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Synthesis and antimicrobial evaluation of some 4- or 6-chloroisatin derivatives

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Two series of chloroisatin-3-semicarbazones and hydrazones have been designed and prepared by condensing 4-chloro and 6-chloroisatin with several substituted semicarbazides and related bioisosteric hydrazides, respectively. Investigation of *in vitro* antimicrobial activity of compounds has been performed by agar double dilution method against nine pathogenic bacteria and four pathogenic fungi. The title compounds are also screened for their antitubercular activity against *Mycobacterium tuberculosis* H_{37} R_v using microplate alamar blue assay. Many of the synthesized compounds exhibit significant antibacterial activity in comparison to sulphamethoxazole, trimethoprim and some compounds show good antifungal activity comparable to clotrimazole. The semicarbazones arised from the 6-chloroisatin series have exhibited good antimicrobial activity than that obtained from 4-chloroisatins. Compounds, 6-chloroisatin-3-(2′-chlorophenyl) semicarbazones **1b** and 6-chloroisatin-3-(4′-bromophenyl) semicarbazone **4b** have shown a promising activity in both antibacterial and antifungal screenings. None of the compounds has been found to be active in antitubercular screening.

Keywords: Chloroisatin-3-semicarbazones, hydrazones, 6-chloroisatin, antitubercular activity, *Mycobacterium tuberculosis*, antibacterial activity

