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Recent Research Assessment in Synthesis & Biological Evaluation of Semicarbazones Derivatives as Anticonvulsant & Antitubercular Agents

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Abstract

A recent research assessment deals with the synthesis & evaluation of Semicarbazones derivatives which are used as Anticonvulsant and Antituberculosis agents. All the semicarbazones derivatives *(A1-A9)* were synthesized by the given schemes and reaction process was monitored by thin layer chromatography method using silica gel-G stationary phase, ethyl acetate: ethanol (2:3) as mobile phase, and detecting the spots with iodine vapours. All the synthesized derivatives were also confirmed by *FTIR, 1H NMR* spectroscopy and elemental analysis method.

Keywords: Semicarbazones; *FTIR; Synthesis*

1. Introduction

Semicarbazone are derived from imines formed via condensation reaction between a ketone or aldehyde and semicarbazide. Semicarbazones are classified under the imine derivatives because Semicarbazones are formed from an aldehyde or ketone with the terminal -NH2 group of semicarbazide, which behaves very similarly to primary amines.

2. Material and methodology

2.1 Method for Synthesis of Semicarbazone Derivatives

2.1.1 Synthesis of heterocyclic aldehydes

A mixture of halopyridine (0.002 mol), p-hyroxybenzaldehyde (0.002 mol), potassium carbonate (0.002 mol) and 10 mL of dry dimethyl formamide were taken in a round bottom flask and heated at 70 ^{0}C for 3-4 hr under reduced pressure. Reaction mixture was poured into water and extract with ethyl acetate. The combined extracts were washed with water and dried over anhydrous sodium sulfate (Shailaja *et al.*, 2010).

4-(Pyridinyl-2-oxy) –benzaldehyde: Molecular Weight: 199.21; Yield: 66.8%; M.P.: 79-81 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (3:2) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.65).

4-(Pyridinyl-3-oxy) –benzaldehyde: Molecular Weight: 199.21; Yield: 61.3%; M.P.: 66-68 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (3:2) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.57).

4-(Pyridinyl-4-oxy) –benzaldehyde: Molecular Weight: 199.21; Yield: 72.5%; M.P.: 127-29 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (3:2) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.71).

2.2 Synthesis of 4-(substituted) aryl semicarbazides

2.2.1 Synthesis of arylurease

Aniline and/or p-substituted aniline (CH_3 & Cl, 0.1 mol) was dissolved in glacial acetic acid (10 mL) and diluted with water (upto 100 mL). To this solution an equimolar (0.1 mol) quantity of sodium cyanate in warm water (50 mL) was added with stirring. The reaction mixture was allowed to stand for 30 min, and then compounds were filtered, washed with water and dried after recrystallization from boiling water.

2.2.2 Synthesis of arylsemicarbazides

The syntheses of arylsemicarbazides were achieved as per scheme A. To an aqueous solution of arylureas (0.1 mol); an equimolar quantity of hydrazine hydrate was added. Ethanol (2 mL) was added to this solution. The reaction mixture was refluxed for 30 min and cooled in ice. For p-substituted phenylsemicarbazides sodium hydroxide (4 g) was added to make the reaction mixture alkaline, before refluxing. The product was filtered under suction and recrystallized from ethanol.

3. Phenylsemicarbazide

Molecular Weight: 151.07; Yield: 65.3 %; M.P.: 121-23 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: Chloroform (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.59).

4- chloro- phenylsemicarbazide: Molecular Weight: 185.61; Yield: 68.1 %; M.P.: 199-201 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: Chloroform (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.68).

4- methyl-phenylsemicarbazide: Molecular Weight: 165.19; Yield: 72.6%; M.P.: 216-218 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: Chloroform (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.63).

4. Synthesis of semicarbazone derivatives

A mixture of heterocyclic benzaldehyde (0.002 mol), aroyl semicarbazides (0.002 mol) and 10 ml of ethanol were taken in around bottom flask. Then few drops of Con. hydrochloric acid was added and refluxed for 2-3 hr. The reaction was monitored by TLC and poured the reaction mixture into ice. The precipitate was filtered, washed with sodium acetate (0.005mol). The crude solid was dried and recrystallized with hot ethanol (Singhal *et al*., 2011).

1-(4-(pyridin-2-yloxy)benzylidene)-4-phenylsemicarbazide (A1):

Molecular Weight: 332.36; Yield: 62.15 %; M.P.: 168-170^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.53).

