

## One pot, green synthesis and biological evaluation of some isatin-based thiocarbohydrazones derivatives

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### ABSTRACT

In the present study, a one-pot, green and three-component route for synthesis of Thiocarbohydrazone (TCS) derivatives is described. This reaction was performed in choline chloride/urea (1:2) as Deep Eutectic Solvent (DES). The structures were characterized by FTIR, <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra and elemental analysis. The derivatives were evaluated for their anti-mycobacterial activity against *Mycobacterium bovis BCG*, and the results revealed that among the synthesized compounds (**1a-1h**, **2a-2h**, **3a-3h**), **3b**, **3c** and **1b** exhibited the highest activity with MIC value of 7.81 and 11.71 µg/mL. Additionally, the target compounds were evaluated for their anti-microbial activity against *E.coli* and *Candida Albicans* and the result indicated that in contrast to the low antifungal activity, the produced derivatives with electron-withdrawing substitution at the ortho, meta and para positions of benzyl ring includes **1d**, **2b** and **3d-3g** demonstrated remarkable anti-mycobacterial activities.

**Keywords:** Hiocarbohydrazone, deep eutectic solvent, one pot and three-component condensation reaction

### INTRODUCTION

The most common method for the synthesis of thiocarbohydrazone derivatives is

condensation reaction of thiocarbohydrazide with carbonyl compounds such as aldehydes or ketones.

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This reaction could be generated Schiff bases that widely have been used as an antifungal [1], anticancer [2] and anti-convulsant [3] agents. Also the wide variety of Thiocarbohydrazone (TCS) containing an appropriate structural framework were found to have antineoplastic [4], antibacterial [5], and antifungal [6] properties.

One of the most important thiocarbohydrazones is thiacetazone as an economical, anti-tubercular and bacteriostatic drug that has been widely used in combination with other antimycobacterial agents like isoniazid. [7] Bermudez and *et al.* Synthesized thiocarbohydrazones with different functional groups on the benzene ring, which had higher activity than thiacetazone [8] In addition, Kobarfard and *et al.* were succeeded in obtaining a new thiosemicarbazone that was both non-toxic and highly active against *M. tuberculosis* [9].

Another widely used heterocyclic in the synthesis of medicinally active Schiff bases is isatin. According to the reports, it had shown many biological properties such as anti-HIV [10], anti-bacterial [11], anti-

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fungal [12], anti-viral [13] and anti-inflammatory [14] activities .

Chinnasamy and *et al* designed and synthesized new isatin-based Schiff bases and investigated their analgesic activity [15].

Volatile organic compounds such as benzene, toluene, xylene and formaldehyde usually were applied in the common organic synthesis reactions. The most common method for synthesis of thiocarbohydrazide derivatives is compounds such as aldehydes or ketones to yield an intermediate compound [16]. This intermediate compound could be generated Schiff bases that widely have been used in biological activities including antifungal, antibacterial and anti-inflammatory properties [17]. In the Schiff base structure, the C=N moiety is important for biological activity.

Minimum inhibitory concentration (MIC) values explain *in vitro* level resistance of specific bacterial strains, so lower MIC values indicate that less of the drug are required to inhibit growth of the organism such as *Mycobacterium bovis BCG*, *E.coli* and *Candida Albicans*. The obtained MIC values have impact on the choice of a therapeutic strategy [18,19,20].

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A one pot synthesis is a strategy to improve efficiency of a chemical reaction that the reactants are subjected to the reaction in just one reactor to produce less waste. Condensation reaction is a type of one pot reaction. In organic chemistry, a reaction that two molecules are combined to form a molecule is known as condensation reaction. Ionic liquids can be used in one pot synthesis instead of organic solvents in green synthesis, so the ionic liquids can be dissolved organometallic compounds and can be reused in the organic reactions and mentined as an environmentally friendly method.

In this article, according to the mentioned biological attractions, 24 different thiocarbohydrazone derivatives had been designed and synthesized using 3 isatin and 8 aldehyde derivatives and evaluated for their biological activity against *Mycobacterium bovis* BCG, *E.coli* and *Candida Albicans*. For this purpose a one-pot, green and three-component route had been used.

## MATERIALS AND METHODS

The chemicals used in this work were purchased from Merck, and Sigma-Aldrich chemical companies. Melting points were measured using

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a capillary tube method by a Barnstead Electrothermal 9200 device. FTIR spectra were recorded using KBr disks on Perkin- Elmer Spectrum RXI FT-IR spectrophotometer. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were measured in DMSO with TMS as an internal standard on a Bruker AMX spectrometer at 300 MHz.

### *General procedure for the preparation of thiocarbohydrazones (1a-1h), (2a-2h), (3a-3h)*

At the first step, an aldehyde (1 mmol) in the 30 min was added to a stirred solution of thiocarbohydrazide (1 mmol) in DES (15 mL). The stirring of the solution was continued at room temperature for 2 h. Then, isatin (1 mmol) was added to this solution and the reaction mixture was refluxed at 80 °C. After the completion of the reaction, ice water was added and the precipitate was collected and recrystallized by ethyl acetate.

## RESULTS

The structures of the synthesized thiocarbohydrazone compounds were confirmed using FTIR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectroscopies are presented below:

(1E,5E)-1-(3-nitrobenzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazid (**1a**)

IR (KBr): 3348 (N–H, Amides), 3107 (C–H, Ar), 1690 (C=O), 1620 (C=N), 1523 (N=O), 1156 (C=S) cm<sup>-1</sup>.