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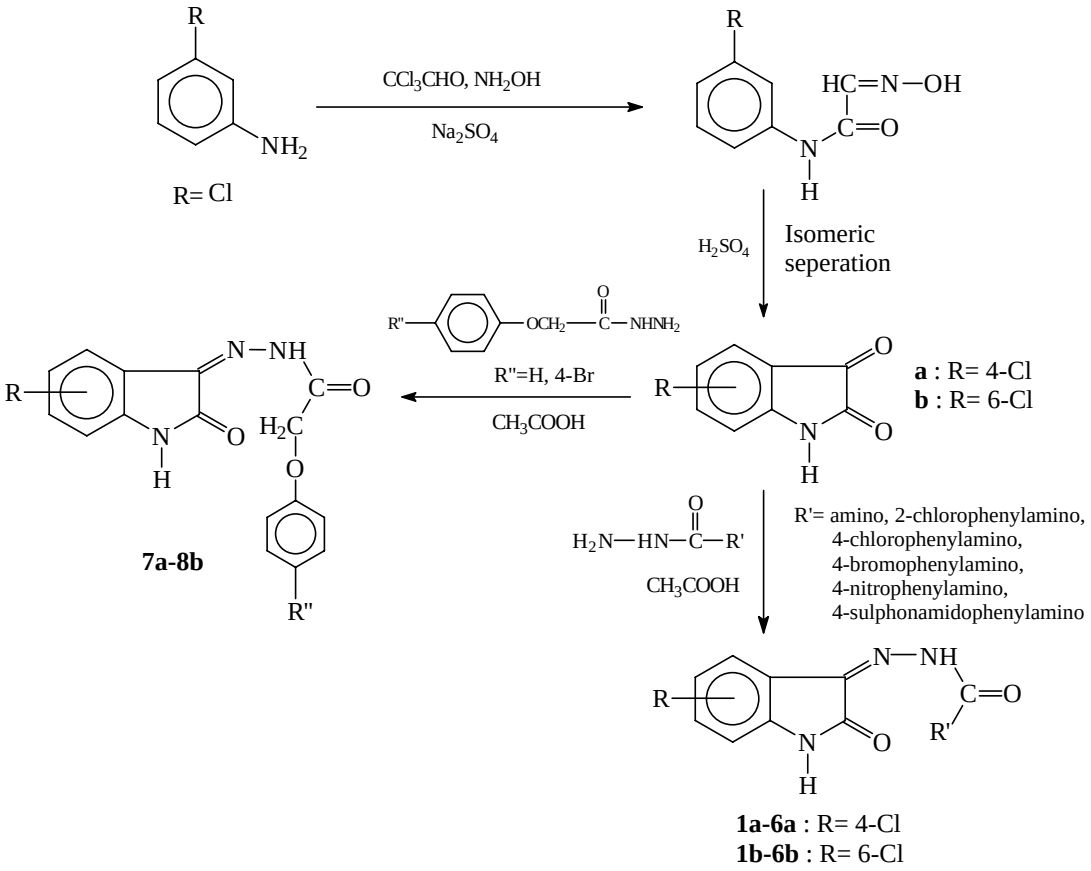
Isatin (indole-2, 3-dione), a versatile heterocyclic compound was identified in animals as a major component of the endogenous monoamine oxidase inhibitor, tribulin[1]. A number of synthetic isatin derivatives were found to have variety of pharmacological properties including anticonvulsant[2], antibacterial[3], antifungal[4], antitubercular[5], antiviral[6] and anti-HIV[7] activities. 5-Substituted isatin-N-Mannich base derivatives[8], especially that of 5-haloisatins were reported to possess both anti-bacterial and antifungal properties. Hydrazides of several nitrogen containing heterocyclic derivatives were reported as potential antitubercular agents[9]. Several semicarbazone derivatives with R-NH-CO-NH-NH-R′ moiety were reported to possess antimicrobial[10] properties. In view of these facts and in continuation of our studies on the synthesis of biologically active isatins, it was considered of interest to synthesize semicarbazones and hydrazones of isatin by incorporating isatin at one end and various substituted phenyl semicarbazides and related hydrazides at the other end of basic moiety. For QSAR study, chloro substituent (π value, 0.71) has been introduced at positions 4 and 6 of isatin residue to increase the lipophilicity. On the other hand, the -NH- of amide group has been replaced with bioisosteric -O-group. The title compounds thus synthesized were evaluated for their antibacterial, antitubercular and antifungal properties.

