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*Original Article*

# Discovery of novel isatin-based thiosemicarbazones: synthesis, antibacterial, antifungal, and antimycobacterial screening

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## *Abstract*

**Background and purpose:** A group of thiosemicarbazones were prepared and their structures were confirmed by spectroscopic methods such as IR and H-NMR, mass spectrometry and also analytical method like elemental analysis. The synthesized semicarbazones were then assessed for their inhibitory activity against bacterial strains including *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Salmonella* species, Enterobacter faecalis, methicillin-resistant *Staphylococcus aureus,* and fungi such as *Candida albicans* and *Aspergillus niger.*

**Experimental approach:** The schiff bases of isatin (**2a-j**) were prepared by a condensation reaction between thiosemicarbazide and substituted N-aryl isatins leading to the desired thiosemicarbazones with exquisite purity.

**Findings / Results:** The results disclosed that all compounds have noticeable inhibitory activity. Compounds **2a**, **2b**, **2c**, **2g**, and **2h** were among the most potent derivatives against Gram negative bacteria and fungi. Besides, the activity of theses compounds were tested against *Mycobacterium bovis* bacillus Calmette-Guerin *(M. bovis* BCG). The antimycobacterial activity indicated compounds **2e** and **2j** are highly active against *M. bovis* BCG (minimum inhibitory concentration < 3.9 µg/mL). Among fluorinated structures, compounds **2a** and **2j** showed the best activities against M. bovis BCG.

**Conclusion and implications:** To sum up, amongst the 10 synthesized compounds, fluorinated derivatives exhibited remarkable activities against both gram negative strains and candida albicans microorganism. Therefore, they should be considered as a clue for further modifications in next investigations. Furthermore, inserting a small/medium size halogen atom with electron-withdrawing and lipophilic properties increases anti-salmonella activity of these compounds and moreover 2-halogenated semithiocarbazones presented promising antimycobacterial activity.

***Keywords:*** Antibacterial agents; Antimycobacterial agents; Isatin; Synthesis, Thiosemicarbazone.

## INTRODUCTION

Isatin analogues have a multiplicity of biological properties such as antifungal (1,2), antibacterial (3,4), antimycobacterial (5-7), analgesic (8), anti-HIV (9,10), anticonvulsant (11), anticancer (12), and antioxidant activity (13). The varied biological activities of isatin derivatives have led to their increasingly comprehensive use as precursors for the synthesis of numerous biologically active compounds. On the other hand, thiosemicarbazone moiety is another privileged structure that is found in several molecules with wide range of biological activities representing several important classes in drug discovery.

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Thiosemicarbazones have been considered for medicinal studies due to their broad range of biological activities including antineoplastic (14), antimycobacterial (15), antibacterial (16), antifungal (17), analgesic, and anticonvulsant effects (18). Also, the flexibility of thiosemicarbazones as nitrogen and sulfur donors consenting them to bring on a great diversity of coordination modes (19).

One of the most prevalent and serious infectious diseases is tuberculosis that happenes mostly by *Mycobacterium tuberculosis (M. Tuberculosis)*. Since there is still no great advance to fight this disease, a serious need exists to produce new antimycobacterial drugs with enhanced features such as increased activity against multidrug resistance with less toxicity. Thiacetazone is an antibiotic which is used in the treatment of tuberculosis in combination with other antimycobacterial drugs like isoniazid (20). Structure activity relationship studies of thiacetazone revealed that the thiosemicarbazone part is an important core for antitubercular activity.

Based on the above considerations and as a continuous work of our previous study (21), we have investigated a series of N-substituted isatin derivatives in combination with thiosemicarbazone moiety to increase the possibility of their antibacterial, antifungal, and antimycobacterial activities. The isatin-thiosemicarbazone hybrids could reveal a promising activity against pathogenic microbial strains.

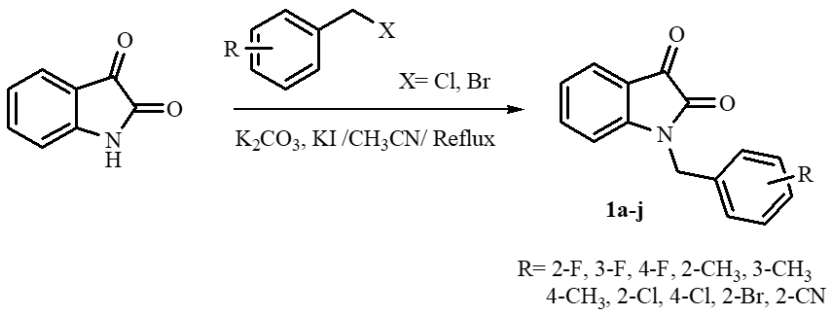
To simplify the antimycobacterial test, bacillus Calmette-Guerin (BCG) was used instead of *M. tuberculosis*. BCG is a weakened strain of *M. bovis* that is not virulent bacillus and is similar to *M. tuberculosis* (22). Hence, the utilization of *M. bovis* is easier that reduces strict biosafety regulations in the labs, therefore, it would be applied in bioassay as an alternative of *M. tuberculosis.* To this end, in this study some N-substituted isatin derivatives bearing thiosemicarbazone moiety were synthesized and assessed against eight types of bacteria, two types of fungi, and *M. bovis.*