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.63 (s, 1H), 12.78 (s, 1H), 11.36 (s, 1H), 8.66 (s, 1H), 8.23-8.33 (m, 3H), 7.70-7.75 (m, 1H), 7.56 (d,  $J=7.5$  Hz, 1H), 7.37 (t,  $J=7.5$  Hz, 1H), 7.12 (t,  $J=7.5$  Hz, 1H), 6.93 (d,  $J=7.5$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.55, 162.75, 148.42, 142.18, 138.27, 135.53, 133.14, 131.55, 130.43, 124.72, 124.34, 122.63, 122.02, 120.93, 119.98, 111.18.

Anal. calcd. For  $\text{C}_{16}\text{H}_{12}\text{N}_6\text{O}_3\text{S}$  (368.07): C, 52.17; H, 3.28; N, 22.81; Found: C, 51.95; H, 3.17; N, 22.50.

(1E,5E)-1-(3-chlorobenzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazide (**1b**)

IR (KBr): 3143 (C-H, Ar), 1704 (C=O), 1620 (C=N), 1156 (C=S)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.61 (s, 1H), 12.69 (s, 1H), 11.37 (s, 1H), 8.16 (s, 1H), 8.01 (s, 1H), 7.80 (d,  $J=6.6$  Hz, 1H), 7.56 (d,  $J=7.2$  Hz, 1H), 7.45-7.50 (m, 2H), 7.37 (t,  $J=7.5$  Hz, 1H), 7.10 (t,  $J=7.5$  Hz, 1H), 6.95 (d,  $J=7.8$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.58, 163.01, 143.48, 142.44, 138.62, 136.08, 134.32, 132.09, 131.28, 130.76, 126.98, 126.84, 123.19, 121.49, 120.21, 111.59.

Anal. calcd. For  $\text{C}_{16}\text{H}_{12}\text{ClN}_5\text{OS}$  (357.82): C, 53.71; H, 3.38; N, 19.57; Found: C, 53.75; H, 3.11; N, 19.68.

(1E,5E)-1-(3-bromobenzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazide (**1c**)

IR (KBr): 3105 (C-H, Ar), 1687 (C=O), 1621 (C=N), 1164 (C=S), 545 (C-Br)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.59 (s, 1H), 12.68 (s, 1H), 11.37 (s, 1H), 8.13 (d,  $J=7.68$  Hz, 2H), 7.85 (d,  $J=7.62$  Hz, 1H), 7.64 (d,  $J=7.8$  Hz, 1H), 7.56 (d,  $J=7.2$  Hz, 1H), 7.34-7.44 (m, 2H), 7.09 (t,  $J=7.2$  Hz, 1H), 6.93 (d,  $J=7.8$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.41, 162.75, 142.72, 142.30, 138.12, 136.04, 133.11, 131.47, 131.01, 129.56, 126.79, 122.59, 122.41, 120.86, 120.01, 111.12.

Anal. calcd. For  $\text{C}_{16}\text{H}_{12}\text{BrN}_5\text{OS}$  (400.99): C, 47.77; H, 3.01; N, 17.41; Found: C, 47.44; H, 3.04; N, 17.73.

(1E,5E)-1-(2, 4-dichlorobenzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazide (**1d**)

IR (KBr): 3154 (C-H, Ar), 1708 (C=O), 1618 (C=N), 1159 (C=S), 792 (C-Cl)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.61 (s, 1H), 12.77 (s, 1H), 11.33 (s, 1H), 8.56 (s, 1H), 8.26 (d,  $J=8.4$  Hz, 1H), 7.75 (d,  $J=2.1$  Hz, 1H), 7.50-7.58 (m, 2H), 7.37 (t,  $J=7.8$  Hz, 1H), 7.10 (t,  $J=7.5$  Hz, 1H), 6.95 (d,  $J=7.8$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.57, 162.99, 142.43, 140.04, 138.76, 136.25, 135.08, 132.17, 130.33, 130.05, 128.37, 128.27, 123.26, 121.55, 120.17, 111.66. Anal. calcd. For  $\text{C}_{16}\text{H}_{11}\text{Cl}_2\text{N}_5\text{OS}$  (391.01): C, 48.99; H, 2.83; N, 17.85; Found: C, 48.86; H, 2.74; N, 17.76.

(1E,5E)-1-(2, 5-dichlorobenzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazide (**1e**)

IR (KBr): 3180 (C-H, Ar), 1666 (C=O), 1621 (C=N), 1146 (C=S), 777 (C-Cl)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.60 (s, 1H), 12.76 (s, 1H), 11.33 (s, 1H), 8.55 (s, 1H), 8.25 (d,  $J=8.7$  Hz, 1H), 7.73 (d,  $J=1.8$  Hz, 1H), 7.49-7.57 (m, 2H), 7.37 (t,  $J=7.8$  Hz, 1H), 7.09 (t,  $J=7.5$  Hz, 1H), 6.94 (d,  $J=7.8$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ): 175.34, 162.72, 142.27, 139.27, 138.26, 135.64, 134.58, 131.53, 130.04, 129.65, 127.92, 127.74, 122.62, 120.90, 119.93, 111.17.

Anal. calcd. For  $\text{C}_{16}\text{H}_{11}\text{Cl}_2\text{N}_5\text{OS}$  (391.01): C, 48.99; H, 2.83; N, 17.85; Found: C, 48.83; H, 2.83; N, 17.49.

