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Uses of 1-Cyanoacetyl-4-Phenyl-3-Thiosemicarbazide in the Synthesis of Antimicrobial and Antifungal Heterocyclic Compounds

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Authors' contributions

This work was carried out in collaboration with all authors. Both the authors read and approved the final manuscript.

Research Article

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ABSTRACT

The thiosemicarbazide synthesized from the reaction of cyanoacetylhydrazine phenylisothiocyanate reacted with acetoacetanilide gave the 2-pyridinone derivative **4**. The reaction of the latter product with 1,3-dicarbonyl and α-halocarbonyl compounds gave the benzo[c]pyridine derivatives **6a,b** and the thiazole derivatives 9a,b, respectively. The reaction with phenylisothiocyanate and elemental sulfur gave the thiazole derivative **14**. The latter compound reacted with α-halocarbonyl compounds to give the thiazole products **16a-c.** The antimicrobial and antifungal evaluations of the newly synthesized products showed that some compounds have high activity.

Keywords: Thiosemicarbazide; pyridine; thiazole; antimicrobial.

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1. INTRODUCTION

In recent years, thiosemicarbazides have attracted much attention because of their fungicidal, Ezabadi et al. (2008): Liesen et al. (2010), bactericidal, Siwek et al. (2011), Plech et al. (2011) and tuberculostatic, Gürsoy et al. (1997) activities. Aryl semicarbazones, Guo et al. (2009), Sun et al. (2009), Deng et al. (2011) and thiosemicarbazones, Taroua et al. (1996), Aly et al. (2010), Yogeeswari et al. (2005) have emerged as structurally novel anticonvulsants. Aryl semicarbazides are reported to display an excellent anticonvulsant activity in mice and rats compared to that of phenytoin, El-Azab et al. (2012), Shindikar et al. (2006), Sheppeck II et al. (2007). The aryl semicarbazones were believed to interact at locations on the putative binding site designated as aryl binding site, a hydrogen bonding domain and an auxiliary aryl binding site, Bialer et al. (2007). The aryl binding site can be phenyl or other hydrophobic moieties with retention of the anticonvulsant activity, Yogeeswari et al. (2003), Ilies et al. (2004), Azam et al. (2009). In this work we demonstrate the uses of the thiosemicarbazide derivative derived from the reaction of cyanoacetylhydrazine with phenylisothiocyanate together with its uses in the synthesis of biologically active heterocyclic compounds.

2. EXPERIMENTAL DETAILS

All melting points are uncorrected. IR spectra were recorded for (KBr) discs on a Pye Unicam SP-1000 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Varian EM-390-200 MHz in CD_3SOCD_3 as solvent using TMS as internal standard, and chemical shifts are expressed as δ. Analytical data were obtained from the Micro analytical Data Unit at Cairo University, Giza, Egypt.

2.1 1-(3-isocyano-4-methyl-2-oxo-6-(phenylamino)pyridine-1(2H)-yl)-3-phenyl-thiourea (4)

To a solution of compound **3** (5 g, 0.021 mol) in benzene (135 mL) containing acetic acid (35 mL) and ammonium acetate (2.0 g), acetoacetanilide (3.78 g, 0.021 mol) was added. The reaction mixture was heated under reflux for 6 hrs using Dean-Stark water separator then evaporated using the oil pump evaporator and poured onto a beaker containing ice/water mixture. The formed solid product was collected by filtration and crystallized from ethanol to give buff crystals (4.50 g, 56%), m.p. 135ºC. *Anal*. Calculated for $C_{20}H_{17}N_5OS$ (375.44): C, 63.98; H, 4.56; N, 18.65; S, 8.53. Found: C, 63.93; H, 5.11; N; 18.17; S, 8.51. IR, υ: 3383-3205 (3NH), 3102 (CH, aromatic), 2926 (CH_3)2260 (CN), 1699 (C=O), 1298 (C=S). ^{1}H-NMR, δ: 2.51 (s, 3H, CH_3), 5.82 (s, 1H, C3-$H_{pyridine}$), 7.09-7.59 (m, 10H, H_{arom}), 8.65, 9.78, 10.30 (br, 3s, 3H, 3NH). ^{13}C NMR, δ: 14.7 (CH_3), 82.6, 112.5, 126.3, 129.1, 129.9, 137.1, 140.5, 141.8, 149.4 (2 C_6H_5, pyridine C), 116.7 (CN), 160.1 (C=O), 181.3 (C=S).