## MATERIALS AND METHODS

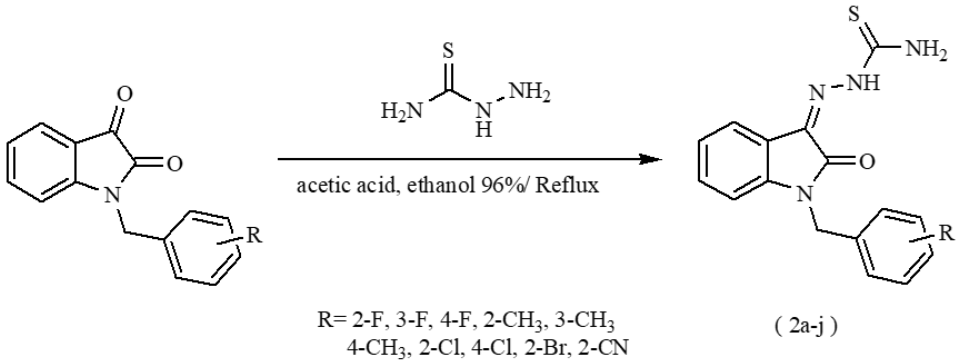
All the solvents and chemicals were purchased from Merck-Millipore Company in Germany. Measurement of the melting points was performed by an Electrothermal 9200 apparatus (United Kingdom) and was uncorrected. The infrared (IR) spectra were attained by a Bruker Tensor27 spectrophotometer and potassium bromide was used as the diluent. Proton nuclear magnetic resonance ($^1$H-NMR) spectra were measured in a 400 MHz Bruker Avance DRX spectrometer that TMS (tetramethylsilane) was the internal standard in this process. Deuterated dimethyl sulfoxide ($d_6$-DMSO) was the solvent to dissolve the synthesized compounds. The mass spectra were obtained by Agilent 6410 LC/MS/MS (USA). The compounds were analyzed for C, H, S, N by a Costech model (4010) and the achieved values were consistent with the suggested structures within ± 0.4% of the theoretical values.

### *General procedure for the synthesis of N-arylisatins (1a-j)*

A mixture of isatin (294 mg, 2 mmol), $K_2CO_3$ (332 mg, 2.4 mmol), and KI (66 mg, 0.4 mmol) in acetonitrile (15 mL) were stirred for 10 min. Afterward, substituted benzyl halides (3 mmol) were added dropwise. The reaction mixture was heated at 80 °C for 4 h and then it was cooled to 20 °C and filtered off. The filtrate was dried with rotary evaporator and the remained crude was recrystallized in water to obtain the titled compounds. This procedure is illustrated in Scheme 1.




**Scheme 1.** Synthesis of N-arylisatins (**1a-j**), reagents, and conditions are described.




**Scheme 2.** Synthesis of thiosemicarbazones (**2a-j**), reagents, and conditions are described.

***General procedure for the synthesis of thiosemicarbazones (2a-j)***

To the same quantities of thiosemicarbazide and N-arylisatins (1 mmol of each), ethanol 96% (20 mL) was added containing 8 drops of glacial acetic acid. The reaction mixture was heated at 90 °C for 6 h and then it was cooled to 20 °C. The subsequent crude was filtered, washed with methanol, and dried. The final prepared derivatives had adequate purity. This procedure is presented in Scheme 2.

***in vitro evaluation of antibacterial and antifungal activities***

Ten mentioned prepared derivatives were evaluated for antimicrobial activity against standard indicator bacteria and fungi by microbroth dilution assay as described in our perivous study (23). *Staphylococcus aureus* PTCC 1337, *S. epidermidis* PTCC 1435, *Bacillus cereus* PTCC 1015, *Escherichia coli* PTCC 1330, *Pseudomonas aeruginosa* PTCC 1310, *Enterococcus faecalis* PTCC 13294, *salmonella* spp., *Methicillin Resistant S. aureus* PTCC, *Candida albicans* PTCC 5027 and *Aspergillus niger* PTCC 5012 were selected as indicator microorganisms. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of synthesized compounds were compared with teicoplanin, amikacin, and amphotericin B as standard antibiotics

***in vitro evaluation of anti mycobacterial activity***

The method for evaluation of antimycobacterial activity was broth microtiter dilution that was performed with BCG (1173P2). To control this process, ethambutol was used as a standard drug. First, a concentration of 1 µg/mL of each compound was prepared in DMSO. Then, 100 µL of synthesized compounds with selected concentrations was added to the wells of microplates in which 100 µL middle broke 7H9 medium had already been added. Different concentrations of each compounds were prepared by serial dilution in the wells. After introducing BCG suspension (100 µL) to wells, microplates were incubated for 4 days at 37 °C. To finalize the process, Tween® 80 (10%, 12 µL) and Alamar Blue® (0.01%, 20 µL) were added to each well. The results were measured after 24 and 48 h (24).

## RESULTS

***Chemistry***

Prediction of drug-likeness was performed by the calculation of molecular properties of the compounds related to the Lipinski's rule (25) which is listed in Table 1. The physicochemical properties were measured by online Toolkit of Molinspiration (https://www.molinspiration.com /cgi-bin/properties).

The structures of final compounds were confirmed using IR, NMR, and mass spectrometry; the elemental analysis and their characterization data are presented below.

*1-(2-fluorobenzyl)indoline-2,3-dione (**1a**)*

Recrystallization solvent: ethanol; yield: 78%; melting point: 148-150 °C; IR (KBr, $\upsilon_{max}$/cm): 767, 1031, 1734 $cm^{-1}$; ($M+H^+$): 256; elemental analysis calculated for $C_{15}H_{10}FNO_2$ (255.07): C, 70.58; H, 3.95; F, 7.44; N, 5.49; O, 12.54. Observed: C, 70.54; H, 3.97; F, 7.41; N, 5.47; O, 12.55.

