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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF NOVEL N-(BENZOXAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDINE) HYDRAZINE CARBOTHIOAMIDES

T. Kranthi Kumar * and R. Sreenivasulu

Mewar University, Chittorgarh - 312901, Rajasthan, India.

Keywords:

Isatin, Benzoxazole, Condensation reaction, Antibacterial, Antifungal, Anti-mycobacterial

Correspondence to Author:
T. Kranthi Kumar

Research Scholar,
Mewar University, Chittorgarh - 312901, Rajasthan, India.

E-mail: kranthikumardoer@gmail.com

ABSTRACT: In view of the biological prominence of the benzoxazole derivatives as well as, isatin derivatives, it was planned to synthesize some novel N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides (VI) as such reports were not available in the literature and were screened for antibacterial, antifungal and anti-mycobacterial activity. Fourteen new compounds were synthesized by condensing different Isatins (V) with N-(Benzoxazol-2-yl) hydrazine carbothioamide (IV). All the prepared compounds were screened for antibacterial, antifungal, and antimycobacterial activities on various microbial strains. The results revealed that all the synthesized compounds were exhibiting antimicrobial properties. Compound VIc, VIe, VIg, VIi, and VIl were declared to possess potent antimicrobial properties in the given bacterial and fungal strains.

INTRODUCTION: Benzoxazole (m.p 27-30 °C; b.p. 182 °C), is a planar molecule with conjugated π electrons sextets in the cyclic system. The chemical properties are aromatic in character [1]. The lone pair of electrons on nitrogen, which is coplanar with the heterocyclic ring and, therefore, not involved in delocalization, confers weakly basic properties. Associated with the aromatic is a degree of stability, but when these are quarternized, the resulting azolium species are significantly activated towards nucleophilic attack [2]. Benzoxazole (pharmacophore) derivatives are biologically active compounds and are known to exhibit various biological activities such as anticancer, antimicrobial, anti-HIV, *etc*.

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Targets containing benzoxazole moiety, either isolated from plants or accessed by total synthesis, have remarkable biological activities. The novel antibacterial agent containing the benzoxazole system is boxazomycin-B reported by Suto and Turner [3]. Benzoxazole ring containing antibiotic, calcymicin reported by Evans [4]. Benzoxazole moieties are also reported to exhibit various biological activities like antihistaminic activity [5], antimicrobial activity, analgesic [6], antiinflammatory activity [7], anti-mycobacterial [8], anticancer [9], anthelmintic activity [10], hypoglycaemic activity [11], antiviral [12], herbicidal [13], diuretic and uricosuric activities [14].

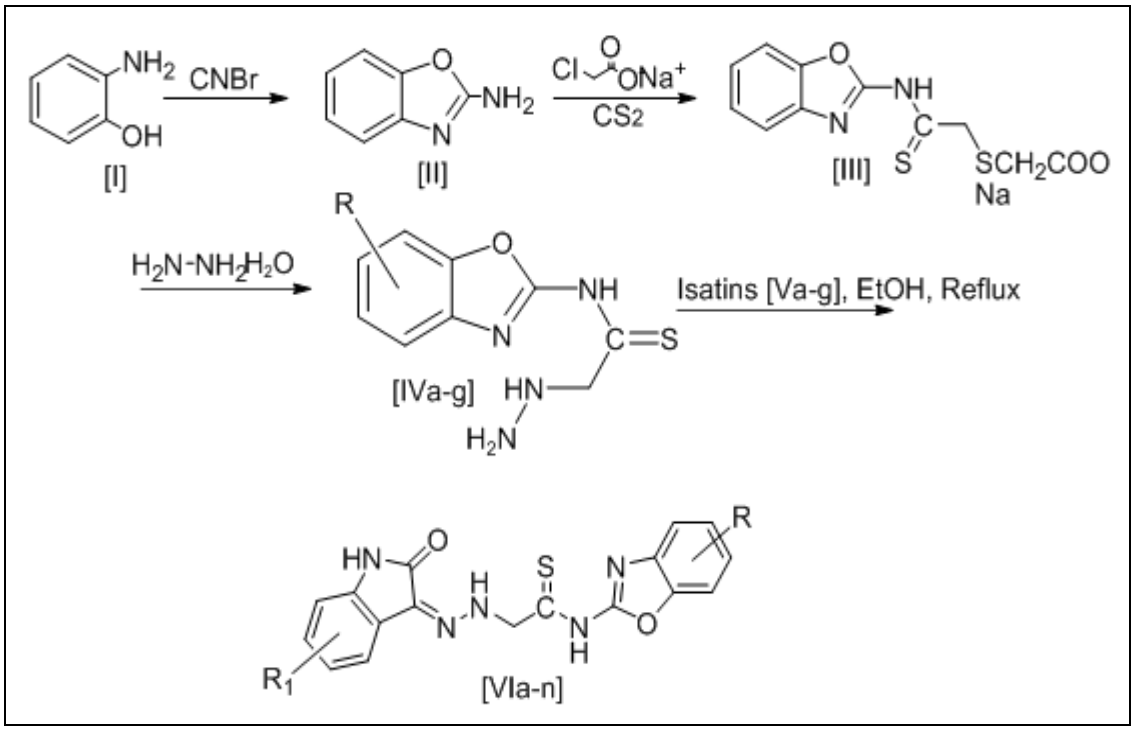
Isatin is a unique molecule (1H-indole-2,3-dione) possessing both amide and keto-carbonyl groups along with an active hydrogen atom attached to nitrogen (or oxygen) and an aromatic ring which should substitute at 5- and 7-positions. Isatin exists in two forms, lactam form, and lactim form. Both are derivatives of 2,3-dihydroindole. This is an example of amido-imidol tautomeric system [16].