2.2 4-Cyano-6,8-dimethyl-3-oxo-1-phenylamino-2N-phenylthiourylbenzo-[*c*]pyridine (6a)

To a solution of compound **4** (3.75 g, 0.01 mol) in ethanol (40 mL) containing piperidine (0.5 mL), acetylacetone (1.00 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 hrs then poured onto a beaker containing ice/water mixture containing a few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from ethanol to give yellow crystals, (2.20 g, 50%), m.p. 75-85ºC. *Anal.* Calculated for $C_{25}H_{21}N_5OS$ (439.53): C, 68.31; H, 4.81; N, 15.93; S, 7.29. Found: C, 68.53;

H, 4.82, N, 16.11; S, 6.88. IR, υ: 3383-3260 (3NH), 3044 (CH, aromatic), 2930, 2885 ($2CH_3$), 2216 (CN), 1685 (C=O), 1229 (C=S). ^{1}H-NMR, δ: 3.30, 3.87 (2s, 6H, $2CH_3$), 7.05-7.74(m, 12H, H_{arom}), 8.11, 9.16, 10.40 (br, 3s, 3H, 3NH). ^{13}C NMR, δ: 22.9, 23.4 (2 CH_3), 100.2, 111.5, 118.8, 122.0, 124.9, 126.4, 129.0, 129.5, 136.2, 138.9 (aromatic C), 116.9 (CN), 160.6 (C=O), 180.7 (C=S).

2.3 1-(4-Cyano-5,6-dihydro-8-methyl-3,6-dioxo-1-(phenylamino)isoquinolin-2(3H)-yl)-3-phenylthiourea (6b)

To a solution of compound **4** (3.75 g, 0.01 mol) in ethanol (40 mL) containing piperidine (0.5 mL), ethyl acetoacetate (1.35 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 hrs then poured onto a beaker containing ice/water mixture containing a few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from ethanol to give pale brown crystals, (2.00 g, 53%), m.p. 210ºC. *Anal.* Calculated for $C_{24}H_{19}N_5O_2S$ (441.50): C, 65.29; H, 4.33; N, 15.86; S, 7.26. Found: C, 65.80; H, 4.80; N, 15.36; S, 6.90. IR υ: 3550-3287 (OH, 3NH), 3059 (CH, aromatic), 2936 (CH_3), 2267 (CN), 1683-1680 (2 C=O), 1233 (C=S). ^{1}H-NMR, δ: 3.39 (s, 3H, CH_3), 6.93-7.47 (m, 12H, H_{arom}), 8.67, 9.48, 10.43 (br, 3s, 3H, 3NH), 11.01 (s, 1H, OH). ^{13}C-NMR, δ: 23.2 (CH_3), 41.0 (CH_2) 101.2, 112.8, 117.2, 120.9, 122.6, 126.4, 128.8, 131.7, 134.6, 136.4, 146.8 (aromatic C), 117.0 (CN), 162.5, 172.3 (2 C=O), 180.7 (C=S).

2.4 7-Amino-1-oxo-3-phenylamino-2-thiourylthieno[3,4-*c*]pyridine (7)

To a solution of compound **4** (1 g, $2.66x10^{-3}$ mol) in ethanol (25 mL) containing triethylamine (0.5 mL), sulfur (0.13 g, $2.66x10^{-3}$ mol) was added. The reaction mixture was heated under reflux for 3 hrs then poured onto a beaker containing ice/water mixture containing a few drops of hydrochloric acid. The solid product was collected by filtration and crystallized from ethanol to give orange crystals, (0.66 g, 60%), m.p. 120-125ºC. *Anal.* Calculated for $C_{20}H_{17}N_5OS_2$ (407.50): C, 58.94; H, 4.2; N, 17.18; S, 15.73. Found: C, 58.47, H, 4.45; N, 16.83; S, 16.30. IR, υ: 3398-3207 (NH_2, NH), 3034 (CH, aromatic), 1685 (C=O), 1634 (C=C), 1236 (C=S). ^{1}H-NMR, δ: 5.18 (br, s, 2H, NH_2), 6.93-7.62 (m, 12H, C5-$H_{thiophene}$, H_{arom}), 9.82, 10.40, 10.98 (br, 3s, 3H, 3NH). ^{13}C NMR δ: 80.9 (pyridine C-3), 119.8, 121.5, 122.4, 123.3, 124.9, 126.5, 129.0, 136.9, 139.0 (aromatic, thiophene C), 158.6 (C=O), 180.6 (C=S).