**Table 1.** Calculated molecular properties related to the Lipinski's Rule of Five.

| Compound | R | logP [a] | HBD [b] | HBA [c] | MW [d] |
|---|---|---|---|---|---|
| **2a** | 2-F | 2.38 | 2 | 2 | 328.36 |
| **2b** | 3-F | 2.38 | 2 | 2 | 328.36 |
| **2c** | 4-F | 2.38 | 2 | 2 | 328.36 |
| **2d** | 2-$CH_3$ | 2.71 | 2 | 2 | 324.40 |
| **2e** | 3-$CH_3$ | 2.71 | 2 | 2 | 324.40 |
| **2f** | 4-$CH_3$ | 2.71 | 2 | 2 | 324.40 |
| **2g** | 2-Cl | 2.78 | 2 | 2 | 344.82 |
| **2h** | 4-Cl | 2.78 | 2 | 2 | 344.82 |
| **2i** | 2-Br | 3.05 | 2 | 2 | 389.27 |
| **2j** | 2-CN | 2.26 | 2 | 3 | 335.38 |

a, logarithm of the octanol/water partition coefficient; b, Number of hydrogen-bond donor atoms; c, Number of hydrogen-bond acceptor atoms; d, molecular weight.

*1-(3-fluorobenzyl)indoline-2,3-dione (**1b**)*

Recrystallization solvent: ethanol; yield: 75%; melting point: 149-151 °C; IR (KBr, $\upsilon_{max}$/cm): 751, 1030, 1732 $cm^{-1}$; ($M+H^+$): 256; elemental analysis calculated for $C_{15}H_{10}FNO_2$ (255.07): C, 70.58; H, 3.95; F, 7.44; N, 5.49; O, 12.54. Observed: C, 70.56; H, 3.95; F, 7.43; N, 5.50; O, 12.57.

*1-(4-fluorobenzyl)indoline-2,3-dione (**1c**)*

Recrystallization solvent: ethanol; yield: 69%; melting point: 152-154 °C; IR (KBr, $\upsilon_{max}$/cm): 847, 1011, 1740 $cm^{-1}$; ($M+H^+$): 256; elemental analysis calculated for $C_{15}H_{10}FNO_2$ (255.07): C, 70.58; H, 3.95; F, 7.44; N, 5.49; O, 12.54. Observed: C, 70.52; H, 3.96; F, 7.42; N, 5.47; O, 12.54.

*1-(2-methylbenzyl)-indoline-2,3-dione (**1d**)*

Recrystallization solvent: ethanol; yield: 81%; melting point: 142-144 °C; IR (KBr, $\upsilon_{max}$/cm): 757, 1739 $cm^{-1}$; ($M+H^+$): 252; elemental analysis calculated for $C_{16}H_{13}NO_2$ (251.09): C, 76.48; H, 5.21; N, 5.57; O, 12.73. Observed: C, 76.45; H, 5.20; N, 5.59; O, 12.71.

*1-(3-methylbenzyl)-indoline-2,3-dione (**1e**)*

Recrystallization solvent: ethanol; yield: 83%; melting point: 140-142 °C; IR (KBr, $\upsilon_{max}$/cm): 762, 1730 $cm^{-1}$; ($M+H^+$): 252; elemental analysis calculated for $C_{16}H_{13}NO_2$ (251.09): C, 76.48; H, 5.21; N, 5.57; O, 12.73. Observed: C, 76.49; H, 5.22; N, 5.55; O, 12.70.

*1-(4-methylbenzyl)-indoline-2,3-dione (**1f**)*

Recrystallization solvent: ethanol; yield: 75%; melting point: 144-146 °C; IR (KBr, $\upsilon_{max}$/cm): 846, 1733 $cm^{-1}$; ($M+H^+$): 252; elemental analysis calculated for $C_{16}H_{13}NO_2$ (251.09): C, 76.48; H, 5.21; N, 5.57; O, 12.73. Observed: C, 76.48; H, 5.23; N, 5.58; O, 12.70.

*1-(2-chlorobenzyl)-indoline-2,3-dione (**1g**)*

Recrystallization solvent: ethanol; yield: 83%; melting point: 176-178 °C; IR (KBr, $\upsilon_{max}$/cm): 598, 753, 1738 $cm^{-1}$; ($M+H^+$): 272; elemental analysis calculated for $C_{15}H_{10}ClNO_2$ (271.04): C, 66.31; H, 3.71; Cl, 13.05; N, 5.16; O, 11.78. Observed: C, 66.29; H, 3.72; Cl, 13.08; N, 5.18; O, 11.75.

*1-(4-chlorobenzyl)-indoline-2,3-dione (**1h**)*

Recrystallization solvent: ethanol; yield: 85%; melting point: 177-179 °C; IR (KBr, $\upsilon_{max}$/cm): 683, 848, 1739 $cm^{-1}$; ($M+H^+$): 272; elemental analysis calculated for $C_{15}H_{10}ClNO_2$ (271.04): C, 66.31; H, 3.71; Cl, 13.05; N, 5.16; O, 11.78. Observed: C, 66.30; H, 3.71; Cl, 13.09; N, 5.17; O, 11.81.

*1-(2-bromobenzyl)-indoline-2,3-dione (**1i**)*

Recrystallization solvent: ethanol; yield: 76%; melting point: 197-199 °C; IR (KBr, $\upsilon_{max}$/cm): 555, 759, 1167, 1738 $cm^{-1}$; ($M+H^+$): 316; elemental analysis calculated for $C_{15}H_{10}BrNO_2$ (314.99): C, 56.99; H, 3.19; Br, 25.27; N, 4.43; O, 10.12. Observed: C, 57.02; H, 3.20; Br, 25.24; N, 4.45; O, 10.13.