Naturally, Isatin is distributed in various plants, animals, and fungi [17]. Various preparation methods like Sandmeyer, Stolle, Gassman, and Martinet have been established, and many more advancements have also been reported in the literature for the synthesis of Isatin derivatives [18].

The current work illustrates the synthesis and antimicrobial activities of N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides **Scheme 1**. Fourteen novel derivatives have been prepared and screened for *in-vitro* antimicrobial studies on various gram positive, gram-negative bacteria, fungal and mycobacterial species.

MATERIALS AND METHODS: All chemicals and solvents used in this work were synthetic grades purchased from Sigma-Aldrich and used without purification. Merck-precoated aluminum TLC plates of silica gel 60 F254 were employed for the reaction monitoring and the spots visualized with iodine vapors and in the UV chamber. Column chromatography was used for the purification and isolation of pure compounds. Melting points were determined by Remi electronic melting point apparatus. ^{1}H NMR recorded on BRUKER DRX – 400 MHz. Chemical shift values (δ) articulated in ppm with reference to internal standard tetramethyl silane (TMS). The splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. MASS recorded on BRUKER ESI-IT MS.

Synthetic Procedure:



SCHEME 1: SYNTHESIS N-(BENZOXAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDINE)HYDRAZINE CARBOTHIOAMIDES (*VIa-n)*

Synthesis of 2-Aminobenzoxazole (II): To a solution of 2-aminophenol (0.1mol) in toluene has added a solution of cyanogen bromide (0.02 mol) in toluene with continuous stirring at room temperature and the stirring was continued for 3 h. The completion of the reaction was monitored by TLC. The solid separated was filtered and washed with carbon tetrachloride (CCl_4) and air-dried to give a purple-colored solid, and recrystallized from ethyl acetate (yield 75%).

Synthesis of n-(benzoxazole-2-yl) hydrazine carbothioamide (III & IVa-g): 2-Amino-benzoxazole (0.1mol) was dissolved in ammonia solution (20 ml). Carbon disulfide (8 ml) was added gradually with stirring in an ice bath. Ethanol (25 ml) was added, and stirring was continued till carbon disulfide was completely dissolved. The reaction mixture was allowed to stand for 3 h while stirring. Sodium chloroacetate solution (0.1mol) was added followed by hydrazine

hydrate (10 ml). The reaction mixture was stirred for 3 h and allowed to stand overnight. Crystals separated were filtered and recrystallized from methanol (yield 70%).

Synthesis of Isatin (Va-g):

Step-1: Isonitroso-acetanilides: In a 5 L R.B. Flask were placed 90g (0.54mol) of chloral hydrate and 1200 ml of water. To this solution, were then added crystallized sodium sulphate (1300 g) followed by a solution of appropriate aniline (0.5 mol) in 300 ml of water, to which 51.2g (43 ml, 0.52 mol) of concentrated Hydrochloric acid has been added to dissolve the aniline. Finally, a solution of hydroxylamine HCl, 110g (1.58mol) in 500 ml of water was added. The contents of the flask were heated on a water bath so that vigorous boiling began in about 40 to 45 min. After 1 to 2 min of vigorous boiling, the reaction was completed. During the heating period, some crystals of isonitroso-acetanilide separated out. On cooling the solution in running water, the remaining crystallized. It was filtered under suction and air-dried.

Step-2: Isatin: Sulphuric acid (600 g, 326 ml, sp.gr. 1.84) was warmed at 50 °C in a 1 liter R.B. flask fitted with an efficient mechanical stirrer, and to this, 0.46 mol of dry finely powdered appropriate isonitroso-acetanilide was added at such a rate so as to maintain the temperature between 60 °C to 70 °C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitroso-acetanilide was completed, the solution was heated to 80 °C and maintained at that temperature for 10 min to complete the reaction. Then the reaction mixture was cooled to room temperature and poured up on 10 to 12 times the volume of crushed ice while stirring. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water, several times to remove sulphuric acid. It was then air-dried.

Synthesis of n-(benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides (VIa-n): To a solution of n-(benzoxazole-2-yl) hydrazine carbothioamide (IV, 0.1mol) in ethanol has added a solution of Isatin derivatives (0.02mol) in ethanol with continuous stirring at room temperature and the stirring was continued for 3 h. The completion of the reaction was monitored by TLC. The solid separated was filtered and washed with sodium sulphate and purified by column chromatography using silica gel 60-120.

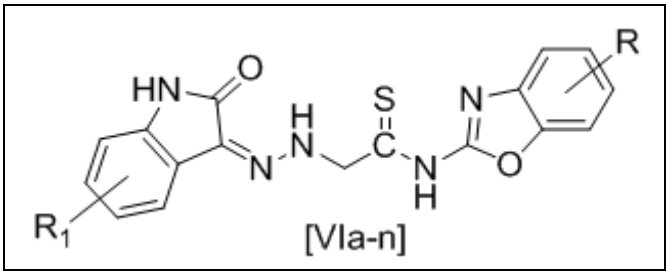


FIG. 1: N-(BENZOXAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDINE) HYDRAZINE CARBOTHIOAMIDES (VIa-n)

Antimicrobial Activity:

Anti-bacterial and Antifungal Activity: The bacterial and fungal strains were obtained from the department of microbiology, Osmania University. They were preserved at 4 ºC.