2.5 Amino-3-cyano-5,7-diphenyl-4-methyl-1-(3‘,4‘-diphenylthiazol-2‘-ylidino)-pyrolo[2,3-*b*]pyridine (9a)

To a solution of compound **4** (3.66 g, $9.76x10^{-3}$ mol) in ethanol (40 mL), phenacyl bromide **(**1.94 g, $9.76x10^{-3}$ mol) was added. The reaction mixture was heated under reflux for 4 hrs then poured onto a beaker containing ice/water mixture and the pH adjusted till 7 using sodium hydroxide. The solid product was collected by filtration and crystallized from ethanol and a few drops of dimethylformamide to give pale yellow crystals, (3.26 g, 58%), m.p. 170-186ºC. *Anal.* Calculated for $C_{36}H_{25}N_5OS$ (575.68): C, 75.10; H, 4.37; N, 12.16; S, 5.56. Found: C, 74.59; H, 5.02; N, 11.65; S, 5.93. IR, υ: 3056 (CH, aromatic), 2927, 2850, 2773 (CH_3, 2CH), 2195 (CN), 1699 (C=O). ^{1}H-NMR, δ: 2.6 (s, 3H, CH_3), 6.45-8.02 (m, 22H, C6-$H_{pyrrole}$, C5-$H_{thiazole}$, H_{arom}). ^{13}C NMR, δ: 16.7 (CH), 116.4 (CN), 118.9, 120.5, 122.2, 125.7, 126.9, 128.3, 129.8, 30.6, 133.1, 138.9, 140.6 (aromatic, thiazole C), 163.7 (C=O), 170.3 (C=N).

2.6 Amino-3-cyano-4,5-dimethyl-7-phenyl-1-4'-methyl-3'-phenylthiazol-2'-ylidino)-pyrolo[2,3-*b*]pyridine (9b)

To a solution of compound **4** (3.66 g, $9.76x10^{-3}$) in ethanol (40 mL), chloroacetone (0.9g, $9.76x10^{-3}$ mol) was added. The reaction mixture was heated under reflux for 4 hrs then poured onto a beaker containing ice/water mixture and the pH adjusted till 7 using sodium hydroxide. The solid product collected by filtration and crystallized from ethanol and a few drops of dimethylformamide to give yellow crystals, (2.22 g, 50%), m.p.170-180ºC. *Anal.* Calculated for $C_{26}H_{21}N_5OS$ (451.54): C, 69.15; H, 4.68; N, 15.50; S, 7.10. Found: C, 69.68; H, 5.23; N, 14.98; S, 6.54. IR, υ: 3043 (CH, aromatic), 2930, 2825, 2848 ($3CH_3$), 2220 (CN), 1680 (C=O), 1643 (C=N). ^{1}H-NMR, δ: 2.49, 2.51, 3.30 (3s, 9H, CH_3), 6.04-7.54(m, 12H, C6-$H_{pyrrole}$, C5-$H_{thiazole}$, H_{arom}).

2.7 4-Benzal-3-cyano-2-oxo-6-phenylamino-1-N-phenylthiourylmethino-pyridine (11)

To a solution of compound **4** (0.5 g, $1.33x10^{-3}$ mol) in ethanol (25 mL) containing piperidine (0.5 mL), benzaldehyde (0.14 g, $1.33x10^{-3}$ mol) was added. The reaction mixture was heated under reflux for 3 hrs then poured onto a beaker containing ice/water mixture together with a few drops of hydrochloric acid. The solid product was collected by filtration and crystallized from ethanol to give pale yellow crystals, (0.5 g, 80%), m.p. 55ºC. *Anal.* Calculated for $C_{27}H_{21}N_5OS$ (463.55): C, 69.95; H, 4.56; N, 15.1; S, 6.91. Found: C, 69.45 H, 4.98; N, 15.47; S, 6.62%. IR, υ: 3383-3259 (3NH), 3043 (CH, aromatic), 2221 (CN), 1682 (C=O), 1634 (C=C), 1193 (C=S). ^{1}H-NMR, δ: 7.06, 7.07 (2d, 2H, 2CH), 7.09 (s, 1H, C3-$H_{pyridine}$), 7.24-8.00 (m, 15H, H_{arom}), 8.65, 9.16, 10.40 (br, 3s, 3H, 3NH). ^{13}C NMR, δ: 82.6 (pyridine C_3), 99.6, 100.4 (C=C), 118.7 (CN), 120.4, 122.6, 124.3, 126.9, 128.0, 129.3, 133.6, 133.9, 134.5, 138.4, 140.6, 144.7 (aromatic, pyridine C), 160.5 (C=O), 180.8 (C=S).

2.8 5-Cyano-2-phenylamino-6-oxo-3[*H*]-1-N-phenylthiouryl-4-phenylhydrazono-methinpyridine (13)

To a solution of compound **4** (1.0 g, $2.66x10^{-3}$ mol) in ethanol (50 mL) containing sodium hydroxide solution (10 mL, 10%) and a solution of benzenediazonium chloride ($2.66x10^{-3}$ mol) [which was prepared by sodium nitrite (0.276 g, $7.36x10^{-4}$ mol) in water, 2 ml was added to a cold solution of aniline (0.24 g, $2.66x10^{-3}$ mol) containing appropriate amount of hydrochloric acid and with continuous stirring] was added with continuous stirring. The formed solid product was collected by filtration and crystallized from dimethylformamide to give reddish brown crystals, (0.64 g, 50%), m.p. 100ºC. *Anal.* Calculated for $C_{26}H_{21}N_7OS$ (479.56): C, 65.11; H, 4.41; N, 20.44; S, 6.68. Found: C, 65.65; H, 4.92; N, 19.84; S, 6.23. IR, υ: 3366-3283 (4NH), 3055 (CH, aromatic), 2217 (CN), 1691 (C=O), 1232 (C=S). ^{1}H-NMR, δ: 6.43-7.70 (m, 17H, C3-$H_{pyridine}$, =CH, H_{arom}), 8.63, 9.79, 9.90, 11.01(br, 4s, 4H, 4NH).