*1-(2-cyanobenzyl)-indoline-2,3-dione (**1j**)*

Recrystallization solvent: ethanol; yield: 81%; melting point: 187-189 °C; IR (KBr, $\upsilon_{max}$/cm): 757, 1346, 1740 $cm^{-1}$; ($M+H^+$): 263; elemental analysis calculated for $C_{16}H_{10}N_2O_2$ (262.07): C, 73.27; H, 3.84; N, 10.68; O, 12.20. Observed: C, 73.29; H, 3.83; N, 10.70; O, 12.20.

*1-(1-(2-fluorobenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2a**)*

Recrystallization solvent: acetonitrile; yield: 56%; melting point: 234- 236 °C; IR (KBr, $\upsilon_{max}$/cm): 748, 1032, 1604, 1681, 3175, 3273, 3420 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 5.05 (s, 2H, $CH_2$) , 7.03 (d, 1H, $J$ = 8 Hz, isatin-H), 7.17 (t, 2H, $J$ = 7.6 Hz, benzyl-H), 7.25 (t, 1H, $J$ = 8 Hz, isatin-H), 7.28-7.42 (m, 3H, aryl-H), 7.45 (d, 1H, $J$ = 7.2 Hz, isatin-H) , 8.79 (s, 1H ,$NH_2$), 9.13 (s, 1H, $NH_2$), 12.38 (s, 1H, NH); ($M+H^+$): 329; elemental analysis calculated for $C_{16}H_{13}FN_4OS$ (328.08): C, 58.52; H, 3.99; F, 5.79; N, 17.06; O, 4.87; S, 9.77. Observed: C, 58.50; H, 4.01; F, 5.79; N, 17.09; O, 4.88; S, 9.75.

*1-(1-(3-fluorobenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2b**)*

Recrystallization solvent: acetonitrile; yield: 58%; melting point: 230-232 °C; IR (KBr, $\upsilon_{max}$/cm): 792, 1029, 1604, 1678, 3171, 3268, 3415 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 5.05 (s, 2H, $CH_2$), 7.03 (d, 1H, $J$ = 8 Hz, isatin-H), 7.17 (t, 2H, $J$ = 7.6 Hz, benzyl-H), 7.25 (t, 1H, $J$ = 8 Hz, isatin-H), 7.27-7.42 (m, 3H, aryl-H) , 7.75 (d, 1H, $J$ = 6.8 Hz, isatin-H), 8.78 (s, 1H, $NH_2$), 9.13 (s, 1H, $NH_2$), 12.39 (s, 1H, NH); ($M+H^+$): 329; elemental analysis calculated for $C_{16}H_{13}FN_4OS$ (328.08): C, 58.52; H, 3.99; F, 5.79; N, 17.06; O, 4.87; S, 9.77. Observed: C, 58.51; H, 3.98; F, 5.80; N, 17.08; O, 4.85; S, 9.79.

*1-(1-(4-fluorobenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2c**)*

Recrystallization solvent: acetonitrile; yield: 60%; melting point: 238- 240 °C; IR (KBr, $\upsilon_{max}$/cm): 841, 1016, 1587, 1690, 3276, 3341, 3459 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 4.98 (s, 2H, $CH_2$), 7.07 (d, 1H, $J$ = 7.6 Hz, isatin-H) , 7.13-7.20 (m, 3H, Aryl-H) , 7.38 (dt, 1H, $J_1$ = 7.6 Hz, $J_2$ = 1.2 Hz, isatin-H), 7.45-7.48 (m, 2H, Benzyl-H), 7.74 (d, 1H, $J$ = 6.8 Hz, isatin-H), 8.77 (s, 1H, $NH_2$), 9.12 (s, 1H, $NH_2$), 12.41 (s, 1H, NH); ($M+H^+$): 329; elemental analysis calculated for $C_{16}H_{13}FN_4OS$ (328.08): C, 58.52; H, 3.99; F, 5.79; N, 17.06; O, 4.87; S, 9.77. Observed: C, 58.54; H, 4.00; F, 5.79; N, 17.07; O, 4.88; S, 9.75.

*1-(1-(2-methylbenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2d**)*

Recrystallization solvent: acetonitrile; yield: 55%; melting point: 232-234 °C; IR (KBr, $\upsilon_{max}$/cm): 748, 1602, 1677, 3175, 3282, 3431 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 2.40 (s, 3H, $CH_3$), 4.98 (s, 2H, $CH_2$) , 7.04 (d, 1H, $J$ = 7.6 Hz, isatin-H) , 7.10-7.22 (m, 3H, benzyl-H), 7.36 (dt, 1H, $J_1$ = 8 Hz, $J_2$ = 1.2 Hz, isatin-H), 7.77 (d, 1H, $J$ = 7.2 Hz, isatin-H), 8.77 (s, 1H, $NH_2$), 9.13 (s, 1H, $NH_2$), 12.39 (s, 1H, NH); ($M+H^+$): 325; elemental analysis calculated for $C_{17}H_{16}N_4OS$ (324.1): C, 62.94; H, 4.97; N, 17.27; O, 4.93; S, 9.88. Observed: C, 62.97; H, 4.98; N, 17.24; O, 4.94; S, 9.90.

*1-(1-(3-methylbenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2e**)*

Recrystallization solvent: acetonitrile; yield: 54%; melting point: 240- 242 °C; IR (KBr, $\upsilon_{max}$/cm): 787, 1599, 1692, 3153, 3251, 3388 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 2.28 (s, 3H, $CH_3$), 4.95 (s, 2H, $CH_2$), 7.04 (d, 1H, $J$ = 7.6 Hz, Benzyl-H), 7.10-7.26 (m, 4H, Aryl-H), 7.37 (dt, 1H, $J_1$ = 8Hz, $J_2$ = 1.2 Hz isatin-H), 7.4 (d, 1H, $J$ = 8 Hz, isatin-H), 8.78 (s, 1H, $NH_2$), 9.12 (s, 1H, $NH_2$), 12.43 (s, 1H, NH); ($M+H^+$): 325; elemental analysis calculated for $C_{17}H_{16}N_4OS$ (324.1): C, 62.94; H, 4.97; N, 17.27; O, 4.93; S, 9.88. Observed: C, 62.95; H, 4.98; N, 17.28; O, 4.95; S, 9.86.