(1E,5E)-1-(2, 6-dichlorobenzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazide (**1f**)

IR (KBr): 3150 (C–H, Ar), 1708 (C=O), 1618 (C=N), 1155 (C=S), 792 (C-Cl)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.18 (s, 1H), 12.75 (s, 1H), 11.12 (s, 1H), 8.49 (s, 1H), 7.53–7.58 (m, 3H), 7.42–7.48 (m, 1H), 7.36 (t,  $J = 7.8$  Hz, 1H), 7.08 (t,  $J = 7.5$  Hz, 1H), 6.92 (d,  $J = 7.5$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 176.02, 162.14, 142.49, 140.48, 138.07, 136.20, 134.30, 131.58, 129.33, 129.15, 127.60, 122.54, 120.91, 120.00, 119.10, 111.11.

Anal. calcd. For  $\text{C}_{16}\text{H}_{11}\text{Cl}_2\text{N}_5\text{OS}$  (391.01): C, 48.99; H, 2.83; N, 17.85; Found: C, 49.16; H, 2.73; N, 17.75.

(1E,5E)-1-(4-chloro-3-nitrobenzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazide (**1g**)

IR (KBr): 3410 (N–H, Amides), 3101 (C–H, Ar), 1696 (C=O), 1621 (C=N), 1529 (N=O), 1152 (C=S)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.62 (s, 1H), 12.61 (s, 1H), 11.34 (s, 1H), 8.50 (s, 1H), 8.21 (s, 1H), 8.15 (d,  $J = 8.4$  Hz, 1H), 7.85 (d,  $J = 8.4$  Hz, 1H), 7.56 (d,  $J = 7.2$  Hz, 1H), 7.36 (t,  $J = 7.5$  Hz, 1H), 7.09 (t,  $J = 7.5$  Hz, 1H), 6.93 (d,  $J = 7.8$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ): 175.49, 162.62, 148.17, 142.30, 140.74, 138.35, 134.43, 132.18, 131.65, 131.51, 125.97, 123.63, 122.60, 120.88, 119.92, 111.15.

Anal. calcd. For  $\text{C}_{16}\text{H}_{11}\text{ClN}_6\text{O}_3\text{S}$  (402.03): C, 47.71; H, 2.75; N, 20.86; Found: C, 47.64; H, 2.61; N, 20.75.

(1E,5E)-1-(3-methoxybenzylidene)-5-(2-oxoindolin-3-ylidene) thiocarbohydrazide (**1h**)

IR (KBr): 3325 (N–H, Amides), 3120 (C–H, Ar), 1695 (C=O), 1623 (C=N), 1166 (C=S)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.63 (s, 1H), 12.61 (s, 1H), 11.30 (s, 1H), 8.15 (s, 1H), 7.55–7.58 (m, 2H), 7.35–7.37 (m, 3H), 6.93 – 7.10 (m, 3H), 3.86 (s, 3H).

$^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ): 175.31, 162.70, 159.68, 144.10, 142.21, 137.89, 135.00, 131.40, 129.98, 122.57, 121.13, 120.82, 120.05, 117.36, 111.11, 110.62, 55.32.

Anal. calcd. For  $\text{C}_{17}\text{H}_{15}\text{N}_5\text{O}_2\text{S}$  (353.4): C, 57.78; H, 4.28; N, 19.82; Found: C, 57.63; H, 4.13; N, 19.63.

(1E,5E)-1-(3-nitrobenzylidene)-5-(5-nitro-2-oxoindolin-3-ylidene) thiocarbohydrazide (**2a**)

IR (KBr): 3361 (N–H, Amides), 3121 (C–H, Ar), 1710 (C=O), 1625 (C=N), 1523 (N=O), 1159 (C=S)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.42 (s, 1H), 12.85 (s, 1H), 11.91 (s, 1H), 8.64 (s, 1H), 8.23–8.29 (m, 5H), 7.32 (t,  $J = 7.8$  Hz, 1H), 7.10 (d,  $J = 8.4$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.11, 162.08, 148.44, 146.34, 142.64, 141.55, 135.45, 133.28, 132.85, 131.82, 127.56, 126.36, 123.83, 121.55, 114.24, 111.31.

Anal. calcd. For  $\text{C}_{16}\text{H}_{11}\text{N}_7\text{O}_5\text{S}$  (413.37): C, 46.49; H, 2.68; N, 23.72; Found: C, 46.37; H, 2.58; N, 23.63.

(1E,5E)-1-(3-chlorobenzylidene)-5-(5-nitro-2-oxoindolin-3-ylidene) thiocarbohydrazide (**2b**)

IR (KBr): 3109 (C–H, Ar), 1711 (C=O), 1624 (C=N), 1521 (N=O), 1157 (C=S), 789 (C-Cl)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.38 (s, 1H), 12.81 (s, 1H), 11.96 (s, 1H), 8.14–8.24 (m, 3H), 7.96 (s, 1H), 7.76 (d,  $J = 6.3$  Hz, 1H), 7.43–7.47 (m, 2H), 7.08 (d,  $J = 8.7$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.27, 163.12, 147.28, 143.11, 142.65, 136.21, 135.66, 133.90, 130.75, 130.31, 127.12, 126.67, 126.49, 120.80, 115.50, 111.33.

Anal. calcd. For  $C_{16}H_{11}ClN_6O_3S$  (402.81): C, 47.71; H, 2.75; N, 20.86; Found: C, 47.72; H, 2.85; N, 20.68.

(1E,5E)-1-(3-bromobenzylidene)-5-(5-nitro-2-oxoindolin-3-ylidene) thiocarbohydrazide (**2c**)

IR (KBr): 3126 (C–H, Ar), 1708 (C=O), 1624 (C=N), 1503 (N=O), 1161 (C=S), 549 (C–Br)  $cm^{-1}$ .