Elemental analysis: Calculated for $C_{19}H_{16}N_4O_2$: C, 68.66; H, 4.85; N, 16.86; O, 9.63 %. Found: C, 68.69; H, 4.83; N, 16.83; O, 9.67 %

Spectral analysis: FT-IR (νmax): 3083 (Aromatic C-H), 2985 & 2877 (Aliphatic CH), 1619 (Phenyl ring stretch.), 1596 (C=N), 1503 & 1483 (Phenyl C-H out of plane bending), 1463 (CH_2 bending), 1276 (C-O), 1181 (in plane aromatic bending), 1129 (C-O-C, asym), 1036 (C-O-C, sym), 926 (ethylene dioxide characteristic peak), 853 (C-Cl) cm^{-1}.

^{1}H-NMR ($CDCl_3$) (ppm): 8.35 (s, 1H, Ph-C<u>H</u>=N at N_1 semicarbazide linkage), 7.78-7.76 (d, 2H, Ortho benzyl ring protons at N_1 semicarbazide linkage), 7.54-7.42 (d, 1H, pyridine ring proton at C_6), 7.32-7.30 (d, 2H, Ortho phenyl ring protons at N_4 semicarbazide linkage), 7.22-7.17 (t, 3H, meta & para phenyl ring protons at N_4 semicarbazide linkage), 6.94-6.80 (m, 3H, pyridine ring proton at C_3 & C_5), 6.66-6.58 (d, 2H, meta benzyl ring protons at N_1 semicarbazide linkage), 6.23 (s, 1H, -C=N-N<u>H</u> semicarbazide proton), 6.21 (s, 1H, O=C-N<u>H</u> semicarbazide proton).

1-(4-(pyridin-2-yloxy)benzylidene)-4-(4-chlorophenyl)semicarbazide (A2) :

Molecular Weight: 366.8; Yield: 68.94 %; M.P.: 168-170 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.58).

Elemental analysis: Calculated for $C_{19}H_{15}ClN_4O_2$: C, 62.21; H, 4.12; Cl, 9.67; N, 15.27; O, 8.72 %. Found: C, 62.24; H, 4.09; Cl, 9.65; N, 15.25; O, 8.76 %.

Spectral analysis: FT-IR (νmax): 3083 (Aromatic C-H), 2985 & 2877 (Aliphatic CH), 1619 (Phenyl ring stretch.), 1596 (C=N), 1503 & 1483 (Phenyl C-H out of plane bending), 1463 (CH_2 bending), 1276 (C-O), 1181 (in plane aromatic bending), 1129 (C-O-C, asym), 1036 (C-O-C, sym), 926 (ethylene dioxide characteristic peak), 853 (C-Cl) cm^{-1}.

^{1}H-NMR ($CDCl_3$) (ppm): 8.59 (s, 1H,Ph-C<u>H</u>=N), 7.94-7.92 (d, 2H, Ortho benzyl ring protons at N_1 semicarbazide linkage), 7.90-7.88 (d, 2H, Ortho phenyl ring protons at N_4 semicarbazide linkage), 7.70-7.62 (t, 1H, pyridine ring proton at C_4), 7.46-7.44 (d, 2H, meta phenyl ring protons at N_4 semicarbazide linkage), 7.24-7.20 (d, 1H, pyridine ring proton at C_6), 7.04-7.02 (d, 2H, meta benzyl ring protons at N_1 semicarbazide linkage), 6.60-6.58 (d, 1H, pyridine ring proton at C_3), 6.39-6.33 (t, 1H, pyridine ring proton at C_5), 6.04 (s, 2H, semicarbazide N<u>H</u> protons)

1-(4-(pyridin-2-yloxy)benzylidene)-4-p-tolylsemicarbazide (A3):

Molecular Weight: 346.38; Yield: 78.86 %; M.P.: 134-136 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.56).

Elemental analysis: Calculated for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17; O, 9.24 %. Found: C, 69.37; H, 5.25; N, 16.12; O, 9.26 %.

Spectral analysis: FT-IR (νmax): 3083 (Aromatic C-H), 2985 & 2877 (Aliphatic CH), 1619 (Phenyl ring stretch.), 1596 (C=N), 1503 & 1483 (Phenyl C-H out of plane bending), 1463 (CH_2

bending), 1276 (C-O), 1181 (in plane aromatic bending), 1129 (C-O-C, asym), 1036 (C-O-C, sym), 926 (ethylene dioxide characteristic peak), 853 (C-Cl) cm^{-1}.

