*Chem Biol Drug Des 2012;* **79:** 950–959

Research Article



doi: 10.1111/j.1747-0285.2012.01360.x

# Microwave-assisted Synthesis and Bioevaluation of Some Semicarbazones

**Laila Jafri[1], Farzana L. Ansari[2], Maryam Jamil[1], Saima Kalsoom[2], Sana Qureishi[2] and Bushra Mirza[1,*]**

*[1]Department of Biochemistry, Qauid-i-Azam University, Islamabad 45320, Pakistan*

*[2]Department of Chemistry, Qauid-i-Azam University Islamabad 45320, Pakistan*

**Corresponding author: Bushra Mirza, bushramirza@qau.edu.pk*

**In continuation to our efforts in finding potential therapeutic agents, a variety of biologically significant semicarbazones were synthesized by the reaction of different carbonyl compounds with phenyl semicarbazides through microwave irradiation. Initially, 18 semicarbazones were studied for their antimicrobial, antitumor, and antioxidant potential. None of the tested compounds showed any antibacterial activity; however, some compounds showed significant antifungal activity. Interestingly, all compounds showed antitumor activity when tested against tumors grown on potato discs. These compounds were also tested for their effect on OH radical-induced oxidative DNA damage. All the compounds showed DNA protection to varying extent. Based on the promising results of antitumor and antioxidant activities, another set of 24 semicarbazones was synthesized, and all of these semicarbazones were evaluated for their antioxidant potential. The results showed that the semicarbazones derived from 2-nitrobenzaldehyde and acetophenone were the most active 2,2-diphenyl-1-picrylhydrazyl 9 (DPPH) free radical scavengers. The overall results have led to the identification of some interesting compounds which seem to have great potential to be developed into effective anticancer drugs.**

**Key words:** antibacterial, antifungal, antioxidant, antitumor, DNA protection, Semicarbazones

Received 2 November 2010, revised 30 December 2011 and accepted for publication 28 January 2012

Semicarbazones, also known as urea derivatives, are the product of the addition–elimination reaction of an aldehyde (or ketone) with semicarbazide ($H_2NNHCONH_2$). They present a wide range of pharmacological applications (1–3). An exhaustive literature search revealed that semicarbazones have shown significant antifungal, antibacterial, antitumor, anticonvulsant, and antioxidant activities (5–9). Metal complexes of semicarbazones have also been found to be active against influenza, protozoa, smallpox, psoriasis, rheumatism, trypanosomiasis, coccidiosis, malaria, and certain kinds of tumors and have been suggested as possible pesticides and fungicides too (4). Present study was planned to synthesize a variety of semicarbazones and to screen them for their biological properties. Initially, synthesis of 18 semicarbazones was carried out including eight benzaldehyde semicarbazones, four acetophenone semicarbazones, five isatin semicarbazones and a ferrocenyl semicarbazone. These semicarbazones were screened against a variety of bioassays. Antibacterial and antifungal activities were determined against four pathogenic bacterial and four fungal strains, respectively. To evaluate the antitumor activity, potato disc antitumor assay was performed. Antioxidant potential of the synthesized compounds was determined by DPPH radical scavenging and DNA protection assay. The results of these bioassays indicated strong potential of these compounds as antitumor and antioxidants. Later, another set of semicarbazones was synthesized, and all these compounds were evaluated for their potential as antioxidant using DPPH free radical scavenging assay. The results indicated that semicarbazones possess a great potential to be developed into anticancerous drugs.

## Materials and Methods

### *Chemicals and instruments*

All chemicals and reagents were purchased from BDH Laboratory Supplies, Poole, UK. Melting points were recorded on a MEL-Temp apparatus. Infrared spectra were recorded on Schimadzu Infrared Spectrophotometer model 270. Samples were taken in KBr pellets. $^1H$ and $^{13}C$-NMR spectra were recorded on Bruker Spectrophotometer at 300 and 75 MHz field strengths, respectively. Tetramethylsilane was used as reference. Chemical shifts are given in $\delta$-scale (ppm). (EI-MS) Mass Spectra were recorded on Agilent GCMS Spectrometer.

### *Synthesis of semicarbazones (general procedure)*

Phenylsemicarbazones were prepared according to literature procedure (10) while semicarbazones were prepared through microwave irradiation according to the procedure reported in literature (11). Phenylsemicarbazide (3 mmol), ethanol (10–20 mL), and few drops of glacial acetic acid were added in equimolar quantity to an appropriate carbonyl compound in ethanol. The reaction mixture was given pulses in a domestic microwave oven Domestic microwave oven (LG, Model: MS-304 A, Microwave: 1250 W, RF outt: 900 W and 2450 Mz) for about 60–120 seconds. The completion of reaction was monitored through TLC (Ethylacetate: n-hexane 4:1) (A). The resultant mixture was cooled, filtered, and dried. Purification of the compounds was

carried out by recrystallization in hot ethanol. Semicarbazones **1–10** are already reported in literature and their physical constants agree with the reported values (11). Following this general procedure, a variety of semicarbazones (**1–42**) were synthesized.

### Compound (11): 1-(1-(3-Aminophenyl) ethylidene)-4-phenylsemicarbazone

m.p.184 °C, $R_f$0.68 (A). Yield 42%. IR (KBr, $\nu$/cm): 3321, 3242, 1760, 1656, 1580, 1420, 1409, 1398.$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$1.55 (s, 3H, $CH_3$), 5.11 (bs, 2H, $NH_2$), 6.20 (bs, 1H, Ar-NH), 7.07–7.66 (m, 9H, aromatic), 8.25 (bs, 1H, =N-NH).

$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 18.9 ($CH_3$), 116.5–151.2 (aromatic), 157.0 (C=N), 169.0 (C=O).m/z (%): 268($M^{+\bullet}$,100).

