4.01	9.37	19.04	29.69	49.37	81.95±8.06
15	2.38	7.68	16.67	26.67	43.68	89.04±7.33
16	2.33	6.67	12.34	27.35	51.02	68.25±8.84
17	6.59	13.14	29.61	52.18	64.65	60.53±11.12
18	6.27	14.18	18.83	33.68	54.23	82.16±8.470
19	4.72	16.58	27.13	42.86	71.88	53.84±11.64
20	8.57	15.43	27.72	56.63	64.73	63.95±11.15
21	3.71	7.54	21.66	25.70	63.59	56.47±10.62
22	8.18	18.13	25.63	36.88	65.89	71.22±9.91
23	28.16	38.65	48.83	56.72	71.75	55.3±7.47
24	5.07	27.45	37.05	37.78	53.32	89.16±7.93
25	9.63	17.63	31.02	40.34	58.63	85.24±8.61
Vitamin C	26.25	46.85	66.86	79.73	92.09	36.75±11.73

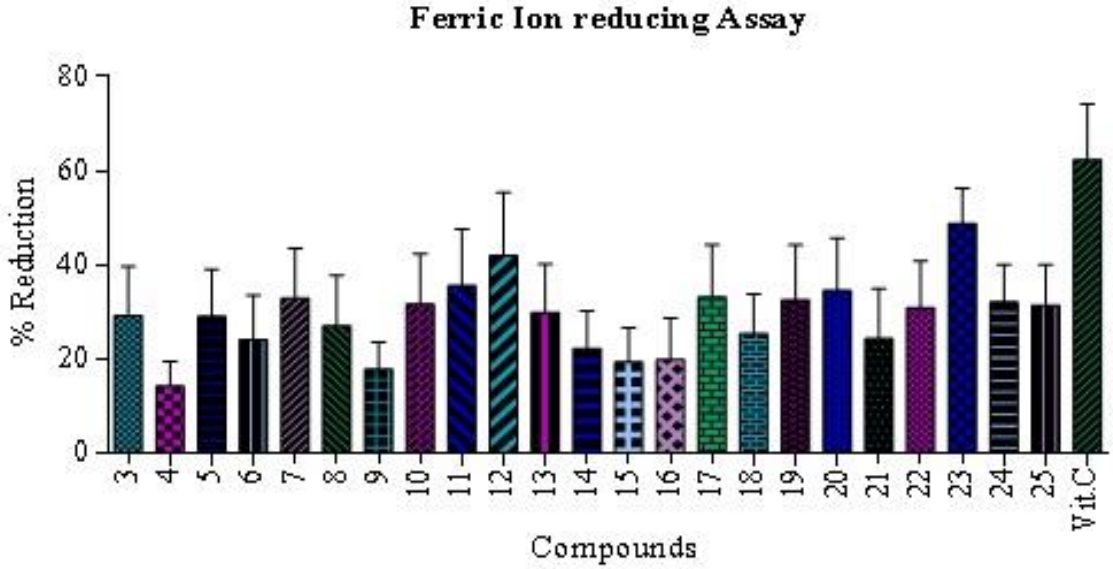


Fig. 2: Ferric ion reducing activity of compounds **3-25**.

Table-3: Ferrous ion chelation activity of compounds.