Antibacterial activity of the compounds, *(VIa-n)* were studied against gram-positive, gram-negative bacterial and fungal strains *Staphylococcus aureus* (NCTC 7447), *Bacillus subtilis* (MTCC-619), *Escherichia coli* (NCTC 6571), *Streptococcus pneumonia*, *Aspergillus niger* and *Candida albicans* (recultured) respectively by disc-diffusion method and Ampicillin (100 µg/ml) Nystatin (10 µg/ml) in DMSO were used as reference antibiotics. Agar media was taken in the pre-sterilized petri-dishes, and the microorganisms were grown. A stock solution of (60 µg/ml) for all the prepared compounds *(VIa-n)* is made by using DMSO. The disc (6 mm in diameter) was impregnated with 200 µg/ml 100 µg/ml, and 50 µg/ml of each test solution, placed on the seeded agar medium and the petri-dishes were incubated at 37 °C for 24 h. DMF alone was used as control at the equal aforementioned concentration. The zone of inhibition of each compound in mm was recorded, and the results were furnished in **Table 3**.

Antimycobacterial Activity: *M. tuberculosis MTB H 37Rv* (ATCC 27294) strains, which are susceptible to rifampicin and isoniazid, were used for the study of the anti-tubercular activity of the synthesized compounds. The bacterial strains were subcultured to have a fresh batch for the study, supplied with Muller Hinton broth at 37 °C for two

weeks. Bacterial suspensions with 0.5 McFarland standard turbidity, equivalents to 10^8 CFU, were prepared by diluting it with normal saline solution. The mixture was vortexed for 30 seconds in a glass vessel, and the particles were allowed to settle [19]. 100µL of the microbial suspension was used for the inoculation.

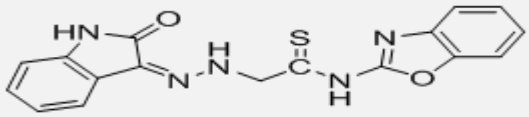
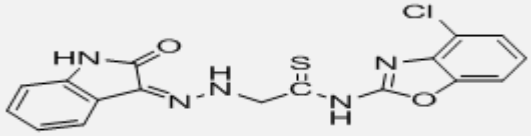
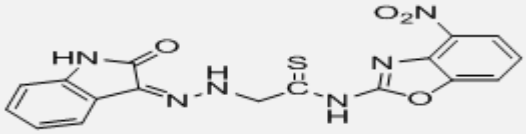
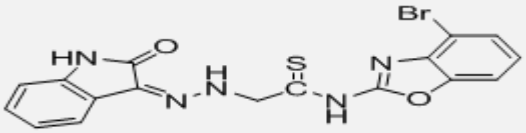
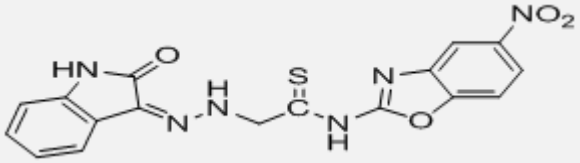
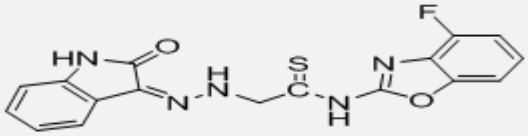
The stock solutions of 100 µg/mL of synthesized compounds were prepared in DMSO. In order to determine the minimum inhibitory concentration of title compounds, a serial dilution of compounds with varying strengths (50, 25, 12.5, 6.25, 3.12, 1.6 and 0.8 µg/mL) was prepared from the respective stock solutions.

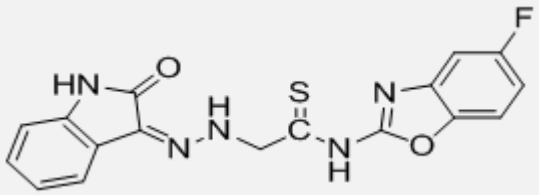
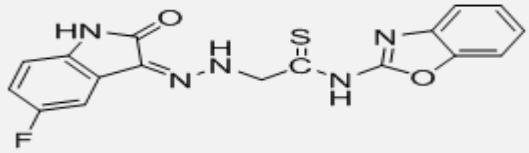
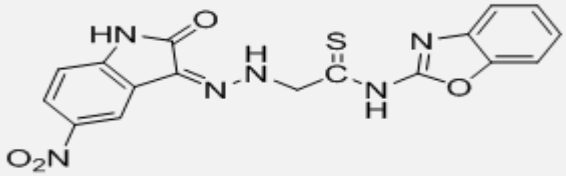
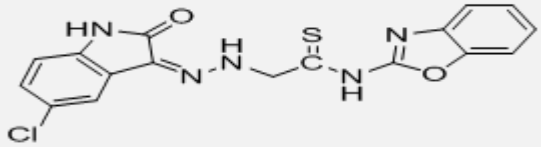
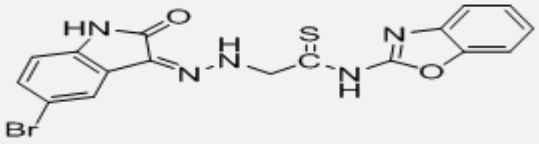
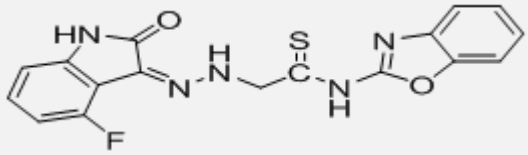
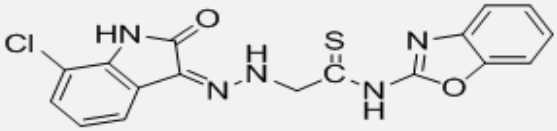
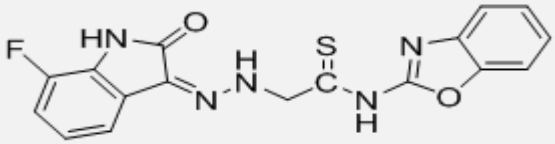
Middle brook 7H11 agar medium was used for growing the mycobacterium, supplemented with Oleic Albumin Dextrose Catalase (OADC), after sterilization under moist heat using autoclave at 121 °C for 15 min. Then the medium was diluted with various strengths (50, 25, 12.5, 6.25, 3.12, 1.6 and 0.8 µg/mL) of synthesized (*VIa-n)* compounds in appropriate volumes. Using aseptic technique, 5-ml of middle brook 7H11 agar medium was dispensed into each labeled quadrants of sterile quad-plates and allowed to solidify under laminar airflow with lids slightly opened.