### Compound (12): 1-(1-(4-Aminophenyl) ethylidene)-4-phenylsemicarbazone

m.p.198 °C, $R_f$ 0.57 (A). Yield 39%. IR (KBr, $\nu$/cm): 3375, 3280, 1756, 1655, 1588, 1445.$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$1.56 (s, 3H, $CH_3$), 5.20 (bs, 2H, $NH_2$), 6.21 (bs, 1H, Ar-NH), 7.07–7.69 (m, 9H, aromatic), 8.18 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 18.9 ($CH_3$), 116.5–151.2 (aromatic-C), 157.0 (C=N), 169.0 (C=O).m/z (%): 268($M^{+\bullet}$,100).

### Compound (13): 1-(1-(Ferrocen-2-y)ethylidene)-4-phenylsemicarbazone

m.p.228 °C, $R_f$ 0.82 (A). Yield 63%. IR (KBr, $\nu$/cm): 3318, 3168, 3023, 1770, 1640, 1612, 1578.$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$5.96 (bs, 1H, Ar-NH), 7.00–7.64 (m, 12H, aromatic), 8.01 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 11.5 ($CH_3$), 69–80 (Ferrocenyl-C), 121.6–135.9 (aromatic-C), 155.6 (C=N), 161.0 (C=O).m/z (%): 361 ($M^{+\bullet}$,100), 296.0 (13.5), 269 (3.29), 185 (6.1).

### 1-(2-Oxoindolin-3-ylidene)-4-phenylsemicarbazone (14–18)

Isatinsemicarbazones have been synthesized according to the reported procedure (Scheme 2). Physical constants of semicarbazones14–18 agree with the literature values (12).

### Compound (18): 4-(2,4-Dinitrophenyl)-1-(2-oxoindolin-3-ylidene) semicarbazone

m.p.187 °C, $R_f$ 0.69 (A). Yield 67%. IR (KBr, $\nu$/cm): 3350, 3218, 3025, 1710, 1665, 1635, 1575, 1560, 1455, 1342.$^1$H NMR (300 MHz, acetone-$d_6$):$\delta$ 5.91 (s, 1H, Ar-NH), 7.00–7.99 (m, 5H, aromatic), 8.01 (bs, 1H, C-NH), 8.56 (bs, 1H, NH, 5-membered ring), 8.56–9.10 (m, 3H, aromatic semicarbazide).$^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta$ 117.0–146.0 (aromatic-C), 132.8 (C=N), 159.0 (C=O amide), 167.5 (C=O, 5-membered ring).m/z(%): 370($M^{+\bullet}$,100).

### Aryl aldehyde/aryl ketone and heteroarylsemicarbazones (19–28)

Semicarbazones **19–21** and **25–27** are already reported and their physical constants agree with the literature values (11).

### Compound (22): 1-(1-(2,4-Dihydroxyphenyl) ethylidene)-4-phenylsemicarbazone

m.p.219 °C, $R_f$ 0.76 (A), Yield 56%. IR (KBr, $\nu$/cm): 3600, 3435, 3241, 3160, 3067, 1759, 1650, 1570, 1428, 1410, 1398. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$1.58 (s, 3H, $CH_3$), 6.15 (bs, 1H, Ar-NH), 6.68–7.57 (m, 8H, aromatic), 7.95 (bs, 2H, OH), 8.30 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.5 ($CH_3$), 110.2–154.5 (aromatic), 157.0 (C=N), 169.1 (C=O). m/z (%): 285 (100).

### Compound (23): 1-(1-(Anthracen-2-yl)ethylidene)-4-phenylsemicarbazone

m.p.176 °C, $R_f$ 0.59 (A). Yield 72%. IR (KBr, $\nu$/cm): 3390, 3257, 3033, 1735, 1655, 1611, 1578, 1438.$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$5.1 (s, 3H, $CH_3$), 5.96 (bs, 1H, Ar-NH), 7.32–7.96 (m, 14H, aromatic), 8.10 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 13.1 ($CH_3$), 121.1–135.9 (aromatic), 153.0 (C=N), 168.7 (C=O).m/z: 353 ($M^{+\bullet}$, 100), 338 (5), 261 (30), 214 (5).

### Compound (28): 4-Phenyl-1-(1-(thiophen-2-yl)ethylidene)semicarbazone

m.p.198 °C, $R_f$ 0.57 (A). Yield 39%. IR (KBr, $\nu$/cm): 3343, 3225, 3018, 1732, 1630, 1600, 1560, 1428.$^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$ 1.56 (s, 3H, $CH_3$), 5.96 (bs, 1H, Ar-NH), 7.00–7.64 (m, 8H, aromatic), 8.01 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$13.8 ($CH_3$), 121.6–154.9 (aromatic), 155.6(C=N), 168.0 (C=O).m/z (%): 259 ($M^{+\bullet}$, 100), 244.0 (3), 167.0 (50), 135 (4).

### 1-(2-Oxoindolin-3-ylidene)-4-phenylsemicarbazone (29–33)

Semicarbazones **29–33** are already reported (Scheme 4) and their physical constants agree with the literature values (11).

### *Semicarbazones derived from hydantoins (34–35)*

### Compound (34):1-(2-Oxo-5-phenylimidazolidin-4-ylidene)-4- phenyl semicarbazone

m.p.210 °C, $R_f$ 0.69(A). Yield 72%.IR (KBr, $\nu$/cm):3255, 3198, 3032, 2980, 1760, 1645, 1590, 1457.$^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$ 2.75 (s, 2H, $CH_2$),6.5 (bs, 1H, Ar-NH), 7.25–7.55 (m, 5H, aromatic), 7.92 (bs, 1H, NH-3), 8.0 (bs, 1H, NH-1), 8.60 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 37.5 ($CH_2$), 121.6–129.0 (aromatic), 156 (C=N), 165 (C=O).m/z (%): 233($M^{+\bullet}$, 100), 98.04 (24), 109 (32).