2.9 5-Amino-2-thioxo-1-phenyl-4-carbo-1-(phenyl-3-thiosemicarbazid-1-yl) thiazole (14)

To a solution of compound **3** (2.34 g, 0.01 mol) in ethanol (40 mL) and dioxan (10 mL) containing triethylamine (2.0 mL), phenylisothiocyanate **(2)** (1.35 g, 0.01 mol), elemental sulfur was added. The reaction mixture was heated under reflux for 4h then left to cool and evaporated under vacuum, the remaining product was triturated with ethanol. The formed

solid product was collected by filtration and crystallized from ethanol to give pale brown crystals, (3.10 g, 79.69%), m.p 95-100ºC. *Anal.* Calculated for $C_{17}H_{15}N_5OS_3$ (401.52): C, 50.85; H, 3.74; N, 17.45; S, 23.94. Found: C, 50.56; H, 3.54; N, 17.25; S, 24.15. IR, υ: 3460-3312 (NH_2, 3NH), 3054 (CH aromatic), 1685 (C=O), 1642 (C=C), 1210-1190 (C=S). ^{1}H-NMR, δ: 4.46 (s, 2H, NH_2), 7.26-7.32 (m, 10H, $2C_6H_5$), 8.20-8.42 (3s, 3H, 3NH). ^{13}C NMR δ: 124.6, 126.4, 126.7, 126.8, 128.1, 129.0, 129.4, 131.7, 134.8 (aromatic, thiazole C), 164.8 (C=O), 176.9, 186.5 (2 C=S).

2.10 5-Amino-1-phenyl-2-thioxo-4-carbohydrazino-(3,4-diphenylthiazol-2-ylidieno)-thiazole (16a)

To a solution of compound **14** (4.01g, 0.01 mol) in ethanol (40 mL), phenacylbromide **(8a)** (1.99 g, 0.01 mol) was added. The reaction mixture was kept at room temperature with stirring for 2 hrs and the formed solid product was collected by filtration and crystallized from ethanol to give pale yellow crystals, (4.11 g, 81.93%), m.p.135-140ºC. *Anal.* Calculated for $C_{25}H_{19}N_5OS_3$ (501.64): C, 59.85; H, 3.81; N, 13.96; S, 19.17. Found: C, 59.39; H, 3.60; N, 13.39; S, 19.01. IR, υ: 3465-3312 (NH_2, NH), 3055 (CH, aromatic), 1686 (C=O), 1655 (exocyclic C=N), 1640 (C=C), 1215-1195 (C=S). ^{1}H-NMR, δ: 4.63 (s, 2H, NH_2), 5.89 (s, 1H, thiazole H-5), 7.23-7.34 (m, 15H, $3C_6H_5$), 8.30 (s, 1H, NH).

2.11 5-Amino-1-phenyl-2-thioxo-4-carbohydrazino-(4-methoxy-3-phenylthiazol-2-ylidieno)thiazole (16b)

To a solution of compound **14** (4.01g, 0.01 mol) in ethanol (40 mL), chloroacetone **(8b)** (0.925 g, 0.01 mol) was added. The reaction mixture was kept at room temperature with stirring for 2 hrs and the formed solid product was collected by filtration and crystallized from ethanol to give yellow crystals, (3.12 g, 71.07%), m.p.50-55ºC. *Anal.* Calculated for $C_{20}H_{17}N_5OS_3$ (439.57): C, 54.64; H, 3.89; N, 15.93; S, 21.88. Found: C, 54.23; H, 3.56; N, 15.46; S, 21.51. IR, υ: 3565-3310 (NH_2, NH), 3055 (CH, aromatic), 1686 (C=O), 1655 (exocyclic C=N), 1640 (C=C), 1212-1195 (C=S). ^{1}H-NMR, δ: 2.72 (s, 3H, CH_3), 4.46(s, 2H, NH_2), 5.63 (s, 1H, thiazole H-5), 7.32-7.44 (m, 10H, $2C_6H_5$), 8.34 (s, 1H, NH).