After solidification, bacterial suspension from the culture broth was inoculated aseptically through a loop (3 mm internal diameter) and incubated for 21 days at 37 °C. The minimum inhibitory concentration (MIC) was determined by counting the colonies formed on the medium by comparing with the controls. DMSO and isoniazid were served as negative and positive controls, respectively [20].

RESULTS AND DISCUSSION: A novel series of fourteen N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides were synthesized by a feasible method **Scheme 1** with adequate yields. The structure elucidation of all the compounds was made using advanced analytical methods like Mass spectrometry and NMR. Structures of all the synthesized compounds along with their yields and melting points have been depicted in the **Table 1**.

TABLE 1: STRUCTURES AND PHYSICAL DATA OF THE TITLED COMPOUNDS *VIa-n*

Compound	R	R_1	Structure	Yield (%)	m.p (°C)
VIa	H	-		89	185-190
VIb	4-Cl	-		84	195-200
VIc	4-NO_2	-		85	175-180
VId	4-Br	-		78	144-147
Vie	3- NO_2	-		80	155-158
VIf	4-F	-		75	140-145

VIg	3-F	-		81	146-149
VIh	-	H		85	146-148
VIi	-	5-Cl		79	150-153
VIj	-	5-NO_2		83	148-150
VIk	-	5-Br		85	150-153
VIl	-	3- F		80	155-160
VIm	-	7-Cl		78	153-154
VIn	-	7-F		79	176-178

(Z)- N- (benzo[d]oxazol-2-yl)-2-(2-(2-oxoindolin-3- ylidene) hydrazineyl) ethanethioamide Compound VI a: Yellow Solid, ^{1}H NMR (500 MHz, DMSO) δ 8.25 (s, 1H), 8.18 (dd, *J* = 8.8, 5.2 Hz, 3H), 8.09 (dd, *J* = 7.8, 6.4 Hz, 3H), 8.05 (s, 1H), 8.01 – 7.99 (m, 1H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.72 – 7.69 (m, 1H), 7.67 (s, 1H), 7.58 (d, *J* = 5.5 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.33 – 7.27 (m, 3H), 6.96 (d, *J* = 7.0 Hz, 1H), 6.03 (s, 2H).^{13}C NMR (125 MHz, DMSO) δ 188.00 (s), 140.78 (s), 139.48 (s), 138.21 (s), 136.76 (s), 134.18 (s), 133.82 (s), 132.96 (s), 132.63 (s), 131.15 (s), 130.89 (s), 129.18 (s), 128.73 (s), 128.16 (s), 127.35 (s), 127.14 (s), 126.64 (s), 126.32 (s), 126.03 (s), 125.26 (s), 123.57 (d), *J* = 18.0 Hz), 122.20 (s), 121.23 (s), 116.03 (s), 113.04 (s), 111.81 (s), 47.97 (s).

(Z)- N- (4- chlorobenzo [d] oxazol-2-yl)-2-(2-(2-oxoindolin- 3- ylidene) hydrazineyl) ethane-thioamide Compound VI b: Yellow Solid, ^{1}H NMR (500 MHz, DMSO) δ 8.25 (s, 1H), 8.20 – 8.16 (m, 3H), 8.09 (dd, *J* = 7.8, 6.4 Hz, 3H), 8.05 (s, 1H), 8.01 – 7.99 (m, 1H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.72 – 7.69 (m, 1H), 7.67 (s, 1H), 7.58 (d, *J* = 5.5 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.33 – 7.27

(m, 3H), 6.96 (d, *J* = 7.0 Hz, 1H), 6.03 (s, 2H).[13]C NMR (125 MHz, DMSO) δ 188.00 (s), 140.78 (s), 139.48 (s), 138.21 (s), 136.76 (s), 134.18 (s), 133.82 (s), 132.96 (s), 132.63 (s), 131.11 (s), 130.89 (s), 129.18 (s), 128.73 (s), 128.16 (s), 127.35 (s), 127.14 (s), 126.64 (s), 126.32 (s), 126.03 (s), 125.26 (s), 123.59 (d, *J* = 12.2 Hz), 122.13 (s), 121.28 (s), 116.03 (s), 113.08 (s), 111.76 (s), 48.02 (s).