### Compound (35): 1-(2-Oxo-5,5-diphenylimidazolidin-4-ylidene)-4-phenyl semicarbazone

m.p.276 °C, $R_f$ 0.55 (A). Yield 58%. IR (KBr, $\nu$/cm): 3300, 3278, 3135, 3045, 1750, 1730, 1635.$^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$ 5.96 (bs, 1H, Ar-NH), 7.00–7.64 (m, 15H, aromatic), 8.10 (bs, 1H, =N-NH), 8.20 (bs, 1H, NH-1), 9.10 (bs, 1H, NH-3).$^{13}$C NMR (75 MHz, DMSO-$d_6$):$\delta$ 59.0 ($4^{\diamond}$C), 121.0–135.0 (aromatic), 159.0 (C=O, ring), 162 (C=N),178.0 (C=O).m/z (%): 385($M^{+\bullet}$,100), 386(24).

### *Semicarbazones derived from miscellaneous carbonyl compounds (36–38)*

#### Compound (36): 4-Phenyl-1-(5-vinyl-1*H*-pyrrol-2 (3*H*)-ylidene)semicarbazone

m.p. 210 °C, $R_f$ 0.87 (A), Yield 76%.IR (KBr, $\nu$/cm): 3210, 3148, 1760, 1686. 1420.$^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$ 2.65 (s, 1H, H-1), 2.68 (d, 2H, H-3), 5.16 (dd, 1H, H-7a), 5.27 (dd, 1H, H-7b), 5.98 (bs, 1H,Ar-NH), 6.25 (dd, 1H, H-6), 6.45 (t, 1H, H-4) 7.23–7.52 (m, 5H, aromatic), 8.80 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 36.8 (C-3), 67.5 (C-2), 95.6 (C-4), 116.4 (C-7), 124.8–136.0 (aromatic-C), 125.0 (C-6), 140.0 (4$^{\diamond}$C), 168.0 (C=N), 171.0 (C=O).m/z (%): 242($M^{+\bullet}$,100).

#### Compound (37): 1-(1*H*-Imidazol-2(3*H*)-ylidene)-4-phenylsemicarbazone

m.p.186 °C, $R_f$ 0.59 (A),Yield 76%.IR (KBr, $\nu$/cm): 3218, 2168, 1760, 1680, 1520, 1453.$^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$ 4.18 (bs, 1H, NH), 5.90 (bs, 1H, Ar-NH), 6.18 (d, 2H, H-4,5), 7.00–7.64 (m, 9H, aromatic), 8.10 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$):$\delta$ 88.7 (C-2), 116.6 (C-4,5), 121.6–135.9 (aromatic), 168.0 (C=N), 172.0(C=O).m/z %): 217.0 ($M^{+\bullet}$,100), 125.0 (80), 92 (25), 161.0 (68), 56 (10).

#### Compound (38): 1-(4-Nitro-1 *H*-benzo[d] imidazol-2(3*H*)-ylidene)-4-phenylsemicarbazone

m.p.256 °C, $R_f$ 0.61 (A),Yield 76%. IR (KBr, $\nu$/cm): 3320, 3244, 3018, 1712, 1686, 1445, 1350.$^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$4.0 (bs, 1H, H-3), 4.2 (bs, 1H, H-1), 5.96 (bs, 1H, Ar-NH), 6.10–7.64 (m, 8H, aromatic), 8.01 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$):$\delta$115.2–137.0 (aromatic-C), 168.0 (C=N),175.0 (C=O amide).m/z %): 312($M^{+\bullet}$,100), 313(15).

### *Benzoheteroazepinone semicarbazones (39–42)*

*Benzoheteroazepinones were synthesized according to literature procedure (13)*

#### Compound (39): 1-(4-Methyl-4,5-dihydro-1 *H*-benzo[b][1,4]diazepin-2(3*H*)-ylidene)-4-phenylsemicarbazone

m.p.197 °C, $R_f$ 0.78 (A),Yield 76%. IR (KBr, $\nu$/cm): 3345, 3230, 3028, 2990, 2850, 1678, 1655, 1580$^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$ 1.27 (d, 3H, $CH_3$), 2.07 (dd, 1H, H-3b), 2.32 (dd, 1H, H-3a), 3.75 (m, 1H, H-4), 4.5 (bs, 1H, NH-5), 4.8 (bs, 1H, NH-1), 5.95 (bs, 1H, Ar-NH), 7.00–8.34 (m, 9H, aromatic), 8.19 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.0 ($CH_3$), 48.6 ($CH_2$), 56.6 (CH), 114.3–135.9 (aromatic), 162.0 (C=N), 171.0, 179.0 (C=O).m/z %): 309($M^{+\bullet}$, 100), 310(20).

#### Compound (40): 1-(4-Methyl-8-nitro-4,5-dihydro-1*H*-benzo[b][1,4]diazepin-2(3*H*)-ylidene)-4-phenylsemicarbazone

m.p.237 °C, $R_f$ 0.67(A), Yield 74%. IR (KBr, $\nu$/cm): 3354, 3242, 3036, 2985, 2865, 1685, 1650, 1589.$^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$ 1.37 (d, $CH_3$), 2.27 (dd, 1H,H-3b), 2.32 (dd, 1HH-3a), 3.55 (m, 1H, 4-H), 4.6 (bs, 1H, NH-1), 5.0 (bs, 1H, NH-5), 5.55 (bs, 1H, Ar-NH), 7.00–8.34 (m, 8H, aromatic), 8.29 (bs, 1H, =N-NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 25.0 ($CH_3$), 49.6 ($CH_2$), 52.6 (CH), 114.3–135.9 (aromatic), 169.0 (C=N), 180 (C=O).m/z %): 354($M^{+\bullet}$, 100), 355 (18).