*1-(1-(4-methylbenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2f**)*

Recrystallization solvent: acetonitrile; yield: 61%; melting point: 242-244 °C; IR (KBr, $\upsilon_{max}$/cm): 826, 1603, 1681, 3175, 3272, 3408 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 2.27 (s, 3H, $CH_3$), 4.94 (s, 2H, $CH_2$), 7.04 (d, 1H, $J$ = 8 Hz, isatin-H), 7.14 (t, 2H, $J$ = 8 Hz, isatin-H), 7.29 (d, 1H, $J$ = 8Hz, isatin-H), 7.37 (dt, 1H, $J_1$ = 8 Hz, $J_2$ = 1.2 Hz, isatin-H), 7.73 (d, 1H, $J$

= 7.2 Hz, isatin-H), 8.76 (s, 1H, $NH_2$), 9.13 (s, 1H, $NH_2$), 12.43 (s, 1H, NH); ($M+H^+$): 325; elemental analysis calculated for $C_{17}H_{16}N_4OS$ (324.1): C, 62.94; H, 4.97; N, 17.27; O, 4.93; S, 9.88. Observed: C, 62.93; H, 4.97; N, 17.30; O, 4.96; S, 9.87.

*1-(1-(2-chlorobenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2g**)*

Recrystallization solvent: acetonitrile; yield: 56%; melting point: 234- 236˚C; IR (KBr, $\upsilon_{max}$/cm): 654, 738, 1611, 1685, 3160, 3258, 3402 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 5.00 (s, 2H, $CH_2$), 7.04 (d, 1H, $J$ = 7.6 Hz, isatin-H) , 7.16 (t, 1H, $J$ = 8 Hz, benzyl-H), 7.35-7.45 (m, 5H, aryl-H), 7.74 (d, 1H, $J$ = 7.6 Hz, isatin-H), 8.79 (s, 1H, $NH_2$), 9.13 (s, 1H, $NH_2$), 12.40 (s, 1H, NH); ($M+H^+$): 345; elemental analysis calculated for $C_{16}H_{13}ClN_4OS$ (344.05): C, 55.73; H, 3.80; Cl, 10.28; N, 16.25; O, 4.64; S, 9.30. Observed: C, 55.76; H, 3.81; Cl, 10.31; N, 16.22; O, 4.62; S, 9.31.

*1-(1-(4-chlorobenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2h**)*

Recrystallization solvent: acetonitrile; yield: 59%; melting point: 242- 244 °C; IR (KBr, $\upsilon_{max}$/cm): 633, 833, 1600, 1681, 3172, 3276, 3435 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 5.06 (s, 2H, $CH_2$), 6.93 (d, 1H, $J$ = 8 Hz, isatin-H), 7.18 (t, 1H, $J$ = 8 Hz, benzyl-H), 7.25-7.40 (m, 4H, Aryl-H) , 7.45 (d, 1H, $J$ = 8 Hz, benzyl-H) , 7.78 (d, 1H, $J$ = 7.2Hz, isatin-H), 8.18 (s, 1H, $NH_2$), 9.14 (s, 1H, $NH_2$), 12.36 (s, 1H, NH); ($M+H^+$): 345; elemental analysis calculated for $C_{16}H_{13}ClN_4OS$ (344.05): C, 55.73; H, 3.80; Cl, 10.28; N, 16.25; O, 4.64; S, 9.30. Observed: C, 55.77; H, 3.82; Cl, 10.27; N, 16.24; O, 4.60; S, 9.28.

*1-(1-(2-bromobenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2i**)*

Recrystallization solvent: acetonitrile; yield: 60%; melting point: 247-249 °C; IR (KBr, $\upsilon_{max}$/cm): 521, 753, 1687, 3143, 3244, 3411 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 5.01 (s, 2H, $CH_2$), 6.89 (d, 1H, $J$ = 8 Hz, isatin-H), 7.16-7.39 (m, 5H, aryl-H), 7.71 (dd, 1H, $J_1$ = 8 Hz, $J_2$ = 1.2 Hz, benzyl-H), 7.78 (d, 1H, $J$ = 7.2 Hz, isatin-H), 8.81 (s, 1H, $NH_2$), 9.14 (s, 1H, $NH_2$), 12.36 (s, 1H, NH); ($M+H^+$): 389; elemental analysis calculated for $C_{16}H_{13}BrN_4OS$ (388): C, 49.37; H, 3.37; Br, 20.53; N, 14.39; O, 4.11; S, 8.24. Observed: C, 49.39; H, 3.37; Br, 20.57; N, 14.41; O, 4.13; S, 8.22.