Compounds	% Chelation Mean (n=3)					$IC_{50}(\mu M)\pm$ SEM
	15μM	30μM	50μM	70μM	100μM	
3	1.99	8.56	18.50	27.83	33.02	126.45±5.77
4	4.768	16.78	32.89	58.38	62.34	63.97±11.28
5	3.85	16.26	33.74	52.25	64.76	60.91±11.19
6	3.55	15.56	31.99	56.64	61.128	62.58±11.22
7	2.98	14.23	36.94	57.23	64.78	57.16±11.92
8	2.69	12.99	34.83	54.21	57.99	64.70±10.94
9	5.76	17.16	36.04	61.70	67.99	58.57±12.12
10	4.22	12.43	30.98	43.73	52.80	78.49±9.15
11	4.71	16.89	35.02	50.12	56.43	77.73±9.76
12	5.92	13.82	26.72	42.93	51.92	84.65±8.61
13	6.21	18.63	24.84	52.85	54.82	80.40±9.61
14	5.21	14.43	28.64	46.93	54.32	77.88±9.31
15	4.73	16.41	32.94	47.32	51.34	87.02±8.90
16	5.10	18.02	36.53	54.31	54.91	79.09±9.86
17	8.52	14.62	24.10	30.10	62.53	75.50±9.40
18	8.08	12.18	28.05	39.24	47.52	99.19±7.58
19	14.34	22.06	41.76	44.98	61.01	77.03±8.36
20	9.17	15.42	27.83	39.33	71.42	61.30±11.0
21	3.02	25.23	31.11	52.62	71.41	59.14±11.74
22	7.23	3.32	12.51	28.01	37.98	109.22±6.56
23	7.11	13.12	21.23	45.92	67.22	56.70±11.23
24	4.82	13.31	24.13	35.52	42.58	109.40±6.92
25	5.23	11.67	18.71	35.53	57.13	71.72±9.18
EDTA	4.25	28.62	54.79	73.98	82.87	53.29±14.53

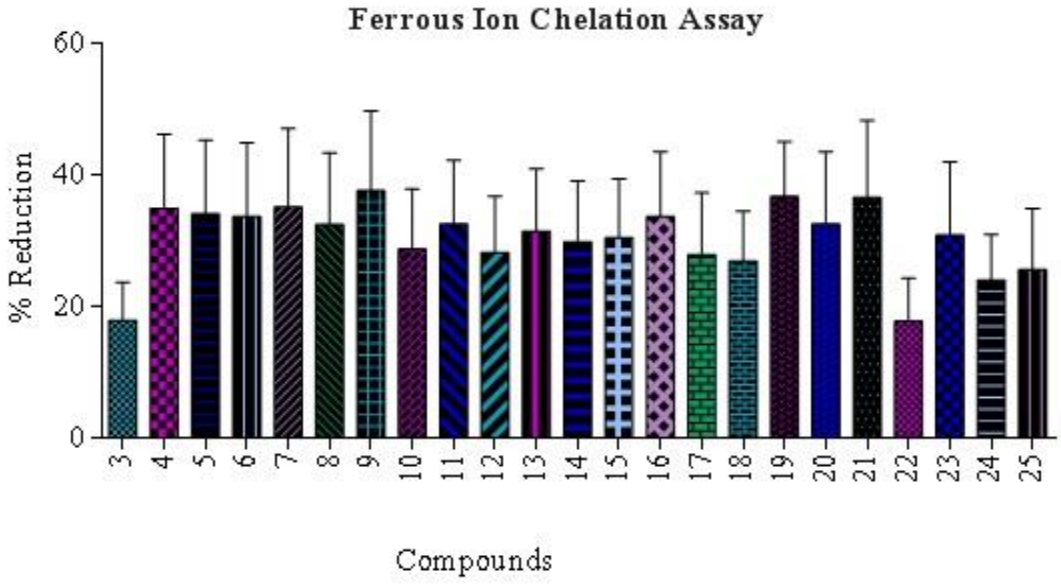


Fig. 3: Ferrous ion chelation activity of compounds **3-25**.

$$\text{Chelating effect (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

All synthesized compounds were scrutinized for their ferrous ion chelation activity (Table 3). All the compounds of Series I displayed a diverse degree of Ferric ion chelation activity. Where IC_{50} values of these compounds comes out in the range of 57.16±11.92μM and 143.40±5.19μM compared with standard EDTA (IC_{50} =53.29±14.53μM). The compounds 3 to 25 show moderate activity compared with the standard EDTA (IC_{50} = 53.29±14.53μM).

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