Results and Discussion

The synthetic protocol followed is outlined in **Scheme I**. The 4-chloro- and 6-chloroisatin**s** were prepared by literature method[14]. The N-substituted semicarbazides and bioisosteric hydrazides were synthesized by using the method described in earlier literatures[15, 16]. All the semicarbazides and related bioisosteric hydrazides were obtained in good quantities except the *para*-sulphonamidophenyl derivative whose yield was poor as compared to other semicarbazides. The condensation of the terminal primary amino group of semicarbazide and N-substituted semicarbazides with the 3-carbonyl group of chloroisatin yielded isatin-3-semicarbazones **1a-6b**. The remaining compounds **7a-8b** were obtained similarly but the condensation reaction of hydrazides with chloroisatin was faster than the previous one. The semicarbazones were crystalline, reddish orange to yellow coloured, whereas hydrazones obtained were amorphous, yellow



Scheme I

Table I — Antibacterial activity of 4-chloro/6-chloroisatin-3-semicarbazones and hydrazones (MIC, μg/mL)

Microbes	**1a**	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**5a**
Shigella flexeneri	>5000	5000	>5000	1250	>5000	>5000	78.12	>5000	5000
Aeromonas hydrophilia	5000	5000	625	78.12	1250	625	1250	625	2500
Enterococcus faecalis	>5000	>5000	1250	5000	>5000	>5000	78.12	>5000	2500
Klebsilla pneumonia	>5000	1250	>5000	156.2	>5000	>5000	>5000	78.12	625
Vibrio cholerae 01 ogawa	>5000	>5000	5000	78.12	>5000	>5000	156.25	>5000	312.5
Escherchia coli ATCC 29212	>5000	>5000	>5000	78.12	>5000	>5000	78.12	1250	5000
Bacillus subtilis	>5000	625	>5000	78.12	>5000	>5000	>5000	625	1250
Staphylococcus aureus ATCC 25923	625	1250	>5000	156.2	>5000	2500	156.25	625	625
Microbes	**5b**	**6a**	**6b**	**7a**	**7b**	**8a**	**8b**	SM	TM
Plesiomonas shigelloids	2500	625	>5000	2500	>5000	78.12	1250	5000	5000
Shigella flexeneri	>5000	>5000	>5000	>5000	>5000	>5000	>5000	2500	156.25
Aeromonas hydrophilia	78.12	2500	1250	5000	78.12	2500	156.25	2500	1250
Enterococcus faecalis	>5000	>5000	625	>5000	>5000	>5000	>5000	1250	78.12
Klebsilla pneumonia	>5000	>5000	>5000	>5000	>5000	>5000	>5000	2500	5000
Vibrio cholerae 01 ogawa	>5000	>5000	>5000	78.12	>5000	>5000	>5000	2500	5000
Escherchia coli ATCC 29212	1250	312.5	>5000	>5000	>5000	312.5	>5000	2500	19.53
Bacillus subtilis	78.12	>5000	>5000	>5000	>5000	>5000	>5000	5000	1250
Staphylococcus aureus ATCC 25923	1250	>5000	2500	2500	>5000	>5000	78.12	5000	>5000

coloured, and both were stable at dry condition. All the synthesized compounds gave satisfactory elemental analysis (within ±0.4%) for C, H and N and the UV, IR and ^{1}H NMR spectra were consistent with the assigned structures. The physicochemical data of the compounds are presented in **Table I**.

The title compounds **1a-8b** were evaluated for their *in vitro* antibacterial activity against nine pathogenic bacteria by agar double dilution method. Most of the compounds exhibited mild to moderate antibacterial activity against all the microbes tested. Compounds **2b, 4a, 4b** and **5b** exhibited broad-spectrum activity showing activity against both gram-positive and gram-negative pathogens at MIC (minimum inhibitory concentration) ranging from 78.12 to 5000 µg/mL. Compounds **2b, 4a, 4b** and **5b** were found to be more active against *Plesiomonas shigelloids ATCC, Aeromonas hydrophilia, Klebsiella pneumonia, Vibrio cholerae, Escherchia coli* and *Staphylococcus aureus* in comparison to the activity of sulphamethoxazole. When compared to trimethoprim compounds **2b, 5b** and **6b** were more active against all microbes. Compounds **2b** and **4b** exhibited better activity than norfloxacin against *Klebsiella pneumonia* and *Staphylococcus aureus*.

The *in vitro* antifungal activity of the compounds was determined against four pathogenic fungi. Most of the compounds showed antifungal activity against all strains tested and was comparable to clotrimazole. Compounds **4b, 5b** and **6b** were very active at MIC 1.56 µg/mL against *Candida albicans* and compounds **2b, 4a, 4b** and **5b** were active against *E. floccusum* at 3.12 µg/mL and the activity of both set of compounds were comparable to clotrimazole. While compound **6b** was very active against *E. floccosum* (1.56 µg/mL) and compounds **4a, 4b, 5a** and **5b** were more active against *M. audouini* (1.56 µg/mL) in comparison to clotrimazole.

From the results of antibacterial and antifungal screening, it was observed that semicarbazones of 6-chloroisatins **1b-6b** exhibited dominating activity over 4-chloro series **1a-6a**. Particularly, the compounds, 6-chloroisatin-3-(4′-chlorophenyl)semicarbazone **2b** and 6-chloroisatin-3-(4′-bromophenyl)-semicarbazone **4b** have been identified as potent antimicrobial agents. This may be attributed to their enhanced electronic character due to the presence of chloro group at the isatin residue favouring greater penetration through microbial membrane.