^{1}H-NMR ($CDCl_3$) (ppm): 8.38 (s, 1H,Ph-CH=N), 7.78-7.76 (d, 2H, Ortho benzyl ring protons at N_1 semicarbazide linkage), 7.66-7.62 (t, 1H, pyridine ring proton at C_4), 7.36-7.34 (d, 2H, meta phenyl ring protons at N_4 semicarbazide linkage), 7.22-7.20 (d, 1H, pyridine ring proton at C_6), 7.04-7.00 (d, 2H, meta benzyl ring protons at N_1 semicarbazide linkage), 6.60-6.58 (d, 1H, pyridine ring proton at C_3), 6.48-6.44 (t, 1H, pyridine ring proton at C_5), 6.11 (s, 2H, semicarbazide NH protons), 2.27 (s, 3H, CH_3 protons at phenyl ring).

1-(4-(pyridin-3-yloxy)benzylidene)-4-phenylsemicarbazide (A4):

Molecular Weight: 332.36; Yield: 51.84 %; M.P.: 129-30 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.43).

Elemental analysis: Calculated for $C_{19}H_{16}N_4O_2$: C, 68.66; H, 4.85; N, 16.86; O, 9.63 %. Found: C, 68.68; H, 4.82; N, 16.83; O, 9.67 %.

Spectral analysis: FT-IR (vmax): 3083 (Aromatic C-H), 2985 & 2877 (Aliphatic CH), 1619 (Phenyl ring stretch.), 1596 (C=N), 1503 & 1483 (Phenyl C-H out of plane bending), 1463 (CH_2 bending), 1276 (C-O), 1181 (in plane aromatic bending), 1129 (C-O-C, asym), 1036 (C-O-C, sym), 926 (ethylene dioxide characteristic peak), 853 (C-Cl) cm^{-1}.

^{1}H-NMR ($CDCl_3$) (ppm): 8.48 (s, 1H, Ph-CH=N at N_1 semicarbazide linkage), 8.30 (s, 1H, pyridine ring proton at C_2), 8.18-8.14 (d, 1H, pyridine ring proton at C_6), 7.80-7.76 (d, 2H, Ortho benzyl ring protons at N_1 semicarbazide linkage), 7.74-7.72 (d, 2H, Ortho phenyl ring protons at N_4 semicarbazide linkage), 7.44-7.42 (t, 2H, meta phenyl ring protons at N_4 semicarbazide linkage), 7.34-7.30 (d, 1H, pyridine ring proton at C_4), 7.24-7.16 (t, 1H, para phenyl ring protons at N_4 semicarbazide linkage), 7.0-6.96 (d, 2H, meta benzyl ring protons at N_1 semicarbazide linkage), 6.01 (s, 2H, semicarbazide NH protons).

1-(4-(pyridin-3-yloxy)benzylidene)-4-(4-chlorophenyl)semicarbazide (A5):

Molecular Weight: 366.8; Yield: 56.54 %; M.P.: 119-121 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.51).

Elemental analysis: Calculated for $C_{19}H_{15}ClN_4O_2$: C, 62.21; H, 4.12; Cl, 9.67; N, 15.27; O, 8.72 %. Found: C, 62.23; H, 4.10; Cl, 9.65; N, 15.24; O, 8.77 %.

Spectral analysis: FT-IR (vmax): 3083 (Aromatic C-H), 2985 & 2877 (Aliphatic CH), 1619 (Phenyl ring stretch.), 1596 (C=N), 1503 & 1483 (Phenyl C-H out of plane bending), 1463 (CH_2 bending), 1276 (C-O), 1181 (in plane aromatic bending), 1129 (C-O-C, asym), 1036 (C-O-C, sym), 926 (ethylene dioxide characteristic peak), 853 (C-Cl) cm^{-1}.

^{1}H-NMR ($CDCl_3$) (ppm): 8.48 (s, 1H, Ph-CH=N at N_1 semicarbazide linkage), 8.30 (s, 1H, pyridine ring proton at C_2), 8.18-8.14 (d, 1H, pyridine ring proton at C_6), 7.80-7.76 (d, 2H, Ortho benzyl ring protons at N_1 semicarbazide linkage), 7.75-7.73 (d, 2H, Ortho phenyl ring protons at N_4 semicarbazide linkage), 7.48-7.46 (d, 2H, meta phenyl ring protons at N_4 semicarbazide linkage), 7.34-7.32 (t, 1H, pyridine ring proton at C_4), 7.04-7.00 (d, 2H, meta benzyl ring protons at N_1 semicarbazide linkage), 6.01 (s, 2H, semicarbazide NH protons).