2.12 5-Amino-1-phenyl-2-thioxo-4-carbohydrazino-(4-hydroxy-3-phenylthiazol-2-ylidieno)thiazole (16c)

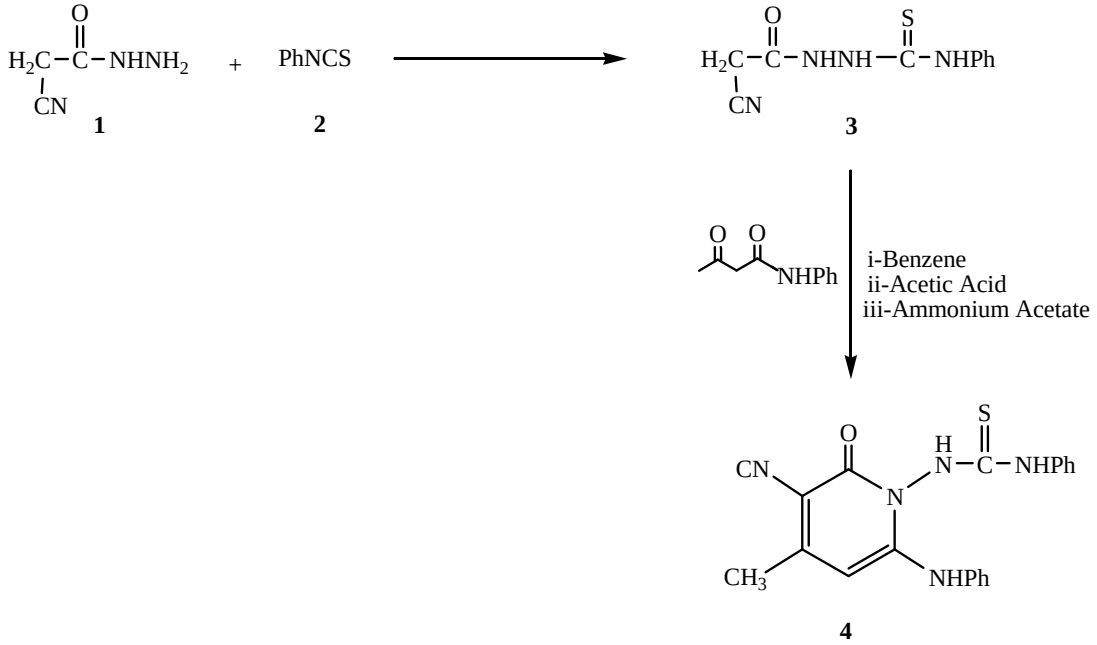
To a solution of compound **14** (4.01g, 0.01 mol) in ethanol (40 mL), ethyl chloroacetate **(8c)** (1.22 g, 0.01 mol) was added. The reaction mixture was kept at room temperature with stirring for 2 hrs and the formed solid product was collected by filtration and crystallized from ethanol to give pale yellow crystals, (3.90 g, 88.43%), m.p.77-80ºC. *Anal.* Calculated for $C_{19}H_{15}N_5O_2S_3$ (441.54): C, 51.68; H, 3.42; N, 15.86; S, 21.78. Found: C, 51.23; H, 3.21; N, 15.43; S, 21.41. IR, υ: 3560-3315 (NH_2, OH, NH), 3060 (CH, aromatic), 1683 (C=O), 1663 (exocyclic C=N), 1642 (C=C), 1215-1203 (C=S). ^{1}H-NMR, δ: 4.49 (s, 2H, NH_2), 6.82 (s, 1H, thiazole H-5), 7.30-7.36 (m, 10H, $2C_6H_5$), 8.26 (s, 1H, NH), 10.34 (s, 1H, OH). ^{13}C NMR, δ: 56.8 (thiazolidene C-5), 119.8, 120.1, 122.5, 123.0, 124.6, 124.8, 129.8, 134.0, 134.7 (2 C6H5, thiazole C), 155.8 (thiazolidene C-2), 169.3 (C=O), 186.3 (C=s), 187.0 (C=N).

3. RESULTS AND DISCUSSION

The reaction of cyanoacetylhydrazine **(1)** with phenylisothiocyanate **(2)** gave the thiosemicarbazide, Mekheimer et al. (1999) derivative 3. The reaction of 3 with

acetoacetanilide in benzene, acetic acid containing ammonium acetate afforded the pyridine derivative **4** (Scheme 1). Structure of compound **4** was based on analytical and spectral data (see experimental section). The ^{13}C NMR showed δ: 14.7 (CH_3), 82.6, 112.5, 126.3, 129.1, 129.9, 137.1, 140.5, 141.8, 149.4 (2 C_6H_5, pyridine C), 116.7 (CN), 160.1 (C=O), 181.3 (C=S). Formation of similar pyridine derivatives were reported by Khidre et al. (2011). Further confirmation for the structure of compound **4** was obtained via studying the reactivity of **4** towards some chemical reagents. Thus, the reaction of **4** with either acetylacetone **(5a)** or ethyl acetoacetate **(5b)** gave the benzo[c]pyridine derivatives **6a** and **6b**, respectively, Khidre et al. (2011).