#### Compound (41): 1-(2-Methyl-2,3-dihydrobenzo[b][1,4]thiazepin-4(5*H*)-ylidene)-4-phenylsemicarbazone

m.p.232 °C, $R_f$ 0.67 (A). Yield 69%. IR(KBr, $\nu$/cm): 3238, 3168, 3040, 1760, 1652, 1428, 1399.$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.23 (d, 3H, $CH_3$), 2.00 (dd, 1H,H-3b), 2.52 (dd, 1H, H-3a), 2.89 (m, 1H, H-4), 5.10 (bs, 1H, Ar-NH), 7.00–8.64 (m, 9H, aromatic-H), 7.96 (bs, 1H, =N-NH), 8.91 (bs, 1H, -NH).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.5 ($CH_3$), 23.6 ($CH_2$), 43.5 (CH), 123.4–144.9 (aromatic-C), 162.8 (C=N), 171.0 (C=O). m/z %): 326($M^{+\bullet}$,100), 327 (20).

#### Compound (42): 4-(2,4-Dinitrophenyl)-1-(4-methyl-4,5-dihydro-1*H*-benzo[b][1,4]diazepin-2(3*H*)-ylidene)-4-phenylsemicarbazone

m.p.292 °C, $R_f$ 0.65 (A), Yield 83%. IR (KBr, $\nu$/cm): 3241, 3162, 3022, 2979, 2855, 1762, 1642, 1560, 1428, 1320, 1220.$^1$H NMR (300 MHz, DMSO-$d_6$):$\delta$ 1.23 (d, 3H, $CH_3$),2.08 (dd, 1H,H-3b), 2.52 (dd, 1H, H-3a), 2.75 (m, 1H, H-4),6.16 (bs, 1H, Ar-NH), 6.52–6.77(m, 4H, aromatic), 7.96 (bs, 1H, =N-NH), 8.16 (s, N-H), 8.16–9.10 (m, 3H, aromatic-H).$^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 21.5 ($CH_3$), 23.6 ($CH_2$), 43.5 (CH), 119.0–148.3 (aromatic-C), 162.8 (C=N), 172.0 (C=O).m/z %): 415($M^{+\bullet}$, 100).

### *Biological screening*

All the synthesized compounds were screened for their antibacterial, antifungal, antitumor, DNA damaging, and DPPH radical scavenging characteristics.

### *Antibacterial activity*

Agar-well diffusion method as reported earlier (14) was used to check antibacterial activity of all synthesized compounds against four bacterial strains namely: *Staphylococcus aurous* (ATTCC 6538), *Bacillus subtilis* (ATTCC 6633), *Escherichia coli* (ATTCC 15224), and *Enterobacter aerogenes* (ATTCC 13048). DMSO and standard antibacterial drugs (Roxithromycin and Cefixim) served as negative and positive controls respectively. The antibacterial activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to negative control.

### *Antifungal activity*

Antifungal activity against four fungal strains namely *Mucor species* (0300), *Aspergillus niger* (0198), *Aspergilus flavus* (0064), and *Aspergilus fumigatus* (66) was determined by using Agar tube dilution method (15). The medium supplemented with DMSO and Terbinafine (200 $\mu$L/mL) was used as negative and positive control, respectively. The growth was determined by measuring linear growth (mm), and growth inhibition was calculated with reference to negative control.

### *Antitumor activity*

Antitumor activity of the synthesized compounds was checked by performing modified potato disc antitumor assay (16). All the synthesized compounds were also tested against *Agrobacterium tumefacians* (AT10) using the agar-well diffusion method as described in antibacterial activity section. All tests were performed in replicate, and percent tumor inhibition was calculated by the following equation

$$\%\,\text{Age inhibition} = 100 - \frac{\text{Average number of tumours of samples}}{\text{Average number of tumours of -ve control}} \times 100$$

### *DNA protection activity*

The determination of antioxidant or prooxidant activity of the synthesized compounds was conducted by hydroxyl-induced DNA damage-based assay (17).

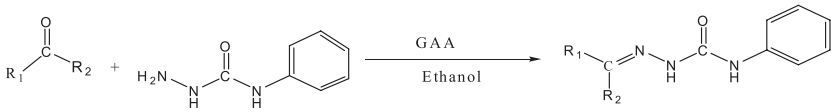
### *DPPH free radical scavenging assay*

The antioxidant effect of semicarbazones was studied using standard DPPH assay (17). Test samples were examined at 100, 50, and 25 $\mu$g/mL as final concentration. Ascorbic acid was used as a positive control and DMSO as negative control. Percentage free radical scavenging was calculated by using following formula

$$\%\,\text{Scavenging} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}}\right] \times 100$$

## Results and Discussion

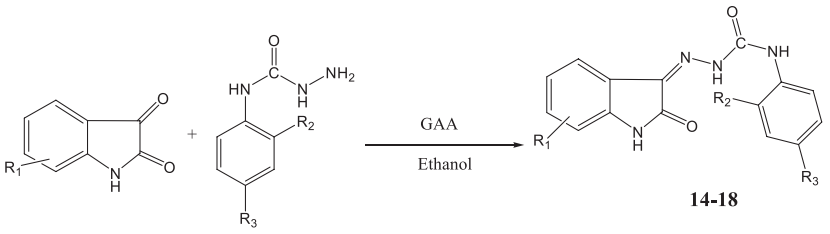
A variety of substituted semicarbazones with the formula $RCNNH_2CONHPh$ were synthesized under microwave radiations, by the reaction of quite diverse carbonyl compounds with phenyl




1-13

**Scheme 1:** Semicarbazones derived from arylaldehydes and ketones.

| No | $R_1$ | $R_2$ | No | $R_1$ | $R_2$ |
|---|---|---|---|---|---|
| **1** | H | $C_6H_5$ | **8** | H | 4-$OCH_3C_6H_4$ |
| **2** | H | 4-$ClC_6H_4$ | **9** | $CH_3$ | $C_6H_5$ |
| **3** | H | 2-$NO_2C_6H_4$ | **10** | $CH_3$ | 4-$OHC_6H_4$ |
| **4** | H | 3-$NO_2C_6H_4$ | **11** | $CH_3$ | 3-$NH_2C_6H_4$ |
| **5** | H | 4-$NO_2C_6H_4$ | **12** | $CH_3$ | 4-$NH_2C_6H_4$ |
| **6** | H | 3-$OHC_6H_4$ | **13** | $CH_3$ | Ferrocenyl |
| **7** | H | 3-$OCH_3C_6H_4$ | | | |