*1-(1-(2-cyanobenzyl)-2-oxoindolin-3-ylidene)-thiosemicarbazide (**2j**)*

Recrystallization solvent: acetonitrile; yield: 55%; melting point: 245- 247 °C; IR (KBr, $\upsilon_{max}$/cm): 750, 1347, 1586, 1688, 3241, 3360, 3454 $cm^{-1}$; H-NMR in DMSO-d6 (400 MHz): $\delta$ 5.20 (s, 2H, $CH_2$), 6.96 (d, 1H, $J$ = 8 Hz, isatin-H), 7.18 (t, 1H, $J$ = 7.6 Hz, isatin-H), 7.38 (dt, 1H, $J_1$ = 7.6 Hz , $J_2$ = 1.2 Hz, isatin-H), 7.46-7.55 (m, 2H, benzyl-H), 7.68 (dt, 1H, $J_1$ = 7.6Hz, $J_2$ = 1.2Hz, isatin-H), 7.78 (d, 1H, $J$ = 7.2 Hz, isatin-H), 7.92 (dd, 1H , $J_1$ = 7.6Hz, $J_2$ = 1.2 Hz, benzyl-H), 8.80 (s, 1H, $NH_2$), 9.14 (s, 1H, $NH_2$), 12.36 (s, 1H, NH); ($M+H^+$): 336; elemental analysis calculated for $C_{17}H_{13}N_5OS$ (335.08): C, 60.88; H, 3.91; N, 20.88; O, 4.77; S, 9.56. Observed: C, 60.85; H, 3.90; N, 20.89; O, 4.78; S, 9.55.

### *Antimicrobial and antimycobacterial evaluation*

MIC all the synthesized compounds were assessed for antimycobacterial activity and the results are summarized in Table 2.

The synthesized isatin derivatives have been screened for *in vitro* antimicrobial activities against ten microorganisms including Gram positive, Gram negative, and two types of fungi. The MIC and MBC of novel compounds are presented in Table 3.

**Table 2**. Evaluation of antimycobacterial activity data of tested compounds against bacillus Calmette-Guerin.

| Compounds | MIC (µg/mL) | |
|---|---|---|
| | **48 (h)** | **72 (h)** |
| **2a** | < 3.9 | < 3.9 |
| **2b** | 500 | 500 |
| **2c** | 250 | 500 |
| **2d** | 500 | 500 |
| **2e** | 500 | 500 |
| **2f** | 500 | 500 |
| **2g** | 500 | 500 |
| **2h** | 500 | 500 |
| **2i** | 500 | 500 |
| **2j** | < 3.9 | < 3.9 |
| Ethambutol | 0.39 | 0.39 |

**Table 3.** Evaluation of antimicrobial activities (MIC, µg/mL; and MBC, µg/mL) of isatin derivatives.

| Compounds | Staphylococcus aureus | | Staphylococcus epidermidis | | Escherichia coli | | Salmonella species | | Bacillus cereus | | Enterobacter faecalis | | Pseudomonas aeruginosa | | MRSA | | Candida albicans | | Aspergillus niger | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** |
| **2a** | 70 | 80 | 70 | NA | 80 | 80 | 50 | 80 | 80 | 80 | 70 | NA | 70 | 80 | 70 | 80 | 30 | 50 | 40 | NA |
| **2b** | 70 | 80 | 70 | NA | 80 | 80 | 50 | 80 | 80 | 80 | 70 | NA | 70 | 80 | 70 | 80 | 30 | 50 | 40 | NA |
| **2c** | 70 | 80 | 70 | NA | 80 | 80 | 50 | 80 | 80 | 80 | 70 | NA | 70 | 80 | 70 | 80 | 30 | 50 | 40 | NA |
| **2d** | 70 | 80 | 70 | NA | 80 | 80 | 70 | 100 | 80 | 80 | 70 | NA | 80 | 100 | 70 | 100 | 60 | 80 | 70 | NA |
| **2e** | 70 | 80 | 70 | NA | 80 | 80 | 70 | 100 | 80 | 80 | 70 | NA | 80 | 100 | 70 | 100 | 60 | 80 | 70 | NA |
| **2f** | 70 | 80 | 70 | NA | 80 | 80 | 70 | 100 | 80 | 80 | 70 | NA | 80 | 100 | 70 | 100 | 60 | 80 | 70 | NA |
| **2g** | 70 | 80 | 70 | NA | 80 | 80 | 60 | 80 | 80 | 80 | 70 | NA | 80 | 80 | 70 | 80 | 30 | 60 | 50 | NA |
| **2h** | 70 | 80 | 70 | NA | 80 | 80 | 60 | 80 | 80 | 80 | 70 | NA | 80 | 80 | 70 | 80 | 30 | 60 | 50 | NA |
| **2i** | 70 | 80 | 70 | NA | 80 | 80 | 70 | 80 | 80 | 80 | 70 | NA | 80 | 80 | 70 | 80 | 50 | 80 | 60 | NA |
| **2j** | 70 | 80 | 70 | NA | 80 | 80 | 70 | NA | 80 | 80 | 70 | NA | 80 | NA | 70 | NA | 60 | 80 | 60 | NA |
| **Amikacin** | 31 | 31 | 31 | 31 | 31 | 31 | 15 | 15 | 31 | 31 | 15 | NA | 15 | 15 | 31 | 31 | - | - | - | - |
| **Teicoplanin** | 7.8 | 7.8 | 7.8 | 7.8 | - | - | - | - | - | - | 3.6 | 3.6 | - | - | 31 | 31 | - | - | - | - |
| **Amphotericin** | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | 0.078 | 0.078 | 0.049 | 0.049 |

MIC, Minimum inhibitory concentration; MBC, minimum bactericidal concentration; MRSA, methicillin resistant Staphylococcus aureus; NA, no activity; symbol (-), not tested,

## DISCUSSION

As described in study conducted by Kalhor *et al.* (26), the synthesis of *N*-benzylated of isatin with different substitutions was done in acetonitrile including potassium carbonate as a weak base (Scheme 1). The final thiosemicarbazone derivatives were prepared through reacting N-substituted isatins with thiosemicarbazide (Scheme 2). The final products had good yields and adequate purity. In the $^{1}$H-NMR spectra of the compounds, the exchangeable hydrogens of the thiosemicarbazone moiety appeared at 8.79, 9.13, and 12.38 ppm. The absence of N-H (isatin ring) is a confirmation for the synthesis of the final products. In the IR spectra, omitting the signal of carbonyl absorptions on site 3 of isatin ring confirmed the preparation of the final thiosemicarbazones as well. The mass of synthesized compounds was also confirmed by electrospray ionization mass spectrometry / mass spectrometry as $M+H^{+}$. The antibacterial results revealed that all compounds are promisingly active against indicator bacteria. Synthesized isatins **2a**, **2b**, **2c**, **2g**, and **2h** showed noticeable activity against gram negative bacteria and fungi.