The structures of the latter products were based on analytical and spectral data. Thus, the ^{1}H-NMR spectrum of **6a** (as an example) showed two singlets at δ 3.30, 3.87 to two CH_3 while in case of compound **6b** showed a singlet at δ 3.39 corresponding to CH_3, and a singlet at 11.01 corresponding to the OH group. On the other hand, the ^{13}C NMR of **6a** showed δ at 22.9, 23.4 (2 CH_3), 100.2, 11.5, 118.8, 122.0, 124.9, 126.4, 129.0, 129.5, 136.2, 138.9 (aromatic C), 116.9 (CN), 160.6 (C=O), 171.7 (C=O), 180.7 (C=S). Compound **6b** exist in keto as well as enol form as this is confirmed from its^{13}C NMR spectrum which showed the presence of a signal at 41.0 corresponing to the -CH_2-CO group and two signals at 162.5, 172.3 for the two CO groups. Formation of 6b goes in a similar manner like the reported work, Maheswara et al. (2006). On the other hand, the reaction of compound **4** with elemental sulfur gave either the thieno[3,4-c]pyridine derivatives **7** Barnes et al. (2006).

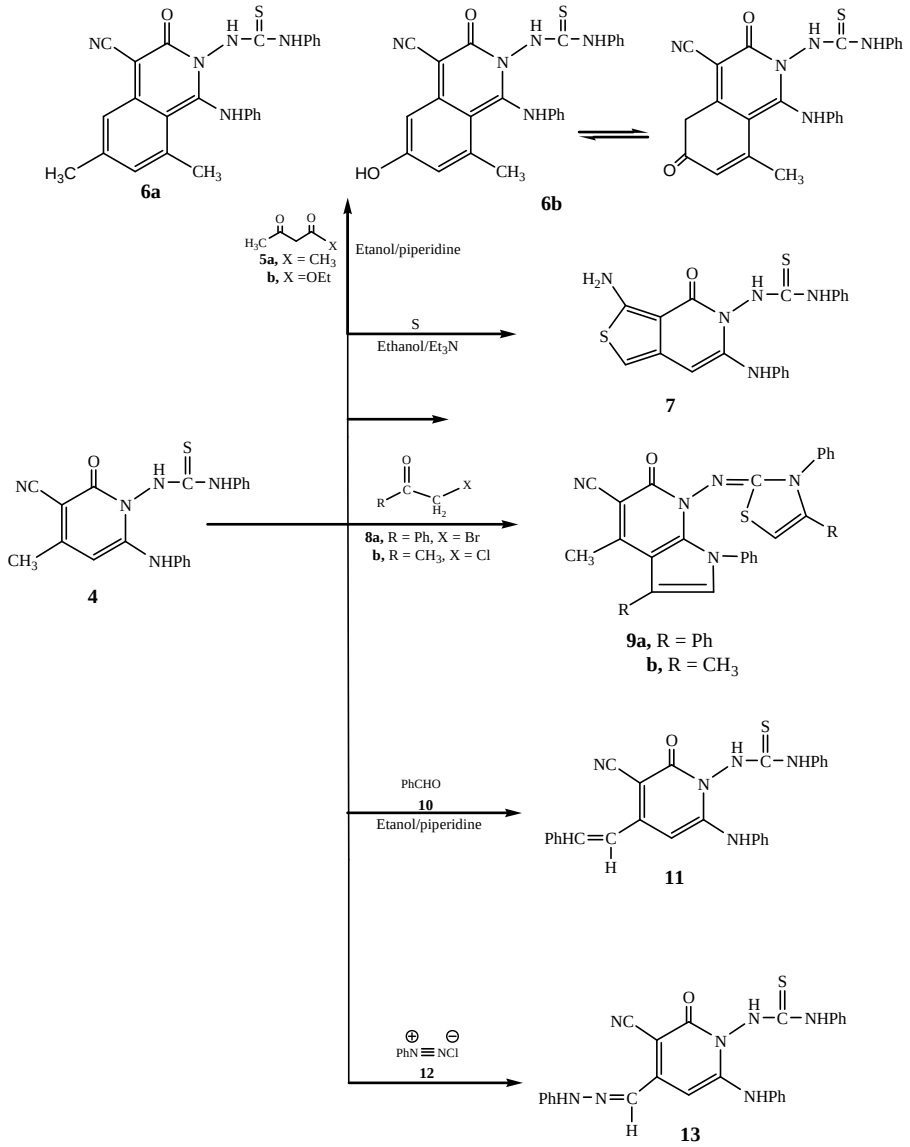


Scheme 1: Synthesis of compounds 3 and 4

Compound **4** as a thiosemicarbazide derivative, reacted with either phenacylbromide **(8a)** or chloroacetone **(8b)** to give the thiazole derivatives **9a** and **9b**, respectively Potewar et al. (2007). Formation of the latter products is explained in terms of the reaction of **4** with two