In antitubercular screening, none of the compounds was found active at 6.25 µg mL^{-1} concentration against *Mycobacterium tuberculosis* H_{37} R_v except compounds **3b** and **4b**, which have shown < 20% inhibition in the primary screening. Trisubstitution on isatin ring preferably with electrophilic groups at C-5, lipophilic groups at N-1 and Schiff bases at C-3 position favoured greater antituberculosis activity as reported earlier[5]. But in the present study, our modifications on isatin ring destroyed the antituberculosis activity. These results suggest that lipophilic and electronic properties play a role in antituberculosis activity isatin derivatives and especially substituents enhancing these properties at N-1 and C-5 are essential for activity.

From the above facts, it can be concluded that (i) the introduction of chloro group on isatin residue, preferably at position-6 increased antimicrobial activity but abolished antituberculosis activity; (ii) the R-NH-CO-NHNH-R′ moiety is essential for maximal action as replacing this with a bioisosteric group R-O-CH_2-CO-NHNH-R′ resulted in poor activity; and (iii) because of the promising *in vitro* antimicrobial properties, the potent compounds **2b** and **4b** merit further studies.

Experimental Section

The reactions were monitored by running TLC on silica gel plates using chloroform-methanol (9:1) as solvent system. Melting points were determined in open capillary tubes by Thomas-Hoover melting point apparatus and are uncorrected. The structures of all the compounds were confirmed by recording UV, IR and ^{1}HMR spectra on 7800 UV/VIS, Jasco FT IR 5300 and Jeol FX 90Q FT NMR Spectrometers, respectively.

4-Chloro/6-chloroisatin-3-semicarbazones (1a to 6b). Equimolar quantities of isatin (0.003 mole) and an appropriate substituted phenyl semicarbazide (0.003 mole) were dissolved in 10 mL of ethanol (95%) containing a few drops of gl. acetic acid. The mixture was refluxed for 45 min and kept in ice. The resultant solid was filtered, dried and recrystallized from ethanol (95%). Percentage yield and melting points are presented in **Table I**.

4-Chloroisatin-3-semicarbazone 1a: Yield 60%, m.p. 208°C, IR (KBr, cm^{-1}): 3447 (hetero NH) 3254 (amide NH) 1741 (C=O), 1608 (C=N), 745 (1, 2, 3-trisubstituted benzene ring); ^{1}H NMR (DMSO-d_6): δ 7.08-7.50 (m, 3H, aromatic CH), 8.37 (s, 1H, N=NH, D_2O exchangeable), 13.80 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_9H_7ClN_4O_2$: C,

45.29; H, 2.95; N, 23.47. Found: C, 45.30; 2.81; 23.42%.

6-Chloroisatin-3-semicarbazone 1b: Yield 67%, m.p. 265°C, IR (KBr, cm^{-1}): 3422 (hetero NH), 3310 (amide NH), 1734 (C=O), 1610 (C=N), 742 (1, 2, 4-trisubstituted benzene ring); ^{1}H NMR (DMSO-d_6): δ 7.12-7.56 (m, 3H, aromatic CH), 8.37 (s, 1H, N=NH, D_2O exchangeable), 14.20 (s, 1H, secondary NH, D_2O exchangeable). Anal. Calcd for $C_9H_7Cl N_4O_2$: C, 45.29; H, 2.95; N, 23.47. Found: C, 45.26; H, 2.86; N, 23.49%.

4-Chloroisatin-3-(2′-chlorophenyl) semicarbazone 2a: Yield 65%, m.p. 222°C, IR (KBr, cm^{-1}): 3442 (hetero NH), 3306 (amide NH), 1734 (C=O), 1612 (C=N), 1655 (NH-CO-NH), 734 (phenyl C-H); ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1H, D_2O exchangeable, N=NH), 7.68-7.92 (m, 7H, hetero-NH), 11.80 (s, 1H, secondary NH, D_2O exchangeable). Anal. Calcd for $C_{15}H_{10}Cl_2 N_4O_2$: C, 51.59; H, 2.88; N, 16.04. Found: C, 51.46; H, 2.91; N, 16.10%.