As expected, the MIC for all compounds against Gram positive and Gram negative bacteria revealed almost the same values due to their structural similarity. The notable point is that the fluorinated compounds **2a**, **2b**, and **2c** which have the lowest MIC against one problematic pathogen, *Pseudomonas aeruginosa*, displayed the close values of MIC and MBC describing the potency of these compounds and necessity of further studies in future.

Beside, all the synthesized compounds were assessed for antimycobacterial activity. Surprisingly, compounds **2a** and **2j** with good electron-withdrawing substitution at the ortho position of benzyl ring demonstrated remarkable activities against *M. bovis* BCG with MIC values comparable with that of isoniazid used as a standard drug. The *in vitro* antimicrobial activity of the isatin derivatives was evaluated against eight clinically important bacterial strains of both Gram positive and Gram negative and also two types of fungi (Table 3). Despite the acceptable antibacterial effects of all derivatives, the antibacterial results obtained were very similar excluding against two pathogen microorganisms, *Salmonella* spp. and *Pseudomonas aeruginosa.* This trend is noticeable for the fluorinated derivatives that showed higher activity against those two mentioned strains. For other strains, it suggests the position and electronic properties of the substitutions on the benzylic ring did not affect the antibacterial activity.

In connection with the results of our previous study (21) which showed that only hydantoin moiety was tolerated in isatin *N*-fluorobenzyl derivatives, in the present work, same fluorinated benzylic substituents when hybridized with thiosemicarbazone moiety revealed noticeable antibacterial activity.

Regarding the antifungal activities (MIC), the test compounds displayed more promising effects on *Candida albicans* compared to *Aspergillus niger*.

According to the previous study (16) which considered the synthesized thiosemicarbazone derivatives based on thiacetazone, the third-line drug of tuberculosis treatment, we also evaluated the antimycobacterial activity of our derivatives. Hopefully, two compounds **2a** and **2j** disclosed considerable activities with MIC values similar to that of thiacetazone.

## CONCLUSION

We assessed the antimicrobial activity of novel isatin derivatives which more fulfilled our previous data and encouraged us for further lead optimization studies. Amongst the ten synthesized compounds, fluorinated derivatives exhibited remarkable activities against both Gram negative strains and *Candida albicans* microorganism. This data is valuable because two Gram negative pathogen microorganisms those were sensitive to our compounds were *Salmonella* spp. and *P. aeruginosa* that usually cause serious infections in human body.

The fluorinated compounds are worth to be considered because two of them (**2a** and **2j)** showed the promising activities against *M. bovis* BCG as well. It is an important finding in terms of conducting new lead optimization studies to discover new isatin-

thiosemicarbazone derivatives with improved antibacterial and antimycobacterial activities simultaneously. Although it is impossible for now to make a relationship between the antitubercular activities of these two compounds and their structures, they should be considered as the clue for further modifications in antituberculosis investigations. It should be noted that all chemical structures in this research have been synthesized for the first time and this novelty makes us to optimize these compounds for better activity in our future studies.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest for this study.

## AUTHORS' CONTRIBUTION

Sh. Mohebbi designed the chemical structures and conducted the synthesis of the derivatives. R. Ghaffari synthesized the derivatives thesis. M. Hassan conducted the antibacterial and antifungal evaluations. S. Sardari and Y. Farmahini Farahani did the antimycobacterial test at Pasteur institute of Iran.