(Z)- N- (4-nitrobenzo[d]oxazol-2-yl)-2-(2-(2-oxo-indolin-3-ylidene)hydrazineyl) ethanethioamide Compound VI c: Yellow Solid, [1]H NMR (500 MHz, DMSO) δ 8.31 (s, 1H), 8.23 – 8.18 (m, 1H), 8.16 (d, *J* = 2.2 Hz, 2H), 8.15 (d, *J* = 2.6 Hz, 1H), 8.12 (d, *J* = 7.1 Hz, 1H), 8.02 – 8.00 (m, 2H), 7.92 (d, *J* = 8.3 Hz, 1H), 7.67 (dd, *J* = 15.1, 9.7 Hz, 1H), 7.62 – 7.61 (m, 2H), 7.46 – 7.42 (m, 2H), 7.31 – 7.28 (m, 3H), 7.03 (d, *J* = 6.7 Hz, 1H), 6.93 (t, *J* = 24.0 Hz, 1H), 6.06 (s, 2H).[13]C NMR (125 MHz, DMSO) δ 188.05 (s), 140.96 (s), 139.36 (s), 139.02 (s), 138.10 (s), 135.86 (d, *J* = 20.0 Hz), 135.07 (s), 133.74 (s), 132.19 (s), 131.04 (s), 130.79 (d, *J* = 12.1 Hz), 129.02 (s), 128.72 (s), 127.27 (d, *J* = 11.5 Hz), 126.95 (s), 126.36 (s), 125.70 (s), 125.28 (s), 123.47 (s), 123.08 (s), 122.66 (s), 122.02 (s), 120.89 (s), 116.09 (s), 113.12 (s), 111.31 (s), 48.15 (s).

(Z)-N-(4-bromobenzo[d]oxazol-2-yl)-2-(2-(2-oxo-indolin-3-ylidene) hydrazineyl) ethanethioamide Compound VI d: Yellow Solid, [1]H NMR (500 MHz, DMSO) δ 7.95 – 7.90 (m, 2H), 7.82 – 7.77 (m, 2H), 7.59 (dd, *J* = 7.2, 6.0 Hz, 2H), 7.56 (d, *J* = 5.0 Hz, 1H), 7.52 – 7.49 (m, 2H), 7.37 – 7.35 (m, 2H), 7.31 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.30 – 7.28 (m, 1H), 7.25 – 7.21 (m, 2H), 6.99 (d, *J* = 5.3 Hz, 1H), 6.96 (d, *J* = 5.0 Hz, 2H), 5.79 (s, 2H). [13]C NMR (125 MHz, DMSO) δ 201.35 (s), 194.10 – 193.94 (m), 185.43 (s), 142.33 – 142.17 (m), 141.13 (s), 138.31 (s), 136.95 (s), 134.27 (s), 133.37 (s), 131.81 (s), 131.07 (s), 129.95 (s), 128.08 (s), 127.27 (s), 126.37 (s), 125.75 (s), 124.26 (s), 124.14 – 123.99 (m), 123.69 (s), 122.04 – 121.88 (m), 121.22 (s), 120.84 (s), 118.98 (s), 118.21 (s), 112.35 (s), 111.59 (s), 47.51 (s).

(Z)- N- (5-nitrobenzo[d]oxazol-2-yl)-2-(2-(2-oxo-indolin-3-ylidene) hydrazineyl) ethanethioamide Compound VI e: Yellow Solid, [1]H NMR (500 MHz, DMSO) δ 8.25 (s, 1H), 8.22 – 8.21 (m, 1H), 8.18 (s, 1H), 8.17 (s, 1H), 8.15 (s, 1H), 8.14 (s, 1H), 8.09 – 8.07 (m, 2H), 8.01 – 7.99 (m, 1H), 7.97 – 7.94 (m, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.67 (s, 1H), 7.45 – 7.40 (m, 2H), 7.31 – 7.27 (m, 3H), 6.96 (d, *J* = 7.0 Hz, 1H), 6.03 (s, 2H) [13]C NMR (125 MHz, DMSO) δ 187.93 (s), 140.96 (s), 139.46 (s), 139.27 (s), 138.20 (s), 136.74 (s), 136.24 (s), 135.51 (s), 133.81 (s), 132.96 (s), 131.25 (dd, *J* = 41.6, 11.2 Hz), 130.88 (s), 129.17 (s), 128.72 (s), 127.70 (d, *J* = 6.3 Hz), 127.13 (s), 126.63 (s), 126.32 (s), 126.02 (s), 125.23 (s), 123.60 (d, *J* = 6.8 Hz), 122.63 (d, *J* = 12.1 Hz), 122.19 (s), 121.24 (s), 115.98 (s), 113.07 (s), 111.75 (s).