$^1H$  NMR (300 MHz, DMSO- $d_6$ ): 14.39 (s, 1H), 12.83 (s, 1H), 11.97 (s, 1H), 8.09 - 8.26 (m, 4H), 7.82 (d,  $J = 7.8$  Hz, 1H), 7.62 (d,  $J = 8$  Hz, 1H), 7.39 (t,  $J = 7.8$  Hz, 1H), 7.08 (d,  $J = 8.7$  Hz, 1H).

$^{13}C$  NMR (75 MHz, DMSO- $d_6$ ): 175.20, 163.12, 147.30, 143.15, 142.67, 136.32, 135.85, 133.21, 130.99, 129.48, 127.13, 126.92, 122.40, 120.83, 115.48, 111.33.

Anal. calcd. For  $C_{16}H_{11}BrN_6O_3S$  (447.27): C, 42.97; H, 2.48; N, 18.79; Found: C, 42.72; H, 2.53; N, 18.67.

(1E,5E)-1-(2,4-dichlorobenzylidene)-5-(5-nitro-2-oxoindolin-3-ylidene) thiocarbohydrazid (**2d**)

IR (KBr): 3460 (N–H, Amides), 3130 (C–H, Ar), 1700 (C=O), 1626 (C=N), 1524 (N=O), 1163 (C=S), 793 (C–Cl)  $cm^{-1}$ .

$^1H$  NMR (300 MHz, DMSO- $d_6$ ): 14.41 (s, 1H), 12.91 (s, 1H), 11.93 (s, 1H), 8.54 (s, 1H), 8.19 - 8.28 (m, 3H), 7.72 (dd,  $J = 6.8$  Hz, 1.9 Hz, 1H), 7.49 (dd,  $J = 8.4$  Hz, 1.6 Hz, 1H), 7.12 (d,  $J = 8.4$  Hz, 1H).

$^{13}C$  NMR (75 MHz, DMSO- $d_6$ ): 175.24, 163.09, 143.55, 142.70, 139.72, 138.69, 135.13, 134.69, 133.97, 131.67, 129.38, 129.68, 127.91, 120.76, 115.51, 111.33.

Anal. calcd. For  $C_{16}H_{10}Cl_2N_6O_3S$  (437.26): C, 43.95; H, 2.31; N, 19.22; Found: C, 43.81; H, 2.41; N, 19.35.

(1E,5E)-1-(2,5-dichlorobenzylidene)-5-(5-nitro-2-oxoindolin-3-ylidene) thiocarbohydrazid (**2e**)

IR (KBr): 3459 (N–H, Amides), 3158 (C–H, Ar), 1701 (C=O), 1624 (C=N), 1525 (N=O), 1159 (C=S), 749 (C–Cl)  $cm^{-1}$ .

$^1H$  NMR (300 MHz, DMSO- $d_6$ ): 14.33 (s, 1H), 12.85 (s, 1H), 11.90 (s, 1H), 8.47 (s, 1H), 8.14-8.23 (m, 3H), 7.67 (m, 1H), 7.42 (d,  $J = 8.7$  Hz, 1H), 7.05 (d,  $J = 8.7$  Hz, 1H).

$^{13}C$  NMR (75 MHz, DMSO): 175.21, 163.11, 147.21, 143.47, 142.71, 139.73, 134.71, 131.32, 129.74, 129.37, 127.91, 127.70, 127.23, 120.72, 115.50, 111.46.

Anal. calcd. For  $C_{16}H_{10}Cl_2N_6O_3S$  (437.26): C, 43.95; H, 2.31; N, 19.22; Found: C, 43.67; H, 2.21; N, 19.14.

(1E,5E)-1-(2,6-dichlorobenzylidene)-5-(5-nitro-2-oxoindolin-3-ylidene) thiocarbohydrazid (**2f**)

IR (KBr): 3372 (N–H, Amides), 3133 (C–H, Ar), 1711 (C=O), 1627 (C=N), 1506 (N=O), 1158 (C=S), 783 (C–Cl)  $cm^{-1}$ .

$^1H$  NMR (300 MHz, DMSO- $d_6$ ): 14.01 (s, 1H), 12.93 (s, 1H), 11.75 (s, 1H), 8.52 (s, 1H), 8.27 (d,  $J = 8.4$  Hz, 1H), 8.22 (s, 1H), 7.58 (m, 2H), 7.41-7.49 (m, 1H), 7.11 (d,  $J = 8.7$  Hz, 1H).

$^{13}C$  NMR (75 MHz, DMSO): 175.72, 163.17, 147.37, 142.65, 141.05, 134.35, 134.07, 131.67, 131.30, 130.52, 129.37, 129.02, 127.28, 120.84, 115.56, 111.33.

Anal. calcd. For  $C_{16}H_{10}Cl_2N_6O_3S$  (437.26): C, 43.95; H, 2.31; N, 19.22; Found: C, 43.81; H, 2.29; N, 19.10.

(1E,5E)-1-(4-chloro-3-nitrobenzylidene)-5-(5-nitro-2-oxoindolin-3-ylidene) thiocarbohydrazide (**2g**)

IR (KBr): 3481 (N–H, Amides), 3114 (C–H, Ar), 1724 (C=O), 1621 (C=N), 1510 (N=O), 1166 (C=S), 791 (C–Cl)  $cm^{-1}$ .