1-(4-(pyridin-3-yloxy)benzylidene)-4-p-tolylsemicarbazide (A6) :

Molecular Weight: 346.38; Yield: 61.32 %; M.P.: 112-115 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.48).

Elemental analysis: Calculated for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17; O, 9.24 %. Found: C, 69.37; H, 5.20; N, 16.15; O, 9.28 %.

Spectral analysis: FT-IR (vmax): 3083 (Aromatic C-H), 2985 & 2877 (Aliphatic CH), 1619 (Phenyl ring stretch.), 1596 (C=N), 1503 & 1483 (Phenyl C-H out of plane bending), 1463 (CH_2 bending), 1276 (C-O), 1181 (in plane aromatic bending), 1129 (C-O-C, asym), 1036 (C-O-C, sym), 926 (ethylene dioxide characteristic peak), 853 (C-Cl) cm^{-1}.

*^{1}H-NMR ($CDCl_3$) (ppm)***:** 8.54 (s, 1H, Ph-C<u>H</u>=N at N_1 semicarbazide linkage), 8.26 (s, 1H, pyridine ring proton at C_2), 8.18-8.15 (d, 1H, pyridine ring proton at C_6), 7.78-7.76 (d, 2H, Ortho benzyl ring protons at N_1 semicarbazide linkage), 7.52-7.50 (d, 2H, Ortho phenyl ring protons at N_4 semicarbazide linkage), 7.33-7.31 (t, 1H, pyridine ring proton at C_4), 7.20-7.18 (d, 2H, meta phenyl ring protons at N_4 semicarbazide linkage), 7.04-7.00 (d, 2H, meta benzyl ring protons at N_1 semicarbazide linkage), 6.08 (s, 2H, semicarbazide N<u>H</u> protons), 2.32 (s, 3H, C<u>H</u>$_3$ protons at phenyl ring).

1-(4-(pyridin-4-yloxy)benzylidene)-4-phenylsemicarbazide (A7):

Molecular Weight: 332.36; Yield: 68.29 %; M.P.: 225-229 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.61).

Elemental analysis: Calculated for $C_{19}H_{16}N_4O_2$: C, 68.66; H, 4.85; N, 16.86; O, 9.63 %. Found: C, 68.67; H, 4.87; N, 16.81; O, 9.65 %.

Spectral analysis: FT-IR (vmax): 3083 (Aromatic C-H), 2985 & 2877 (Aliphatic CH), 1619 (Phenyl ring stretch.), 1596 (C=N), 1503 & 1483 (Phenyl C-H out of plane bending), 1463 (CH_2 bending), 1276 (C-O), 1181 (in plane aromatic bending), 1129 (C-O-C, asym), 1036 (C-O-C, sym), 926 (ethylene dioxide characteristic peak), 853 (C-Cl) cm^{-1}.

^{1}H-NMR ($CDCl_3$) (ppm): 8.48 (s, 1H, Ph-C<u>H</u>=N at N_1 semicarbazide linkage), 8.36-8.33 (d, 2H, pyridine ring protons at C_2 & C_6), 7.78-7.76 (d, 2H, Ortho benzyl ring protons at N_1 semicarbazide linkage), 7.64-7.62 (d, 2H, Ortho phenyl ring protons at N_4 semicarbazide linkage), 7.41-7.39 (t, 2H, meta phenyl ring protons at N_4 semicarbazide linkage), 7.18-7.14 (t, 1H, para phenyl ring protons at N_4 semicarbazide linkage), 7.04-7.03 (d, 2H, pyridine ring protons at C_3 & C_5), 7.02-7.01 (d, 2H, meta benzyl ring protons at N_1 semicarbazide linkage), 6.00 (s, 2H, semicarbazide N<u>H</u> protons).

1-(4-(pyridin-4-yloxy)benzylidene)-4-(4-chlorophenyl)semicarbazide (A8):

Molecular Weight: 366.8; Yield: 72.63 %; M.P.: 202-204 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.65).

Elemental analysis: Calculated for $C_{19}H_{15}ClN_4O_2$: C, 62.21; H, 4.12; Cl, 9.67; N, 15.27; O, 8.72 %. Found: C, 62.24; H, 4.14; Cl, 9.63; N, 15.23; O, 8.74 %.