(Z)-N-(4-fluorobenzo[d]oxazol-2-yl)-2-(2-(2-oxo-indolin-3-ylidene) hydrazineyl) ethanethioamide Compound VI f: Yellow Solid, [1]H NMR (500 MHz, DMSO) δ 8.21 – 8.16 (m, 1H), 8.03 – 7.98 (m, 2H), 7.95 (dd, *J* = 7.5, 4.2 Hz, 1H), 7.92 – 7.88 (m, 2H), 7.88 – 7.85 (m, 1H), 7.81 (dt, *J* = 12.4, 7.2 Hz, 1H), 7.75 – 7.68 (m, 1H), 7.62 – 7.52 (m, 1H), 7.54 – 7.48 (m, 2H), 7.47 – 7.42 (m, 1H), 7.39 – 7.31 (m, 1H), 7.31 – 7.04 (m, 3H), 7.03 (t, *J* = 6.3 Hz, 1H), 6.98 – 6.94 (m, 1H), 5.88 (s, 2H), 3.92 – 3.63 (s, 3H). [13]C NMR (125 MHz, DMSO) δ 184.69 (s), 140.33 (s), 138.04 (s), 137.78 (s), 137.58 (s), 134.83 (s), 133.77 (s), 131.64 (s), 130.88 (s), 130.57 (s), 129.03 (d, *J* = 5.1 Hz), 128.70 (s), 126.98 (d, *J* = 13.8 Hz), 126.37 (d, *J* = 9.6 Hz), 125.69 (s), 125.28 (s), 124.12 (s), 123.33 (s), 123.00 (s), 121.81 (s), 120.83 (s), 118.20 (s), 116.68 (s), 113.97 (s), 113.16 (s), 111.14 (s), 55.62 (s), 48.40 (s).

(Z)-N-(5-fluorobenzo[d]oxazol-2-yl)-2-(2-(2-oxo-indolin-3-ylidene) hydrazineyl) ethanethioamide Compound VI g: Yellow Solid, [1]H NMR (500 MHz, DMSO) δ 8.21 (s, 1H), 8.17 (dd, *J* = 8.3, 2.9 Hz, 2H), 8.06 (d, *J* = 8.4 Hz, 3H), 8.00 (t, 4.3 Hz, 2H), 7.92 (t, 3H), 7.66 (m, 4H), 7.44 (t, 1H), 7.29 (dt, *J* = 11.1, 3.6 Hz, 2H), 6.97 (d, *J* = 6.9 Hz, 1H), 6.02 (s, 2H). [13]C NMR (125 MHz, DMSO) δ 146.12 (d, *J* = 10.8 Hz), 142.64 (s), 138.60 (s), 137.29 (s), 135.71 (s), 133.90 (d, *J* = 5.3 Hz), 131.61 (d, *J* = 65.0 Hz), 131.34 – 131.20 (m), 130.58 (d, *J* = 35.4 Hz), 130.37 – 130.36 (m), 129.95 (s), 129.00 (s), 128.27 (s), 127.91 (s), 126.43 (s), 122.80 (d, *J* = 10.4 Hz), 116.63 (s), 116.53 – 116.37 (m), 52.94 – 52.78 (m).

(Z)- N- (benzo[d]oxazol-2-yl)- 2- (2-(5-fluoro-2-oxoindolin- 3- ylidene) hydrazineyl) ethanethioamide Compound VI h: Yellow Solid, ^{1}H NMR (500 MHz, DMSO) δ 8.19 – 8.16 (m, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.99 – 7.96 (m, 1H), 7.95 (d, *J* = 2.6 Hz, 1H), 7.92 (s, 2H), 7.89 (dd, *J* = 8.7, 4.5 Hz, 2H), 7.81 (t, *J* = 9.9 Hz, 1H), 7.61 – 7.53 (m, 1H), 7.39 – 7.36 (m, 2H), 7.24 (dd, *J* = 5.8, 2.6 Hz, 3H), 7.08 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.96 (d, *J* = 7.0 Hz, 1H), 5.91 (s, 2H), 3.84 – 3.78 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 184.70 (s), 159.85 (s), 140.37 (d, *J* = 6.9 Hz), 138.58 (s), 138.08 (s), 137.78 (s), 135.27 (s), 133.77 (s), 132.12 (s), 131.56 (s), 130.84 (s), 129.80 (s), 128.95 (s), 128.72 (s), 127.04 (s), 126.41 (s), 125.69 (s), 125.28 (s), 124.13 (s), 123.41 (s), 122.97 (s), 121.82 (s), 120.80 (s), 118.20 (s), 116.85 (s), 112.99 (s), 111.12 – 110.96 (m), 48.39 – 48.23 (m).

(Z)-N-(benzo[d]oxazol-2-yl)-2-(2-(5-nitro-2-oxo-indolin-3-ylidene) hydrazineyl) ethanethioamide Compound VI i: Yellow Solid, ^{1}H NMR (500 MHz, DMSO) δ 8.24 – 8.20 (m, 3H), 8.19 – 8.16 (m, 2H), 8.05 (s, 1H), 8.01 (d, *J* = 3.0 Hz, 1H), 7.99 (d, *J* = 1.7 Hz, 1H), 7.90 (d, *J* = 8.3 Hz, 1H), 7.69 (s, 1H), 7.64 – 7.57 (m, 3H), 7.45 – 7.41 (m, 1H), 7.40 – 7.36 (m, 2H), 7.32 – 7.26 (m, 2H), 6.97 (d, *J* = 7.0 Hz, 1H), 6.02 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 185.37 (s), 141.40 (s), 137.89 (s), 133.84 (s), 132.54 (s), 131.63 (d, *J* = 9.7 Hz), 130.92 (s), 129.21 (s), 128.90 (s), 127.21 (s), 126.69 (s), 126.04 (s), 125.60 (s), 125.20 (s), 124.26 (s), 123.57 (s), 123.11 (s), 121.59 (s), 118.07 (s), 111.85 (s).