$^1H$  NMR (300 MHz, DMSO- $d_6$ ): 14.43 (s, 1H), 12.99 (s, 1H), 11.98 (s, 1H), 8.50 (s, 1H), 8.19-8.27 (m, 1H), 8.19-8.21 (m, 3H), 8.13 (dd,  $J = 8.3, 1.5$  Hz, 1H), 7.85 (d,  $J = 8.4$  Hz, 1H), 7.10 (d,  $J = 8.4$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ): 175.23, 163.08, 148.16, 147.34, 142.71, 141.22, 136.55, 134.27, 132.25, 131.83, 127.22, 126.17, 123.60, 120.74, 115.54, 111.41.

Anal. calcd. For  $\text{C}_{16}\text{H}_{10}\text{ClN}_7\text{O}_5\text{S}$  (447.81): C, 42.91; H, 2.25; N, 21.89; Found: C, 42.78; H, 2.17; N, 21.72.

(1E,5E)-1-(3-methoxybenzylidene)-5-(5-nitro-2-oxoindolin-3-ylidene) thiocarbohydrazide (**2h**)

IR (KBr): 3197 (C–H, Ar), 1705 (C=O), 1626 (C=N), 1498 (N=O), 1159 (C=S)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.46 (s, 1H), 12.79 (s, 1H), 11.92 (s, 1H), 8.21–8.28 (m, 2H), 8.15 (s, 1H), 7.57 (s, 1H), 7.34–7.39 (m, 2H), 7.13 (d,  $J=8.7$  Hz, 1H), 7.00–7.04 (m, 1H), 3.86 (s, 3H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.16, 163.09, 159.67, 147.25, 144.57, 142.68, 136.08, 134.83, 129.99, 127.08, 121.29, 120.88, 117.56, 115.46, 111.35, 110.46, 55.30.

Anal. calcd. For  $\text{C}_{17}\text{H}_{14}\text{N}_6\text{O}_4\text{S}$  (398.4): C, 51.25; H, 3.54; N, 21.09; Found: C, 51.37; H, 3.46; N, 20.96.

(1E,5E)-1-(3-nitrobenzylidene)-5-(5-bromo-2-oxoindolin-3-ylidene) thiocarbohydrazide (**3a**)

IR (KBr): 3402 (N–H, Amides), 3126 (C–H, Ar), 1701 (C=O), 1616 (C=N), 1502 (N=O), 1159 (C=S), 534 (C-Br)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.56 (s, 1H), 12.85 (s, 1H), 11.46 (s, 1H), 8.65 (s, 1H), 8.23–8.28 (m, 3H), 7.73 (m, 1H), 7.50–7.59 (m, 2H), 6.89 (d,  $J=7.8$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ): 175.44, 162.35, 148.35, 142.29, 141.30, 137.02, 135.40, 133.54, 133.14, 130.37, 124.70, 122.88, 122.12, 121.95, 114.19, 113.09.

Anal. calcd. For  $\text{C}_{16}\text{H}_{11}\text{BrN}_6\text{O}_3\text{S}$  (447.27): C, 42.95; H, 2.48; N, 18.79; Found: C, 42.94; H, 2.41; N, 18.59.

(1E,5E)-1-(3-chlorobenzylidene)-5-(5-bromo-2-oxoindolin-3-ylidene) thiocarbohydrazide (**3b**)

IR (KBr): 3131 (C–H, Ar), 1701 (C=O), 1617 (C=N), 1163 (C=S), 790 (C-Cl), 534 (C-Br)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.52 (s, 1H), 12.76 (s, 1H), 11.48 (s, 1H), 7.77–8.15 (m, 4H), 7.48–7.59 (m, 3H), 6.88 (s, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.36, 162.40, 142.95, 141.29, 136.95, 135.75, 133.91, 133.54, 130.76, 130.26, 129.77, 126.56, 122.94, 122.18, 114.23, 113.08.

Anal. calcd. For  $\text{C}_{16}\text{H}_{11}\text{BrClN}_5\text{OS}$  (436.71): C, 44.00; H, 2.54; N, 16.04; Found: C, 43.90; H, 2.43; N, 15.91.

(1E,5E)-1-(3-bromobenzylidene)-5-(5-bromo-2-oxoindolin-3-ylidene) thiocarbohydrazide (**3c**)

IR (KBr): 3399 (N–H, Amides), 3151 (C–H, Ar), 1701 (C=O), 1617 (C=N), 1162 (C=S), 534 (C-Br)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.52 (s, 1H), 12.76 (s, 1H), 11.48 (s, 1H), 8.12 (d,  $J=9.9$  Hz, 2H), 7.82–7.85 (m, 1H), 7.38–7.64 (m, 4H), 6.88 (d,  $J=8.1$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.34, 162.40, 142.91, 141.28, 136.93, 135.95, 133.51, 133.14, 130.98, 129.55, 126.83, 122.89, 122.40, 122.20, 114.20, 113.09.

Anal. calcd. For  $\text{C}_{16}\text{H}_{11}\text{Br}_2\text{N}_5\text{OS}$  (481.16): C, 39.94; H, 2.30; N, 14.56; Found: C, 39.68; H, 2.23; N, 14.35.

(1E,5E)-1-(2,4-dichlorobenzylidene)-5-(5-bromo-2-oxoindolin-3-ylidene) thiocarbohydrazide (**3d**)

IR (KBr): 3410 (N–H, Amides), 3120 (C–H, Ar), 1697 (C=O), 1617 (C=N), 1162 (C=S), 793 (C-Cl), 524 (C-Br)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.49 (s, 1H), 12.79 (s, 1H), 11.40 (s, 1H), 8.52 (s, 1H), 8.20

(d,  $J = 6.3$  Hz, 1H), 7.49-7.69 (m, 4H), 6.89 (s, 1H), 7.21-7.35 (m, 2H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.23, 162.35, 141.27, 139.52, 137.05, 135.69, 134.62, 133.59, 129.96, 129.65, 127.92, 127.77, 122.95, 122.07, 114.26, 113.11.