6-Chloroisatin-3-(2′-chlorophenyl) semicarbazone 2b: Yield 68%, m.p. 157°C, IR (KBr, cm^{-1}): 3437 (hetero NH), 3317 (amide NH), 1734 (C=O), 1614 (C=N), 1650 (NH-CO-NH), 734 (phenyl C-H); ^{1}H NMR (DMSO-d_6): δ 8.38 (s, 1H, D_2O exchangeable, N=NH), 7.60-7.90 (m, 7H, hetero-NH), 11.80 (s, 1H, secondary NH, D_2O exchangeable). Anal. Calcd for $C_{15}H_{10}Cl_2 N_4O_2$: C, 51.59; H, 2.88; N, 16.04. Found: C, 51.42; H, 2.67; N, 16.31%.

4-Chloroisatin-3-(4′-chlorophenyl) semicarbazone 3a: Yield 65%, m.p. 166°C, IR (KBr, cm^{-1}): 3448 (hetero NH), 3297 (amide NH), 1744 (C=O), 1610 (C=N), 1646 (NH-CO-NH), 734 (phenyl C-H); ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1H, D_2O exchangeable, N=NH), 7.68-7.88 (m, 7H, hetero-NH), 11.85 (s, 1H, secondary NH, D_2O exchangeable). Anal. Calcd for $C_{15}H_{10}Cl_2 N_4O_2$: C, 51.59; H, 2.88; N, 16.04. Found: C, 51.62; H, 2.79; N, 16.14%.

6-Chloroisatin-3-(4′-chlorophenyl) semicarbazone 3b: Yield 71%, m.p. 182°C, IR (KBr, cm^{-1}): 3436 (hetero NH), 3312 (amide NH), 1736 (C=O), 1614 (C=N), 1650 (NH-CO-NH), 752 (phenyl C-H); ^{1}H NMR (DMSO-d_6): δ 8.38 (s, 1H, D_2O exchangeable, N=NH), 7.72-7.92 (m, 7H, hetero-NH), 11.70 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{15}H_{10}Cl_2 N_4O_2$: C, 51.59; H, 2.88; N, 16.04. Found: C, 51.67; H, 2.90; N, 16.16%.

4-Chloroisatin-3-(4′-bromophenyl) semicarbazone 4a: Yield 72%, m.p. 225°C, IR (KBr, cm^{-1}): 3347 (hetero NH), 3254 (amide NH), 1739 (C=O), 1609 (C=N), 1645 (NH-CO-NH), 792 (phenyl C-H); ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1H, D_2O exchangeable, N=NH), 7.66-7.90 (m, 7H, hetero-NH), 11.72 (s, 1H, secondary NH, D_2O exchangeable). Anal. Calcd for $C_{15}H_{10}BrClN_4O_2$: C, 45.77; H, 2.56; N, 14.23. Found: C, 45.70; H, 2.51; N, 14.17%.

6-Chloroisatin-3-(4′-bromophenyl) semicarbazone 4b: Yield 76%, m.p. 230°C, IR (KBr, cm^{-1}): 3422 (hetero NH), 3310 (amide NH), 1734 (C=O), 1610 (C=N), 1648 (NH-CO-NH), 802 (phenyl C-H); ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1H, D_2O exchangeable, N=NH), 7.60-7.88 (m, 7H, hetero-NH), 11.70 (s, 1H, secondary NH, D_2O exchangeable). Anal. Calcd for $C_{15}H_{10}BrClN_4O_2$: C, 45.77; H, 2.56; N, 14.23. Found: C, 45.71; H, 2.41; N, 14.19%.