(Z)- N- (benzo[d]oxazol-2-yl)- 2- (2-(5-chloro-2-oxoindolin- 3- ylidene) hydrazineyl) ethanethioamide Compound VI j: Yellow Solid, ^{1}H NMR (500 MHz, DMSO) δ 8.31 (s, 2H), 8.18 – 8.17 (m, 2H), 8.15 (s, 1H), 8.06 (dd, *J* = 23.0, 8.1 Hz, 1H), 8.02 – 7.99 (m, 2H), 7.92 (d, *J* = 8.3 Hz, 2H), 7.85 (s, 1H), 7.60 (s, 2H), 7.53 (d, *J* = 6.6 Hz, 1H), 7.43 (d, *J* = 6.8 Hz, 1H), 7.33 (d, *J* = 4.6 Hz, 1H), 7.29 – 7.29 (m, 1H), 7.02 (d, *J* = 7.0 Hz, 2H), 6.06 (s, 2H), 2.53 (s, *J* = 15.4 Hz, 3H). ^{13}C NMR (125 MHz, DMSO) δ 185.38 (s), 143.99 (s), 141.39 (s), 137.89 (s), 133.85 (s), 132.53 (s), 130.89 (s), 129.68 (s), 129.04 (d, *J* = 34.1 Hz), 128.81 (s), 127.21 (s), 126.64 (s), 126.03 (s), 125.62 (s), 125.20 (s), 124.27 (s), 123.56 (s), 123.17 (s), 121.66 (s), 118.07 (s), 111.84 (s), 65.58 (s).

(Z)- N- (benzo[d]oxazol-2-yl)- 2- (2-(5-bromo-2-oxoindolin- 3- ylidene) hydrazineyl) ethanethioamide Compound VI k: Yellow Solid, ^{1}H NMR (500 MHz, DMSO) δ 8.47 (s, 1H), 8.27 (d, *J* = 48.0 Hz, 1H), 8.17 – 8.09 (m, 2H), 8.08 – 7.80 (m, 1H), 7.64 (dd, *J* = 15.2, 12.1 Hz, 2H), 7.59 – 7.52 (m, 3H), 7.31 – 7.13 (m, 5H), 5.49 (dd, *J* = 29.2, 14.8 Hz, 2H). ^{13}C NMR (125 MHz, DMSO) δ 185.19 (s), 141.36 (s), 139.06 (s), 137.76 (d, *J* = 13.0 Hz), 137.53 (s), 137.37 (s), 137.06 (s), 136.68 (s), 136.45 (s), 132.10 (d, *J* = 5.0 Hz), 130.56 (s), 129.97 (d, *J* = 18.1 Hz), 129.24 (s), 126.37 (s), 124.17 (s), 123.54 (s), 123.10 (s), 122.13 (s), 121.39 (dd, *J* = 35.2, 17.8 Hz), 118.02 (s), 116.19 (s), 113.01 (s), 111.80 (s), 49.62 (s).

(Z)-N-(benzo[d]oxazol-2-yl)-2-(2-(4-fluoro-2-oxoindolin-3-ylidene)hydrazineyl) ethanethioamide Compound VI l: Yellow Solid, ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.29 (s, 5H), 8.10 (dd, *J* = 5.5, 3.5 Hz, 6H), 8.04 (d, *J* = 8.2 Hz, 13H), 8.00 (s, 3H), 7.70 – 7.65 (m, 6H), 7.55 – 7.52 (m, 13H), 7.37 (d, *J* = 8.0 Hz, 10H), 7.25 (dd, *J* = 11.4, 5.8 Hz, 22H), 5.49 (s, 10H), 2.41 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 185.18 (s), 143.20 (s), 138.08 (s), 137.66 (s), 137.15 (s), 136.31 (s), 135.88 (s), 132.10 (d, *J* = 6.6 Hz), 130.04 (s), 129.80 (d, *J* = 19.6 Hz), 128.75 (s), 126.43 (s), 125.33 (s), 124.17 (s), 123.43 (s), 122.01 (s), 121.28 (s), 121.05 (s), 116.69 (s), 112.99 (s), 111.66 (s), 49.34 (s).

(Z)- N- (benzo[d]oxazol-2-yl)- 2- (2-(7-chloro-2-oxoindolin-3-ylidene) hydrazineyl) ethanethioamide Compound VI m: Yellow Solid, ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.31 (s, 1H), 8.11 (d, *J* = 3.1 Hz, 1H), 8.07 (d, *J* = 8.2 Hz, 3H), 7.90 – 7.85 (m, 2H), 7.79 – 7.75 (m, 3H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.64 (d, *J* = 15.5 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 3H), 7.29 – 7.26 (m, 3H), 7.24 (t, *J* = 6.6 Hz, 3H), 5.49 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 188.34 (s), 143.33 (s), 138.99 (s), 138.33 – 137.61 (m), 136.49 (s), 133.16 (s), 132.20 (s), 130.71 (s), 128.42 (s), 126.94 (s), 126.35 (s), 123.66 (s), 122.21 (s), 121.23 (s), 119.05 (s), 116.38 (s), 113.20 (s), 111.64 (s), 110.97 (s), 49.57 (s).