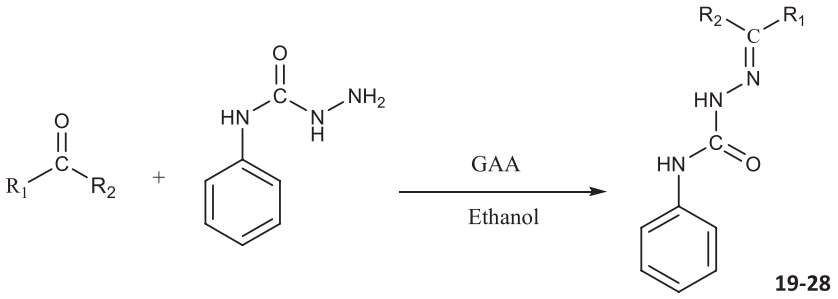

14-18

| No. | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **14** | H | H | H |
| **15** | 4-$NO_2$ | H | H |
| **16** | 3-OH | H | H |
| **17** | 4-OH | H | H |
| **18** | H | $NO_2$ | $NO_2$ |

**Scheme 2:** Isatin semicarbazones derived from phenyl and dinitro phenyl semicarbazide.

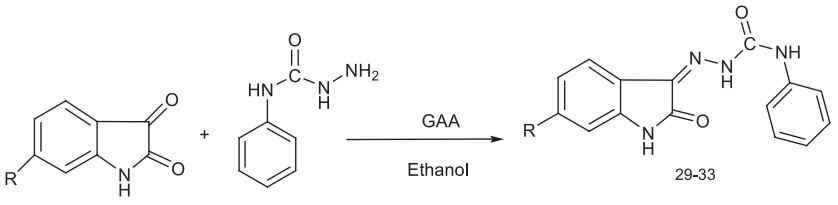
semicarbazide which in turn was synthesized by the reaction of phenyl hydrazine with potassium cyanate. Semicarbazone **1–12** was synthesized by the reaction of commercially available aryl aldehydes or ketones, while compound **13** was synthesized by using acetylferrocene as the carbonyl compound (Scheme 1). Semicarbazones **14–17** were obtained by the reaction of various substituted isatins with phenyl semicarbazide (Schemes 2 and 3). In all isatins, only the exocyclic carbonyl group at C-3 undergoes a condensation reaction as the C-2 carbonyl functionality is involved in amide linkage with the ring nitrogen.

A few semicarbazones were also derived from heteroaryl carbonyl derivatives (Scheme 3). Quite a few semicarbazones were synthesized by the reaction of phenyl semicarbazide with different 5-member heterocycles such as hydantoins, imidazolones, and pyrrolidinone (Scheme 3–6). Few exocyclic semicarbazones **39–42** derived from 7-member heteroazepinones such as 1,5-benzodiazepinone and 1,5-benzothiazepinones have been shown in Scheme 7. Semicarbazones **18** and **42** were derived from 2,4-dinitrophenyl semicarbazide, which in turn was synthesized by the reaction of, 4-dinitrophenyl hydrazine with potassium cyanate. The yields of the products vary from 40% to 80%.



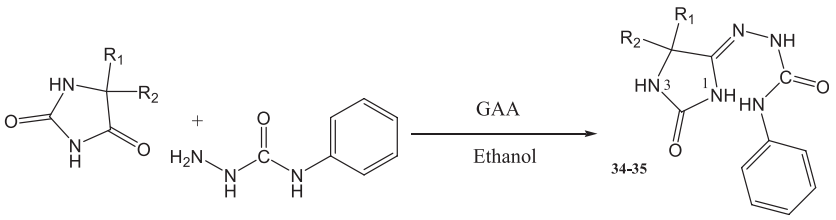

| No | $R_1$ | $R_2$ | No | $R_1$ | $R_2$ |
|---|---|---|---|---|---|
| **19** | H | 2-$ClC_6H_4$ | **24** | $CH_3$ | Biphenyl |
| **20** | H | 4-$OHC_6H_4$ | **25** | $CH_3$ | 2-Pyridyl |
| **21** | $CH_3$ | 3-$OHC_6H_4$ | **26** | $CH_3$ | 3-Pyridyl |
| **22** | $CH_3$ | 2,4-$(OH)_2C_6H_4$ | **27** | $CH_3$ | Chalcone |
| **23** | $CH_3$ | Anthracenyl | **28** | $CH_3$ | 2-Thienyl |

**Scheme 3:** Semicarbazones derived from diverse carbonyl compounds.



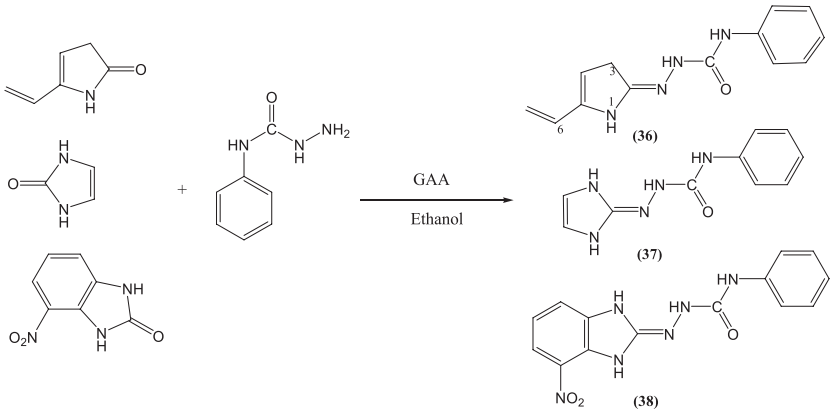

| No. | R |
|---|---|
| **29** | 2-$NO_2$ |
| **30** | 2-OH |
| **31** | 2-$CH_3$ |
| **32** | 4-$CH_3$ |
| **33** | 2-COOH |