Anal. calcd. For  $\text{C}_{16}\text{H}_{10}\text{BrCl}_2\text{N}_5\text{OS}$  (471.16): C, 40.79; H, 2.14; N, 14.86; Found: C, 40.83; H, 2.03; N, 14.77.

(1E,5E)-1-(2,5-dichlorobenzylidene)-5-(5-bromo-2-oxoindolin-3-ylidene) thiocarbohydrazide (**3e**)

IR (KBr): 3150 (C–H, Ar), 1708 (C=O), 1618 (C=N), 1155 (C=S), 792 (C-Cl), 519 (C-Br)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.51 (s, 1H), 12.81 (s, 1H), 11.41 (s, 1H), 8.55 (s, 1H), 8.22 (d,  $J = 8.1$  Hz, 1H), 7.48-7.54 (m, 2H), 7.60-7.72 (m, 2H), 6.90 (d,  $J = 8.1$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO): 175.19, 162.20, 141.10, 139.54, 137.04, 135.72, 134.63, 133.61, 129.97, 129.64, 127.91, 127.76, 122.94, 122.07, 114.27, 113.11.

Anal. calcd. For  $\text{C}_{16}\text{H}_{10}\text{BrCl}_2\text{N}_5\text{OS}$  (471.16): C, 40.79; H, 2.14; N, 14.86; Found: C, 40.71; H, 2.13; N, 14.74.

(1E,5E)-1-(2,6-dichlorobenzylidene)-5-(5-bromo-2-oxoindolin-3-ylidene) thiocarbohydrazide (**3f**)

IR (KBr): 3486 (N–H, Amides), 3139 (C–H, Ar), 1696 (C=O), 1619 (C=N), 1147 (C=S), 776 (C-Cl), 536 (C-Br)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.09 (s, 1H), 12.81 (s, 1H), 11.22 (s, 1H), 8.50 (s, 1H), 7.44–7.57 (m, 5H), 6.88 (d,  $J = 6.9$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ): 175.96, 161.74, 146.22, 141.45, 140.70, 136.82, 134.29, 133.59, 131.51, 129.27, 129.00, 128.65, 122.89, 122.17, 114.11, 113.06.

Anal. calcd. For  $\text{C}_{16}\text{H}_{10}\text{BrCl}_2\text{N}_5\text{OS}$  (471.16): C: 40.79; H: 2.14; N: 14.86; Found: C: 40.61; H: 2.04; N: 14.75.

(1E,5E)-1-(4-chloro-3-nitrobenzylidene)-5-(5-bromo-2-oxoindolin-3-ylidene) thiocarbohydrazide (**3g**)

IR (KBr): 3420 (N–H, Amides), 3119 (C–H, Ar), 1693 (C=O), 1617 (C=N), 1529 (N=O), 1172 (C=S), 789 (C-Cl), 527 (C-Br)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.48 (s, 1H), 12.87 (s, 1H), 11.45 (s, 1H), 8.44 (s, 1H), 8.16 (s, 1H), 8.10 (d,  $J = 7.5$  Hz, 1H), 7.78 (d,  $J = 7.5$  Hz, 1H), 7.54 (s, 1H), 7.47 (d,  $J = 7.5$  Hz, 1H), 6.84 (d,  $J = 7.5$  Hz, 1H).

$^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ): 175.37, 162.28, 148.04, 141.24, 140.79, 137.04, 134.32, 133.50, 132.13, 131.62, 126.07, 123.68, 122.89, 122.05, 114.22, 113.07.

Anal. calcd. For  $\text{C}_{16}\text{H}_{10}\text{BrClN}_6\text{O}_3\text{S}$  (481.71) C: 39.89; H: 2.09; N: 17.45; Found: C: 40.02; H: 1.99; N: 17.34.

(1E,5E)-1-(3-methoxybenzylidene)-5-(5-bromo-2-oxoindolin-3-ylidene) thiocarbohydrazide (**3h**)

IR (KBr): 3124 (C–H, Ar), 1699 (C=O), 1618 (C=N), 1160 (C=S), 536 (C-Br)  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ): 14.54 (s, 1H), 12.68 (s, 1H), 11.39 (s, 1H), 8.13 (s, 1H), 7.33–7.57 (m, 5H), 6.87–7.00 (m, 2H), 3.84 (s, 3H).