## REFERENCES

1. Raj AA, Raghunathan R, Sridevikumaria MR, Raman N. Synthesis, antimicrobial and antifungal activity of a new class of spiro pyrrolidines. Bioorg Med Chem. 2003;11:407-419. DOI: 10.1016/s0968-0896(02)00439-x.
2. Rodriguez-Arguelles MC, Mosquera-Vazquez S, Touron-Touceda P, Sanmartin-Matalobos J, Garcia-Deibe AM, Belicchi-Ferraris M, *et al*. Complexes of 2-thiophenecarbonyl and isonicotinoyl hydrazones of 3-(*N*-methyl)isatin: a study of their antimicrobial activity. J Inorg Biochem. 2007;101(1):138-147. DOI: 10.1016/j.jinorgbio.2006.09.004.
3. Pakravan P, Kashanian S, Khodaei MM, Harding FJ. Biochemical and pharmacological characterization of isatin and its derivatives: from structure to activity. Pharmacol Rep. 2013;65(2):313-335. DOI: 10.1016/s1734-1140(13)71007-7.
4. Mohebbi S, Hassan M, Gorji E. Preparation and evaluation of antibacterial and antifungal effects of novel isatin and indole derivatives bearing a hydantoin moiety. J Adv Med Biomed Res. 2017;25(113):113-121.
5. Tran VH, Nguyen QD, Le NV. Study on the antituberculosis effect of some thiosemicarbazones and isonicotinyl hydrazone derivatives of isatin and 5-haloisatin. Tap Chi Dou Hoc. 2002;8:15-17.
6. Hussein MA, Aboul-Fadl T, Hussein A. Synthesis and antitubercular activity of mannich bases derived from isatin, isonicotinic acid hydrazone. Pharm Sci. 2005;28(1):131-136. DOI: 10.21608/BFSA.2005.65240.
7. Aboul-Fadl T, Bin-Jubair S. Anti-tubercular activity of isatin derivatives. Int J Res Pharm Sci. 2010;1(2):113-126.
8. Figueiredo GS, Zardo RS, Silva BV, Violante FA, Pinto AC, Fernandes PD. Convolutamydine A and synthetic analogues have antinociceptive properties in mice. Pharmacol Biochem Behav. 2013;103(3):431-439. DOI: 10.1016/j.pbb.2012.09.023.
9. Sriram D, Yogeeswari, Meena K. Synthesis, anti-HIV and antitubercular activities of isatin derivatives. Pharmazie. 2006;61(4):274-277. DOI: 10.1002/chin.200629154.
10. Banerjee D, Yogeeswari P, Bhat P, Thomas A, Srividya M, Sriram D. Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection. Eur J Med Chem. 2011;46(1):106-121. DOI: 10.1016/j.ejmech.2010.10.020.
11. Verma M, Pandeya SN, Singh KN, Stables JP. Anticonvulsant activity of Schiff bases of isatin derivatives. Acta Pharm. 2004;54(1):49-56.
12. Taher AT, Khalil NA, Ahmed EM. Synthesis of novel isatin-thiazoline and isatin-benzimidazole conjugates as anti-breast cancer agents. Arch Pharm Res. 2011;34(10):1615-1621. DOI: 10.1007/s12272-011-1005-3.
13. Andreani A, Burnelli S, Granaiola M, Leoni A, Locatelli A, Morigi R, *et al*. New isatin derivatives with antioxidant activity. Eur J Med Chem. 2010;45(4):1374-1378. DOI: 10.1016/j.ejmech.2009.12.035.
14. Singh NK, Srivastava A, Sodhi A, Ranjan P. *In vitro* and *in vivo* antitumor studies of a new thiosemicarbazide derivative and its complexes with 3d-metal ions. Trans Met Chem. 2000;25:133-140. DOI: 10.1023/A:1007081218000.
15. Shahlaei M, Fassihi A, Nezami A. QSAR study of some 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione-3-thiosemicarbazone derivatives as anti-tubercular agents. Res Pharm Sci. 2009;4(2):123-131.
16. Haj Mohammad Ebrahim Tehrani K, Kobarfard F, Azerang P, Mehravar M, Soleimani Z, Ghavami G, *et al*. Synthesis and antimycobacterial activity of symmetric thiocarbohydrazone derivatives against mycobacterium bovis BCG. Iranian J Pharm Res. 2013;12(2):331-346.

17. Altıntop MD, Atlı O, Ilgın S, Demirel R, Ozdemir A, Kaplancıklı ZK. Synthesis and biological evaluation of new naphthalene substituted thiosemicarbazone derivatives as potent antifungal and anticancer agents. Eur J Med Chem. 2016;108:406-414. DOI: 10.1016/j.ejmech.2015.11.041.
18. Aly MM, Mohamed YA, El-Bayouki KA, Basyouni WM, Abbas SY. Synthesis of some new 4(3*H*)-quinazolinone-2-carboxaldehyde thiosemicarbazones and their metal complexes and a study on their anticonvulsant, analgesic, cytotoxic and antimicrobial activities, part-1. Eur J Med Chem. 2010;45(8):3365-3373. DOI: 10.1016/j.ejmech.2010.04.020.
19. Yu Y, Kalinowski DS, Kovacevic Z, Siafakas AR, Jansson PJ, Stefani C, *et al*. Thiosemicarbazones from the old to new: iron chelators that are more than just ribonucleotide reductase inhibitors. J Med Chem. 2009;52(17):5271-5294.
20. Rabahi MF, Júnior JLRS, Ferreira ACG, Tannus-Silva DGS, Conde MB, DOI: 10.1590/S1806-37562016000000388
21. Tehrani KHME, Hashemi M, Hassan M, Kobarfard F, Mohebbi S. Synthesis and antibacterial activity of Schiff bases of 5-substituted isatins. Chin Chem Lett. 2016;27(2):221-225. DOI: 10.1016/j.cclet.2015.10.027.
22. Sedighi V, Azerang P, Sardari S. Antimycobacterial evaluation of novel [4,5-dihydro-1*H*-pyrazole-1-carbonyl]pyridine derivatives synthesized by microwave-mediated Michael addition. Drug Test Anal. 2015;7(6):550-554. DOI: 10.1002/dta.1712.
23. Hassan M, Javadzadeh Y, Lotfipour F, Badomchi R. Determination of comparative minimum inhibitory concentration (MIC) of bacteriocins produced by enterococci for selected isolates of multi-antibiotic resistant *Enterococcus* spp. Adv Pharm Bull. 2011;1(2):75-79. DOI: 10.5681/apb.2011.011.
24. Sardari S, Feizi S, Rezayan AH, Azerang P, Shahcheragh SM, Ghavami G, *et al*. Synthesis and biological evaluation of thiosemicarbazide derivatives endowed with high activity toward *Mycobacterium* bovis. Iranian J Pharm Res. 2017;16(3):1128-1140.
25. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001;46(1-3):3-26. DOI: 10.1016/s0169-409x(00)00129-0.
26. Kalhor N, Mardani M, Abdollahzadeh S, Vakof M, Esfahani Zadeh M, Tehrani KHME, *et al*. Novel *N*-Substituted ((1*H*-indol-3-yl) methylene) benzo-hydrazides and ((1*H*-indol-3-yl) methylene)-2-phenylhydrazines: synthesis and antiplatelet aggregation activity. Bull Korean Chem Soc. 2015;36(11):2632-2639. DOI: 10.1002/bkcs.10531.