**Scheme 4:** Isatin derived from phenyl semicarbazones.

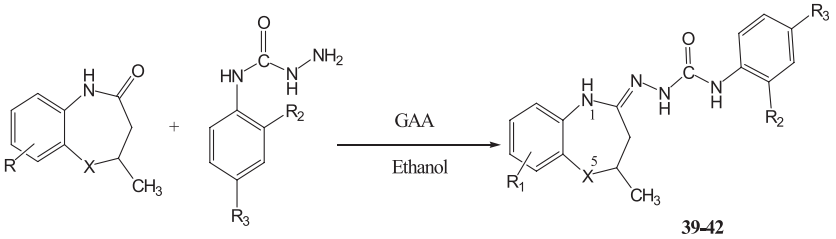



| No. | $R_1$ | $R_2$ |
|---|---|---|
| **34** | H | H |
| **35** | Ph | Ph |

**Scheme 5:** Synthesis of hydantoin semicarbazones.




**Scheme 6:** Semicarbazones derived from heteroazepinones.




| No. | X | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| **39** | NH | H | H | H |
| **40** | NH | 7-$NO_2$ | H | H |
| **41** | S | H | H | H |
| **42** | NH | H | $NO_2$ | $NO_2$ |

**Scheme 7:** Semicarbazones derived from heteroazepinones.

### *Antibacterial activity*

Four strains of bacteria, that is, *S. aurous, E. aerogenes, E. coli* and *B. subtilis*, were used to assess the antibacterial activity of 18 synthesized compounds by the agar-well diffusion protocol at a concentration of 1 mg/mL (1000 ppm). None of the tested compounds showed any significant antibacterial activity. These results are consistent with a previous report (18).

### *Antifungal activity*

A set of 18 semicarbazones were screened against four fungal strains; *Mucor species* (0300), *A. niger* (0198), *A. flavus* (0064), and *A. fumigatus* (66) using tube diffusion method. Linear growth inhibition was observed in each case. Terbinafine which were used as standard drug showed 100% inhibition against all strains. The criteria for activity were based on percent growth inhibition; more than

70% growth inhibition was considered as significant, 60–70% inhibition as good, 50–60% inhibition as moderate and below 50% as non-significant activity. Compound **7** showed quite significant activity against *A. fumigatus* and *A. flavus* (69% against both strains) while moderate activity was seen against *A. niger* and *Mucor*. Three compounds showed significant activity against only one fungal strain, for example, semicarbazone **4** showed 68.4% inhibition against *A. niger* while compounds **14** and **17** showed significant antifungal activity against *A. fumigatus* only. Other compounds showed varying degree of percentage inhibition (Table 1). These results agree with the two previous reports (7,8) that semicarbazones usually have significant antifungal activity.

**Table 1:** Antifungal activity of semicarbazones **1–18**

| | Percentage growth inhibition | | | |
|---|---|---|---|---|
| Sample code | *Aspergillus niger* | *Aspergilus fumigatus* | *Aspergilus flavus* | *Mucor* |
| **1** | 52.62 | 48.94 | 42.20 | 16.85 |
| **2** | 54.20 | 12.91 | 45.30 | 18.77 |
| **3** | 43.15 | 34.43 | 36.01 | 18.77 |
| **4** | 68.41 | 34.93 | 41.17 | 21.83 |
| **5** | 56.83 | 40.44 | 42.20 | 19.15 |
| **6** | 44.73 | 2.90 | 29.30 | 18.00 |
| **7** | 44.73 | 69.96 | 64.91 | 56.32 |
| **8** | 36.83 | 40.44 | 52.52 | 13.02 |
| **9** | 32.10 | 25.42 | 39.62 | 21.83 |
| **10** | 48.41 | 46.44 | 40.14 | 21.83 |
| **11** | 23.24 | 53.33 | 34.08 | 9.890 |
| **12** | 37.20 | 51.11 | 43.63 | 36.39 |
| **13** | 39.52 | 58.66 | 52.27 | 20.49 |
| **14** | 16.27 | 67.55 | 45.90 | 22.25 |
| **15** | 9.29 | 46.66 | 54.08 | 20.49 |
| **16** | 13.94 | 46.66 | 31.81 | 16.95 |
| **17** | 9.29 | 60 | 27.26 | 18.72 |
| **18** | 2.31 | 51.11 | 54.54 | 16.95 |

**Table 2:** Antitumor activity of semicarbazones **1–18**

| | Percentage tumor inhibition (ppm) | | | |
|---|---|---|---|---|
| Organic compounds | 1000 | 100 | 10 | $IC_{50}$ $\mu$g/mL |
| **1** | 20.88 | 15.11 | –0.88>1000 | |
| **2** | 56.92 | 65.12 | 38.97 | 38.96 |
| **3** | 64.10 | 62.05 | 49.23 | 7.28 |
| **4** | 31.14 | 38.18 | 35.05 | >1000 |
| **5** | 53.05 | 36.61 | 22.53 | 669.37 |
| **6** | 65.57 | 49.92 | 20.97 | 159.35 |
| **7** | 47.40 | 12.59 | 38.51 | >1000 |
| **8** | 40 | 40 | 25.18 | >1000 |
| **9** | 64.72 | 58.52 | 39.53 | 45.87 |
| **10** | 63.70 | 44.44 | 25.92 | 191.01 |
| **11** | 75.58 | 56.97 | 34.49 | 52.88 |
| **12** | 77.51 | 53.48 | 36.43 | 52.03 |
| **13** | 64.72 | 56.97 | 37.98 | 57.34 |
| **14** | 67.84 | 56.69 | 35.36 | 62.66 |
| **15** | 67.84 | 43.89 | 25.85 | 157.46 |
| **16** | 50.78 | 41.60 | 25.19 | 699.61 |
| **17** | 70.80 | 41.27 | 10.76 | 200.26 |
| **18** | 81.29 | 58.16 | 32 | 51 |
| Vincristine | 100 | 100 | 100 | 0.003 |