4-Chloroisatin-3-(4′-nitrophenyl) semicarbazone 5a: Yield 69%, m.p. 130°C, IR (KBr, cm^{-1}): 3443 (hetero NH), 3362 (amide NH), 1741 (C=O), 1602 (Amide C=O), 1610 (C=N), 721 (phenyl CH); ^{1}H NMR (DMSO-d_6): δ 6.57 (s, 1H, D_2O exchangeable, aryl NH), 7-7.7 (m, 7H, aromatic CH), 8.38 (s, 1H, D_2O exchangeable, N=NH), 11.8 (s, 1H, secondary NH, D_2O exchangeable). Anal. Calcd for $C_{15}H_{10}Cl N_5O_4$: C, 50.08; H, 2.80; N, 19.46. Found: C, 50.17; H, 2.98; N, 19.61%.

6-Chloroisatin-3-(4′-nitrophenyl) semicarbazone 5b: Yield 68%, m.p. 201°C, IR (KBr, cm^{-1}): 3422 (hetero NH), 3310 (amide NH), 1734 (C=O), 1653 (Amide C=O), 1610 (C=N), 729 (phenyl CH); ^{1}H NMR (DMSO-d_6): δ 6.57 (s, 1H, D_2O exchangeable, aryl NH), 7.2-7.7 (m, 7H, aromatic CH), 8.37 (s, 1H, D_2O exchangeable, N=NH), 11.79 (s, 1H, D_2O exchangeable, hetero NH). Anal. Calcd for $C_{15}H_{10}Cl N_5O_4$: C, 50.08; H, 2.80; N, 19.46. Found: C, 50.13; H, 2.78; N, 19.50%.

4-Chloro/6-chloroisatin-3-hydrazones (7a to 8b). Equimolar quantities of isatin (0.003 mole) and *para*-bromo or unsubstituted phenoxy acetyl hydrazide (0.003 mole) were dissolved in 10 mL of warm ethanol (95%) containing a few drops of glacial acetic acid. The mixture was refluxed for 30 min and kept in ice. The resultant solid was filtered, dried and recrystallized from ethanol (95%). Percentage yield and melting points of title compounds are presented in **Table I**.

4-Chloroisatin-3-(phenoxyacetyl) hydrazone 7a: Yield 79%, m.p. 168°C, IR (KBr, cm^{-1}): 3345 (hetero NH), 2922 (CH_2), 1734 (C=O), 1608 (C=N), 748 (phenyl CH); ^{1}H NMR (DMSO-d_6): δ 5.3 (s, 2H, O-CH_2), 7.1-7.9 (m, 7H, aromatic CH), 8.3 (s, 1H, D_2O

exchangeable, N=NH), 11.84 (s, 1H, D_2O exchangeable, heterocyclic NH). Anal. Calcd for $C_{16}H_{12}Cl\ N_3\ O_3$: C, 58.28; H, 3.66; N, 12.74. Found: C, 58.22; H, 3.60; N, 12.75%.

6-Chloroisatin-3-(phenoxyacetyl) hydrazone 7b: Yield 80%, m.p. 262°C, IR (KBr, cm^{-1}): 3355 (hetero NH), 2926 (CH_2), 1734 (Keto C=O), 1602 (C=N), 762 (phenyl CH); ^{1}H NMR (DMSO-d_6): δ 5.2 (s, 2H, O-CH_2), 7-7.8 (m, 7H, aromatic CH), 8.3 (s, 1H, D_2O exchangeable, N=NH), 11.75 (s, 1H, D_2O exchangeable, heterocyclic NH). Anal. Calcd for $C_{16}H_{12}Cl\ N_3\ O_3$: C, 58.28; H, 3.66; N, 12.74. Found: C, 58.35; H, 3.58; N, 12.63%.