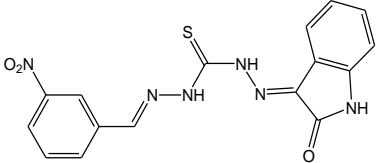
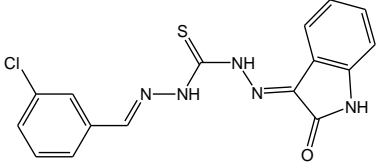
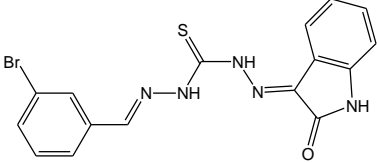
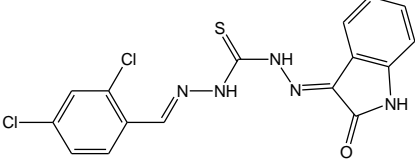
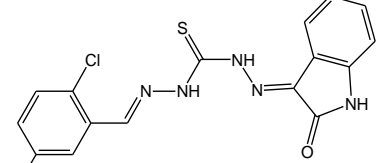
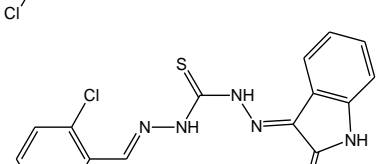
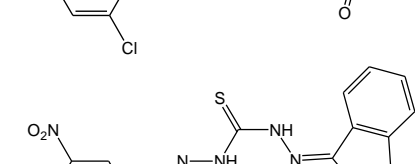
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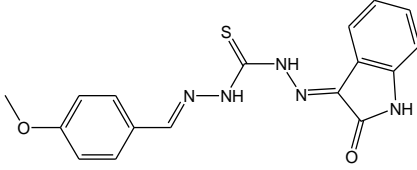
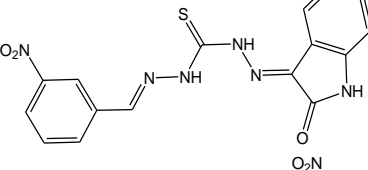
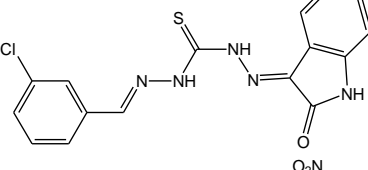
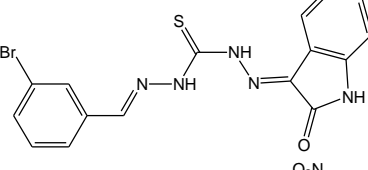
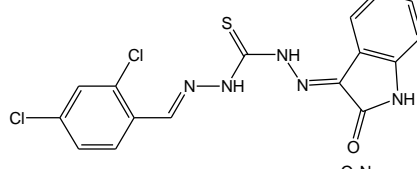
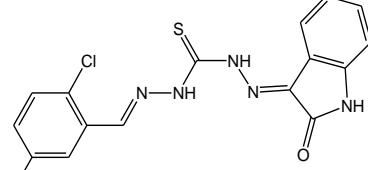
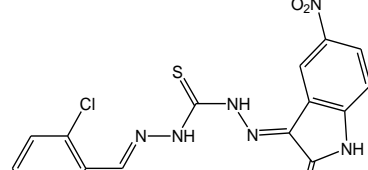
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The MIC values of the synthesized thiocarbohydrazone compounds are shown in Table 1.

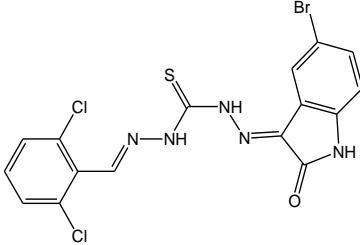
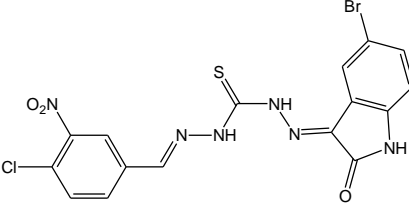
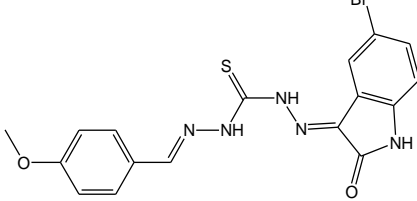
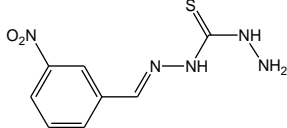
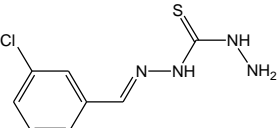
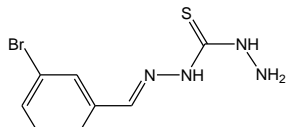
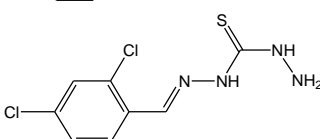
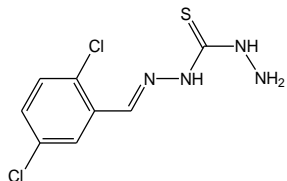


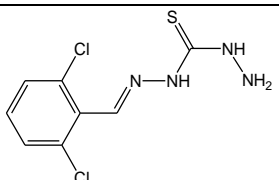
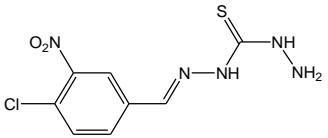
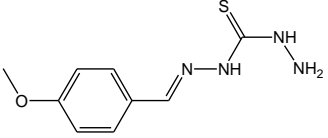
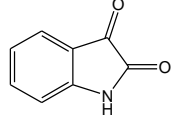
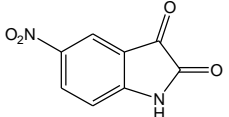
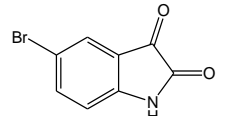
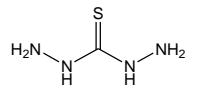
**Table 1.** The MIC values of the synthesized thiocarbohydrazone compounds

Entry	Code	Product	Time (h)	Yield (%)	Mp (°C)	MIC (µg/ml) BCG (48 h)	MIC (µg/ml) E.Coli (10 h/16h)	MIC (µg/ml) C. albicans (48 h)
1	1a		4	90	227-229	31.25	250	>1000
2	1b		4	85	230-233	7.81	125	750
3	1c		5	78	253-256	15.625	125	1000
4	1d		4	85	266-268	31.25	62.5	500
5	1e		3	80	256-258	15.625	125	500
6	1f		3	90	252-254	31.25	125	1000
7	1g		4	90	224-226	31.25	125	1000