### *Antitumor activity*

Potato disc antitumor assay is a reliable bench-top assay used to detect antitumor behavior of compounds (19). All the synthesized semicarbazones were investigated for their antitumor activity. The

**Table 3:** DNA protection activity of semicarbazones **1–18**

| Compound | Concentration | Protection | Damage |
|---|---|---|---|
| **1** | 1000 | + | |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **2** | 1000 | ++ | – |
| | 100 | + | – |
| | 10 | ++ | – |
| **3** | 1000 | ++ | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **4** | 1000 | ++ | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **5** | 1000 | ++ | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **6** | 1000 | + | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **7** | 1000 | ++ | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **8** | 1000 | ++ | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **9** | 1000 | ++ | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **10** | 1000 | ++ | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **11** | 1000 | ++ | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **12** | 1000 | + | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **13** | 1000 | + | – |
| | 100 | + | – |
| | 10 | ++ | – |
| **14** | 1000 | – | +++ |
| | 100 | – | +++ |
| | 10 | – | +++ |
| **15** | 1000 | – | +++ |
| | 100 | + | – |
| | 10 | ++ | – |
| **16** | 1000 | ++ | – |
| | 100 | ++ | – |
| | 10 | ++ | – |
| **17** | 1000 | – | +++ |
| | 100 | + | |
| | 10 | ++ | – |
| **18** | 1000 | – | +++ |
| | 100 | ++ | – |
| | 10 | ++ | – |

+ Weakly effective, ++ Moderately effective, +++ Strongly effective.

experiment was carried out at different concentrations of semicarbazones to investigate whether an increase in concentration influenced the tumor formation efficiency; $IC_{50}$ values were calculated later. The solvent DMSO served as a negative control for calculating percentage inhibition as mentioned in Table 2. Results showed that all the tested compounds had significant ($p < 0.05$) impact on tumor formation at three different concentrations, that is, 10, 100, and 1000 ppm (Table 2). In our experiments, compound **3** was found to have the highest inhibitory activity ($IC_{50}$ value 7.28 $\mu g/mL$). As compounds with $IC_{50}$ values <10 $\mu g/mL$ are usually considered highly active antitumor agents (20), none of compounds have shown antibacterial activity against *A. tumefacians*, which is evidence that tumor inhibition is solely because of the intrinsic antitumor potential of the tested compounds. Hence, they can be investigated for further studies against different cancer cell lines.

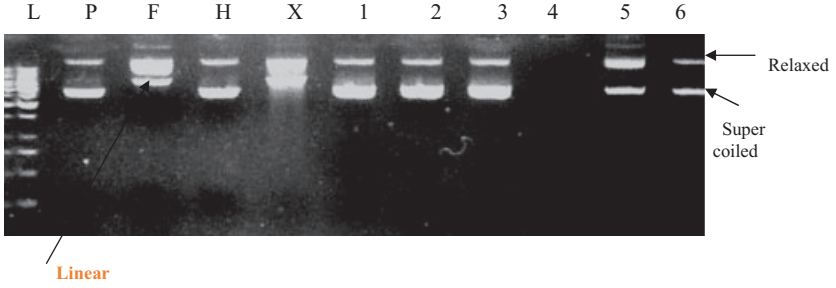
### *DNA damaging studies*

The ability of the semicarbazones to affect Fenton-mediated single- and double-strand breaks in plasmid pBR322 DNA was assessed. In a Fenton reaction, $Fe^{2+}$ metal iron reduces hydrogen peroxide resulting in the production of hydroxyl radical which attack supercoiled plasmid DNA (SC) to break it into two forms, namely, open circular (OC) and linear form (Linear) representing single-stranded and double-stranded breaks, respectively (17). A control run in the assay showed changes from SC DNA to the open circular (OC) and linear form (Linear) indicating the cleavage of DNA when treated with $H_2O_2$ in the presence of $FeSO_4$. DNA was also treated either with $FeSO_4$ or with $H_2O_2$ alone which indicated that $H_2O_2$ and $FeSO_4$ together caused much more damage to DNA than either of them alone, and this observation was consistent with a previously reported results (21). It was also found that most of the semicarbazones like benzaldehyde semicarbazones (**1–8**) and acetophenone semicarbazones (**9–12**) and two isatinsemicarbazones (**13** and **16**) protected DNA against the attack of hydroxyl radical (Table 3). However, the series of isatinsemicarbazone (**15–17**) and a ferrocenylsemicarbazone (**18**) caused cleavage of DNA at one or more concentrations (Figure 1). Compounds **15, 17, and 18** showed damaging effect at 1000 ppm, but protective effect at 100 and 10 ppm was observed. It has been reported in literature that several chemopreventive agents that are antioxidant at some concentrations may behave as prooxidant at other concentrations (22).

The results of these two assays indicated the potential of the tested semicarbazones for antitumor and antioxidant activities. Therefore, another set of 24 compounds was synthesized, and all 42 compounds were then tested for their potential as free radical scavengers.