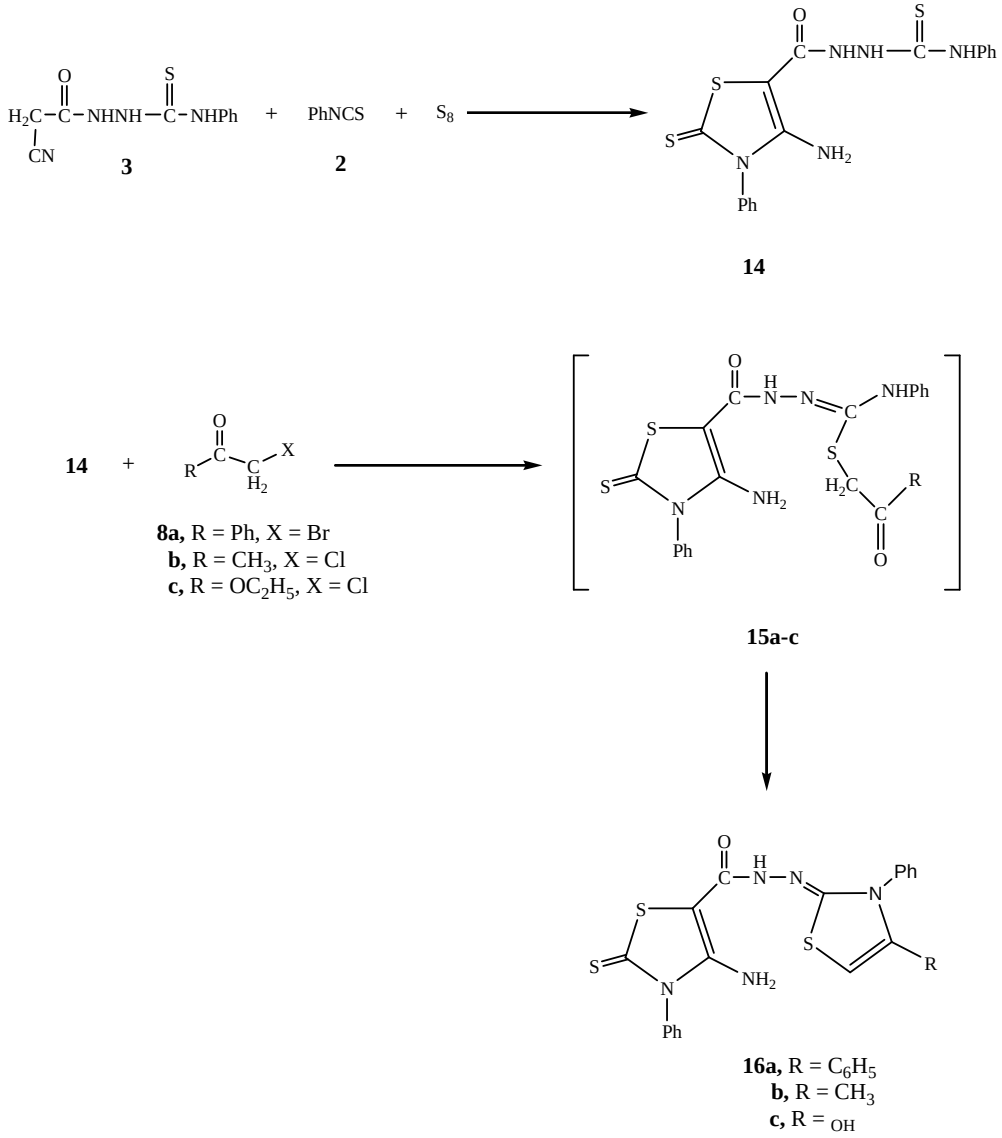
molecules of the α-haloketone, on two active centers, the thioamide moiety and the 2-phenyl amino group followed by cyclization at the two active centers.

On the other hand, the reaction of compound **4** with benzaldehyde **(10)** gave the 1-N-1-phenylthiouryl-4-benzalmethinopyridine derivative **11**, Konieczny et al. (2005). The structure of **11** was established on the basis of analytical and spectral data where the IR spectrum showed CN group stretching band at δ 2221 cm^{-1} and C=S at δ 1193 cm^{-1}. The reaction of **4** with benzenediazonium chloride **(12)** gave the aryl hydrazone derivative **13**, Radwan et al. (2007), (Scheme 2). The analytical and spectral data was in agree with the proposed structure.