Entry	Code	Product	Time (h)	Yield (%)	Mp (°C)	MIC (µg/ml) BCG (48 h)	MIC (µg/ml) E.Coli (10 h/16h)	MIC (µg/ml) C. albicans (48 h)
8	1h		5	80	232-234	15.625	250	750
9	2a		3	85	227-229	23.43	250	1000
10	2b		3	90	222-225	15.625	62.5	>1000
11	2c		4	76	219-221	23.43	250	500
12	2d		5	87	212-214	46.87	250	750
13	2e		5	76	249-251	31.25	250	1000
14	2f		3	85	239-242	15.625	125	750

Entry	Code	Product	Time (h)	Yield (%)	Mp (°C)	MIC (µg/ml) BCG (48 h)	MIC (µg/ml) E.Coli (10 h/16h)	MIC (µg/ml) C. albicans (48 h)
15	2g		4	90	237-240	62.5	250	750
16	2h		3	75	214-220	31.25	250	500
17	3a		4	82	234-236	31.25	125	750
18	3b		4	90	228-230	11.71	125	750
19	3c		5	85	222-225	11.71	250	>1000
20	3d		3	80	249-251	62.5	125	1000
21	3e		3	92	247-249	62.5	62.5	500

Entry	Code	Product	Time (h)	Yield (%)	Mp (°C)	MIC (µg/ml) BCG (48 h)	MIC (µg/ml) E.Coli (10 h/16h)	MIC (µg/ml) C. albicans (48 h)
22	3f		4	90	250-252	15.625	62.5/62.5	500
23	3g		4	85	242-244	31.25	62.5/62.5	750
24	3h		4	80	241-243	31.25	250/250	1000
25	4a		1	90	232-235	31.25	1000/1000	375
26	4b		1	85	210-213	31.25	1000/1000	500
27	4c		1	85	215-217	31.25	500/1000	1000
28	4d		1	90	224-227	62.5	250/250	1000
29	4e		1	87	237-240	62.5	250/500	750

Entry	Code	Product	Time (h)	Yield (%)	Mp (°C)	MIC (µg/ml) BCG (48 h)	MIC (µg/ml) E.Coli (10 h/16h)	MIC (µg/ml) C. albicans (48 h)
30	4f		1	80	210-214	7.81	250/500	500
31	4g		1	75	-	15.625	500/500	500
32	4h		1	82	198-200	11.71	1000/1000	500
33	4i		-	-	-	125	-	-
34	4j		-	-	-	125	-	-
35	4k		-	-	-	125	-	-
36	4l		-	-	-	31.25	-	-
37	5a	Amphotericin B	-	-	-	-	-	11.7
38	5b	Fluconazole	-	-	-	-	-	12.5
39	5c	Ethambutol	-	-	-	0.39	-	-
40	5d	Isoniazid	-	-	-	0.39	-	-
41	5e	Streptomycin	-	-	-	-	0.78/1.562	-
42	5f	DMSO	-	-	-	5% v/v	5% v/v	5% v/v

## DISCUSSION

Thiocarbohydrazone derivatives are used to prepare heterocycles and their biological activities are important [21,22,23]. There are many reactions to synthesize thiocarbohydrazone derivatives with organic solvents, but in this study, we used Deep Eutectic Solvents (DES) that is similar to ionic liquids in the organic reactions. Due to low solubility of many drugs in the common solvents, we used DES to improve the dissolution behavior. The FTIR, H-NMR and  $^{13}\text{C}$ -NMR spectra of the synthesized thiocarbohydrazone derivatives were obtained and we found that the related condensation reaction with an aldehyde, was conducted to yield an intermediate compound. This intermediate compound could be generated Schiff base that widely have been used in biological activities [24,25,26]. The synthesis was carried out in one pot and three-component route including thiocarbohydrazide, isatin (2,3 benzopyrrole substituted with oxygen at carbon position 2 and 3) and aldehyde in the presence of DES to yield Schiff base of isatin. We found that the intensity of C=O stretching vibrations at  $1700\text{ cm}^{-1}$ , related to the functional group of the thiocarbohydrazone derivative was weak

and revealed the formation of Schiff base of isatin [27,28].

The MIC values of the synthesized thiocarbohydrazone derivatives showed noticeable activity against *Mycobacterium bovis BCG* and *E.coli*. The results of this study represented noticeable activity for synthesized thiocarbohydrazone derivatives **1a to 1h, 2a to 2h, 3a to 3h** against *Mycobacterium bovis BCG* and for synthesized thiocarbohydrazone derivatives **1d, 2b, 3d to 3g** against *E.coli*. These thiocarbohydrazone derivatives compounds with good electron-withdrawing substitution at the ortho, meta and para positions of benzyl ring demonstrated remarkable activities against *Mycobacterium bovis BCG* and *E.coli*.

## CONCLUSION

We assessed the related antimicrobial activity of the synthesized thiocarbohydrazone compounds in this study. Also we found a relationship between the antimicrobial activity of these compounds and their structures. These compounds were evaluated for their anti-mycobacterial activity against *Mycobacterium bovis BCG*, and the obtained results showed that some of the synthesized compounds (**1a-1h, 2a-2h, 3a-3h**), **3b, 3c** and **1b** had the highest activity with MIC value of 7.81 and 11.71  $\mu\text{g/mL}$ . Regarding to the

structure of the synthesized compounds with electron-withdrawing substitution at the ortho, meta and para positions of benzyl ring, some of them showed remarkable anti-micobacterial activities.

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