### *DPPH radical scavenging assay*

DPPH radical scavenge assay is a non-enzymatic method currently used to provide basic information about the ability of compounds to scavenge free radicals. Reduction of DPPH by an antioxidant results in the loss of absorbance at 517 nm (23). The resulting decrease in color from purple to yellow is observed as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule. The antioxidant activity of the synthesized semicarbazones was determined spectrophotometrically by monitoring the disappearance of DPPH at 517 nm. The results indicated that although most of the compounds were antioxidant (except **12** and **17**), however, the level of activity varied considerably. Some compounds like **1**, **3**, **7**, **9**–**10**, **22**, **25**, **27**, **29**, **34**, **39,** and **41**–**42** were found to be strong antioxidant and showed $IC_{50}$ values ranging from 24 to 30 $\mu g/mL$ while others showed low antioxidant activity (Table 4). The compounds (e.g., **3** and **9**) with good antitumor activity were found to be strong antioxidants as well, and these results are consistent with the previous reports of some correlations found in these two activities (24). Semicarbazones **12** and **17** behaved as prooxidant, and this behavior is also reported in




| | |
|---|---|
| **L** | 1 kb ladder |
| **P** | pBR322 plasmid |
| **F** | pBR322 plasmid treated with $FeSO_4$ |
| **H** | pBR322 plasmid treated with $H_2O_2$ |
| **X** | (positive control) pBR322 plasmid treated with $FeSO_4$ and $H_2O_2$ |
| **Lane 1** | pBR322 plasmid treated with $FeSO_4$ and $H_2O_2$ + compound 16(1000 ppm) |
| **Lane 2** | pBR322 plasmid treated with $FeSO_4$ and $H_2O_2$ + compound 16(100 ppm) |
| **Lane 3** | pBR322 plasmid treated with $FeSO_4$ and $H_2O_2$ + compound 16(10 ppm) |
| **Lane 4** | pBR322 plasmid treated with $FeSO_4$ and $H_2O_2$ + compound 17(1000 ppm) |
| **Lane 5** | pBR322 plasmid treated with $FeSO_4$ and $H_2O_2$ + compound 17(100 ppm) |
| **Lane 6** | pBR322 plasmid treated with $FeSO_4$ and $H_2O_2$ + compound 17(10 ppm) |

**Figure 1:** Effect of compounds **16** and **17** on pBR322 plasmid DNA.

**Table 4:** DPPH free radical scavenging activity of the semicarbazones **1–42**

| | Percentage scavenging (ppm) | | | | |
|---|---|---|---|---|---|
| Sample code | 100 | 50 | 25 | $IC_{50}$ $\mu g/mL$ | Remarks |
| **1** | 82.07 | 75.47 | 34.40 | 33.29 | Antioxidant |
| **2** | 11 | 4.9 | 11 | >100 | |
| **3** | 86.6 | 86.1 | 41.87 | 25.65 | Antioxidant |
| **4** | 17.61 | 13.55 | 1.96 | >100 | |
| **5** | 15.80 | 15.46 | 14.49 | >100 | |
| **6** | 11.76 | 13.29 | 15.04 | >100 | |
| **7** | 91.29 | 93.27 | 93 | <25 | Antioxidant |
| **8** | 7.06 | .136 | 7.05 | >100 | |
| **9** | 91.0 | 86.50 | 43.88 | 24.82 | Antioxidant |
| **10** | 51 | 59 | 57.92 | <25 | Antioxidant |
| **11** | 15.99 | 16.78 | 10.96 | >100 | |
| **12** | 12.39 | 2.40 | −88.03 | >100 | Prooxidant |
| **13** | 72.11 | 47.10 | 1.96 | 60.45 | Antioxidant |
| **14** | 25.90 | 13.79 | 6.75 | >100 | |
| **15** | 4.2 | 4.57 | 8.58 | >100 | |
| **16** | 73.38 | 63.18 | 28.36 | 42.90 | Antioxidant |
| **17** | −18.16 | −6.62 | −17.45 | – | Prooxidant |
| **18** | 5.043 | 22.61 | 37.01 | >100 | |
| **19** | 55.76 | 36.30 | 23.45 | 81.85 | |
| **20** | 34.7 | 31.7 | 23.3 | >100 | |
| **21** | 41.7 | 43.04 | 42.9 | >100 | |
| **22** | 89 | 85 | 39.78 | 27.46 | Antioxidant |
| **23** | 31.8 | 5.3 | 11.50 | >100 | |
| **24** | 14.8 | 2.98 | 1.34 | >100 | |
| **25** | 65 | 56.7 | 50.41 | 24.80 | Antioxidant |
| **26** | 33 | 20 | 9.87 | >100 | |
| **27** | 53.79 | 76.9 | 81.2 | <25 | Antioxidant |
| **28** | 17 | 18.88 | 12.77 | >100 | |
| **29** | 58.5 | 70.9 | 72.66 | <25 | Antioxidant |
| **30** | 32.69 | 28.48 | 23.00 | >100 | |
| **31** | 67 | 27.93 | 30.95 | | |
| **32** | 35.91 | 21.64 | 38.16 | >100 | |
| **33** | 32.8 | 38.04 | 15.07 | >100 | |
| **34** | 85 | 86 | 85 | <25 | Antioxidant |
| **35** | 23.41 | 12.60 | 10.2 | >100 | |
| **36** | 39.0 | 37.49 | 35.49 | >100 | |
| **37** | 35.15 | 37.40 | 33.72 | >100 | |
| **38** | 1.20 | 7.42 | 8.89 | >100 | |
| **39** | 73.27 | 73.68 | 40.16 | 29.78 | Antioxidant |
| **40** | 51.38 | 50.05 | 16.71 | 75.44 | |
| **41** | 75.71 | 67.51 | 40.12 | 32.33 | Antioxidant |
| **42** | 75.14 | 76.60 | 85.76 | <25 | Antioxidant |
| Ascorbic acid | 100 | 100 | 100 | 9.9 | Antioxidant |

literature (25,26). Furthermore, it was noted that these two compounds (**12** and **17**) have shown DNA protective activity but no antioxidant activity (27). On the basis of these findings, it has been suggested that the compounds showing DNA protective behavior are not always antioxidant as well.

## Conclusion and Future Prospects

Biological evaluation of the synthesized semicarbazones was carried out by testing them as antimicrobial, antitumor, and as antioxidant agents. None of the compounds showed any antibacterial activity against any tested bacterial strain. However, some of the compounds showed variable activity against different fungi. All compounds showed significant inhibition against tumor formation on potato discs induced by AT 10. DNA protective activity was observed for all semicarbazones. The overall results showed that these semicarbazones have significant potential to be developed into antioxidants and anticancer drugs.

## Acknowledgments

The authors acknowledge the Higher Education Commission, Pakistan for providing financial support.

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