Scheme 2. Synthesis of compounds 6a,b-13

On the other hand, the reaction of compound **3** with phenylisothiocyanate **2** and elemental sulfur gave the thiazole derivative **14**, the ^{1}H-NMR spectrum showed a singlet at δ 4.46 corresponding to NH_2 group, a multiplet at δ 7.26-7.32 corresponding to two phenyl groups and three singlets at δ 8.20-8.42 corresponding to three NH groups. The reaction of compound **14** with either phenacyl bromide **(8a),** chloroacetone **(8b)** or ethyl chloroacetate **(8c)** gave the thiazole derivatives **16a-c**, which are formed through the intermediate formation of **15a-c** followed by cyclization Mohareb et al. (1994), Bondock et al. (2010), (Scheme 3). The analytical and spectral data of the latter are in agreement with the proposed structures (see the experimental section).



Scheme 3. Synthesis of compounds 13 and 16a-c

3.1 *In vitro* Antimicrobial and Antifungal Evaluation of the Newly Synthesized Compounds

An evaluation of the antibacterial activity using two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive bacteria (*Bacillus subtilis* and *Bacillus cereus*) and the antifungal activity using *Candida albicans* as a representative species of fungi was assessed for compounds. The minimal inhibitory concentration (MIC in µg/mL) was determined using an adaptation of agar streak dilution method based on radial diffusion. In the same conditions different concentrated solutions of ampicillin (antibacterial) and cycloheximide (antifungal) were used as standards. The MIC was considered to be the lowest concentration of the tested compound which inhibits growth of bacteria or fungi on the plate. The diameters of the inhibition zones corresponding to the MICs are presented in (Table 1) the tested compounds are not active against *Pseudomonas aeruginosa* starting from DMSO solutions of 1000 µg/mL of each compound.

Table 1. Biological activity of the tested compounds

Compound	**MIC in µg/mL (zone of inhibition in mm)**			
	***E. coli* ECT 101**	***B. Cereus* CECT 148**	***B. subtilis* CECT 498**	***C. albicans* CECT 1394**
4	Not active	0.08 (3)	5.23 (8)	8.44 (6)
6a	Not active	7.03 (8)	0.68 (2)	4.50 (5)
6b	Not active	18.25 (15)	20 (8)	30 (6)
7	12.50 (6)	20 (8)	6.25 (4)	8.65 (4)
9a	Not active	22.01 (3)	0.48	25.60 (6)
9b	Not active	18.32 (5)	6.22 (2)	0.40 (10)
11	Not active	20.15 (4)	23.16 (9)	100 (5)
13	Not active	12.32 (3)	16.32 (8)	14.40 (4)
14	16.64	0.06 (2)	6.33 (5)	50 (11)
16a	Not active	12.30 (4)	4.22 (6)	12.55 (12)
16b	Not active	6.05 (6)	12.42 (2)	2.55 (10)
16c	Not active	22.01 (4)	20.13 (10)	25.61 (6)
Ampicillin	6.25	3.13	12.50 (10)	-
Cycloheximide	-	-	-	12.50

From the analysis of Table 1 it is possible to establish some SARs. The only active compounds against *E. coli* in the concentrations tested are 7 and 14 (MIC 12.5 µg/mL), the substituted pyrazole moiety being the responsible for the activity. However, the dithiazole derivative 16b showed the highest activity. Against Gram + bacteria the MICs for 16b are much higher than those for 16a and 16c. Comparing 6a with 6b (both of them are benzopyridine derivatives), compound 6a (with the CH_3 group) shows to be more active against B. cereus (MIC 3.13 µg/mL) than 6b (with the OH substituent). It is obvious from Table 1 that the pyridine derivative 4 showed the highest activity against *B. Cereus* CECT 148 *B. subtilis* CECT 498. It is clear also that compounds 6a and 16b showed the most activity towards *C. albicans* CECT 1394.

For the *in vitro* antimicrobial activity, suspensions of the microorganism were prepared to contain approximately 108 cfu/mL and the plates were inoculated. A stock solution of the synthesized compound (1000 µg/mL) in DMSO was prepared and graded dilutions of the tested compounds were incorporated in a cavity (depth 3 mm, diameter 4 mm) made in the

center of the Petridis (nutrient agar for antibacterial activity and Sabouraud vs dextrose agar medium for antifungal activity). The plates were incubated at 37ºC (for bacteria) and at 30ºC (for fungi) for 24 h in duplicate. Positive control using only inoculation and negative control using only DMSO in the cavity were carried out.

4. CONCLUSION

The present work showed the synthesis of the polyfunctionally substituted pyridine derivative 4. The latter underwent a series of heterocyclic reaction to form pyridine, benzo[c]pyridine, thieno[3,4-c]pyridine, pyrolo[2,3-b]pyridine and thiazole derivatives with antimicrobial and antifungal activities.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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