Spectral analysis: FT-IR (vmax): 3083 (Aromatic C-H), 2985 & 2877 (Aliphatic CH), 1619 (Phenyl ring stretch.), 1596 (C=N), 1503 & 1483 (Phenyl C-H out of plane bending), 1463 (CH_2 bending), 1276 (C-O), 1181 (in plane aromatic bending), 1129 (C-O-C, asym), 1036 (C-O-C, sym), 926 (ethylene dioxide characteristic peak), 853 (C-Cl) cm^{-1}.

^{1}H-NMR ($CDCl_3$) (ppm): 8.48 (s, 1H, Ph-C<u>H</u>=N at N_1 semicarbazide linkage), 8.36-8.33 (d, 2H, pyridine ring protons at C_2 & C_6), 7.78-7.76 (d, 2H, Ortho benzyl ring protons at N_1 semicarbazide linkage), 7.74-7.72 (d, 2H, Ortho phenyl ring protons at N_4 semicarbazide linkage), 7.49-7.47 (d, 2H, meta phenyl ring protons at N_4 semicarbazide linkage), 7.10-7.09 (d, 2H, pyridine ring protons at C_3 & C_5), 7.05-7.03 (d, 2H, meta benzyl ring protons at N_1 semicarbazide linkage), 6.01 (s, 2H, semicarbazide N<u>H</u> protons).

1-(4-(pyridin-4-yloxy)benzylidene)-4-p-tolylsemicarbazide (A9):

Molecular Weight: 346.38; Yield: 76.95 %; M.P.: 189-192 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (2:3) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.62).

Elemental analysis: Calculated for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17; O, 9.24 %. Found: C, 69.38; H, 5.25; N, 16.15; O, 9.22 %.

Spectral analysis: FT-IR (vmax): 3083 (Aromatic C-H), 2985 & 2877 (Aliphatic CH), 1619 (Phenyl ring stretch.), 1596 (C=N), 1503 & 1483 (Phenyl C-H out of plane bending), 1463 (CH_2 bending), 1276 (C-O), 1181 (in plane aromatic bending), 1129 (C-O-C, asym), 1036 (C-O-C, sym), 926 (ethylene dioxide characteristic peak), 853 (C-Cl) cm^{-1}.

^{1}H-NMR ($CDCl_3$) (ppm): 8.54 (s, 1H, Ph-C<u>H</u>=N at N_1 semicarbazide linkage), 8.33-8.31 (d, 2H, pyridine ring protons at C_2 & C_6), 7.78-7.76 (d, 2H, Ortho benzyl ring protons at N_1 semicarbazide linkage), 7.56-7.52 (d, 2H, Ortho phenyl ring protons at N_4 semicarbazide linkage), 7.21-7.19 (d, 2H, meta phenyl ring protons at N_4 semicarbazide linkage), 7.04-7.03 (d, 2H, pyridine ring protons at C_3 & C_5), 7.02-7.01 (d, 2H, meta benzyl ring protons at N_1 semicarbazide linkage), 6.08 (s, 2H, semicarbazide N<u>H</u> protons), 2.31 (s, 3H, C<u>H</u>$_3$ protons at phenyl ring).

5. Result and Discussion

The present study deals with the preparation of Semicarbazones derivatives:-

1-(4-(pyridin-2-yloxy)benzylidene)-4-phenylsemicarbazide (A1), 1-(4-(pyridin- loxy) benzylidene)-4-(4-chlorophenyl)semicarbazide (A2), 1-(4-(pyridin-2-yloxy)benzylidene)-4-p-tolylsemicarbazide (A3),1-(4-(pyridin-3-yloxy)benzylidene)-4-phenylsemicarbazide (A4), 1-(4-(pyridin-3-yloxy)benzylidene)-4-(4-chlorophenyl)semicarbazide(A5),1-(4-(pyridin-3-yloxy) benzylidene)-4-p-tolylsemicarbazide(A6),1-(4-(pyridin-4-yloxy)benzylidene)-4-phenylsemicarbazide (A7) 1-(4-(pyridin-4-yloxy)benzylidene)-4-(4-chlorophenyl)semicarbazide (A8),1-(4-(pyridin-4-yloxy)benzylidene)-4-p-tolylsemicarbazide (A9) are prepared by using extraction method. Characterization was done by element analysis and FTIR ,^{1}H-NMR & TLC methods. The yields of carbazones derivatives was found respectively (62.15), (68.94), (78.86), (51.84), (56.54), (61.32), (68.29), (72.73), (76.95).

6. Conclusion

The semicarbazones derivatives are prepared were successfully from carbazone by extraction method. These derivatives shows anticonvulsant and antituberculosis activity. characterization was done by FTIR , ^{1}H-NMR & TLC and element analysis.

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