4-Chloroisatin-3-(4′-bromophenoxyacetyl) hydrazone 8a: Yield 73%, m.p. 220°C, IR (KBr, cm^{-1}): 3419 (hetero NH), 3282 (amide NH), 2925 (-CH_2-O-), 1734 (C=O), 1653 (amide C=O), 1610 (C=N); ^{1}H NMR (DMSO-d_6): δ 5.0 (s, 1H, -O-CH_2), 7.1-7.8 (m, 7H, aromatic CH), 8.5 (s, 1H, D_2O exchangeable, =N-NH), 11.82 (s, 1H, D_2O exchangeable, heterocyclic-NH). Anal. Calcd for $C_{16}H_{11}BrClN_3O_3$: C, 47.02; H, 2.71; N, 10.28. Found: C, 46.97; H, 2.73; N, 10.35%.

6-Chloroisatin-3-(4′-bromophenoxyacetyl) hydrazone 8b: Yield 78%, m.p. 256°C, IR (KBr, cm^{-1}): 3419 (hetero NH), 3282 (amide NH), 2920 (-CH_2-O-), 1734 (C=O), 1658 (amide C=O), 1610 (C=N); ^{1}H NMR (DMSO-d_6): δ 5.2 (s, 1H, -O-CH_2), 7.3-7.8 (m, 7H, aromatic CH), 8.6 (s, 1H, D_2O exchangeable, =N-NH), 11.70 (s, 1H, D_2O exchangeable, heterocyclic-NH). Anal. Calcd for $C_{16}H_{11}BrClN_3O_3$: C, 47.02; H, 2.71; N, 10.28. Found: C, 47.21; H, 2.76; N, 10.47%.

Antimicrobial Evaluation

In vitro antibacterial and antifungal screening

The *in vitro* antibacterial activity against nine pathogenic bacteria was performed by agar double dilution technique[11]. The compounds were evaluated for their *in vitro* antifungal activity against four pathogenic fungi using agar dilution method[12] with Sabaraud's dextrose agar (HI-media). The microbial strains used in testing both antibacterial and antifungal activities were procured from the Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi. The stock solution of standard and test compounds was prepared in DMSO and subsequent dilutions were made with the same solvent. Mueller Hint Agar Media (HI Media) was used to subculture various strains of microbes. Normal saline was used to prepare the inoculums of the bacteria to be used for the antibacterial study. Under aseptic conditions, the diluted test solutions with different concentrations (5000 to 78.12 μg/mL) were added to the vials (previously sterilized) containing 100 discs and the discs were placed on the numbered plates. Then, the plates were incubated at 37°C for 24 hr. The MIC, the lowest concentration of the test drug that completely inhibited the growth of microbe was noted. The standard drugs used for antibacterial and antifungal screening were sulphamethoxazole (SM), trimethoprim (TM) and clotrimazole, respectively. The MIC of test compounds and standard drugs are presented in **Tables I** and **II**.

Table II — Antifungal activity of 4-chloro/6-chloroisatin-3-semicarbazones and hydrazones (MIC, μg/mL)

Compd	*A.niger*	*C.albicans*	*E.floccosum*	*M.audouini*
1a	50	25	6.25	12.5
1b	12.5	12.5	25	50
2a	12.5	50	12.5	25
2b	12.5	6.25	3.12	12.5
3a	25	25	6.25	25
3b	12.5	12.5	6.25	12.5
4a	12.5	6.25	3.12	1.56
4b	6.25	1.56	3.12	1.56
5a	6.25	12.5	12.5	6.25
5b	3.12	1.56	3.12	1.56
6a	25	50	6.25	3.12
6b	12.5	1.56	1.56	3.12
7a	50	50	12.5	100
7b	50	6.25	6.25	25
8a	25	12.5	25	12.5
8b	25	12.5	6.25	12.5
Clotrimazole	2.44	0.3	2.44	4.88

In vitro antitubercular screening

The *in vitro* antitubercular screening was carried out at Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, Alabama. Primary screening conducted at 6.25μg mL^{-1} against *Mycobacterium tuberculosis* $H_{37}\ R_v$ (ATCC 27294) in BACTEC 12 B medium using a broth micro dilution assay, the Microplate Alamar Blue Assay (MABA)[13]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system.

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