(Z)- N- (benzo[d]oxazol-2-yl)-2-(2-(7-fluoro-2-oxoindolin- 3- ylidene) hydrazineyl) ethane-thioamide Compound VI n: Yellow Solid, ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.47 (s, 1H), 8.27 (d, *J* = 48.0 Hz, 1H), 8.17 – 8.09 (m, 2H), 8.08 – 7.80 (m, 1H), 7.64 (dd, *J* = 15.2, 12.1 Hz, 2H), 7.59 – 7.52 (m, 3H), 7.31 – 7.13 (m, 5H), 5.49 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 188.18 (s), 143.32 (s), 142.92 (s), 141.50 (s), 138.98 (s), 138.20 – 137.32 (m), 137.32 – 137.27 (m), 136.47 (s), 133.15 (s), 130.57 (s), 129.25 (s), 128.48 (d, *J* = 14.4 Hz), 126.35 (s), 124.30 (s), 123.66 (s), 123.20 (s), 122.23 (s), 121.65 (s), 121.23 (s), 119.05 (s), 116.41 (s), 113.19 (s), 111.67 (d, *J* = 9.7 Hz), 110.96 (s), 49.57 (s).

Antimicrobial Activity: Antibacterial activity of the compounds, (VIa-n) were screened against gram positive, gram negative bacterial and fungal strains *Staphylococcus aureus* (NCTC 7447), *Bacillus subtilis* (MTCC-619), *E. coli* (NCTC 6571), *Streptococcus pneumonia*, *Aspergillus niger* and *Candida albicans* (recultured) respectively by disc-diffusion method and Ampicillin (100 µg/ml) Nystatin (10 µg/ml) in DMSO were used as reference antibiotics **Table 2** and **3**.

TABLE 2: ANTIBACTERIAL ACTIVITY OF N-(BENZOXAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDINE) HYDRAZINE CARBOTHIOAMIDES

Sample	Zone of inhibition											
	E. coli			*S. aureus*			*B. subtilis*			*S. pneumonia*		
	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
VIa	7	10	13	6	11	13	10	11	15	7	10	14
VIb	8	10	14	6	12	12	10	12	16	7	11	16
VIc	13	17	20	12	15	19	18	21	24	12	15	20
VId	7	11	13	5	12	11	12	12	15	6	9	13
Vie	12	16	19	10	14	18	18	19	20	11	15	17
VIf	6	9	12	7	9	12	10	12	16	6	11	15
VIg	11	16	19	10	14	17	15	17	19	10	12	14
VIh	6	11	15	5	9	14	10	14	17	6	9	13
VIi	11	15	18	12	15	16	15	17	19	10	13	17
VIj	5	9	14	6	8	14	10	16	20	5	8	11
VIk	6	8	15	4	8	14	9	14	18	6	9	13
VIl	10	13	17	10	10	14	12	18	19	9	11	14
VIm	8	12	16	7	11	15	8	14	18	7	10	12
VIn	7	11	15	7	11	13	12	16	19	5	9	11
Ampicillin (100 µg/ml)		21			20			24			22	

TABLE 3: ANTIFUNGAL ACTIVITY OF N-(BENZOXAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDINE) HYDRAZINE CARBOTHIOAMIDES

Sample	Zone of inhibition					
	A. niger			*C. albicans*		
	50µg/ml	100µg/ml	200µg/ml	50µg/ml	100µg/ml	200µg/ml
VIa	10	13	18	12	16	18
VIb	9	10	14	10	14	17
VIc	12	16	22	14	17	22
VId	8	10	13	9	13	16
Vie	11	15	21	13	16	21
VIf	8	11	12	9	12	15
VIg	10	15	20	12	15	20
VIh	9	12	17	11	13	15
VIi	10	14	19	12	15	20
VIj	8	11	16	8	14	17
VIk	7	10	15	9	13	16
VIl	11	13	19	11	14	19
VIm	7	9	10	9	12	14
VIn	6	8	11	8	11	13
Nystatin		26			24	

From the above data, it is coherent that *VIc* is highly active amongst the synthesized compounds, as it displayed better inhibition against all the bacterial and fungal species followed by Compound *VIe, VIg, VIi,* and *VIl*. Among all the species, *B. subtilis* displayed better sensitivity towards the prepared molecules.

Antimycobacterial Activity: All the fourteen compounds (*VIa-n*) were screened for anti-tubercular activity against *M. tuberculosis* MTB H 37Rv (ATCC 27294) which are susceptible to isoniazid at various concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.6 and 0.8 µg/mL) using the middle brook 7H11 medium. The anti-tubercular activity was expressed as MIC (the minimum concentration of the test sample **Table 4** that can inhibit the complete growth of the culture) and are compared with the standard drugs Isoniazid.

TABLE 4: ANTIMYCOBACTERIAL ACTIVITY OF N-(BENZOXAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDINE) HYDRAZINE CARBOTHIOAMIDES

Compound	MIC against *MTB* H37Rv (µg/mL)
VIa	12.5
VIb	25
VIc	3.12
VId	25
Vie	3.12
VIf	12.5
VIg	3.12
VIh	50
VIi	6.25
VIj	50
VIk	25
VIl	6.25
VIm	50
VIn	50
Isoniazid	0.36

The results indicated that few of the synthesized compounds exhibited comparatively good anti-tubercular activity against isoniazid sensitive *M. tuberculosis* MTB H 37Rv (ATCC 27294) strain. The results are depicted in the Table 4. From the results it is evident that the compounds *VIc*, *Vie* and *VIg* (MIC - 3.12 µg/mL) has excellent anti-tubercular activity followed by compounds *VIi* and *VIl* (MIC – 6.25 µg/mL) showed significant anti-tubercular activity against *M. tuberculosis* MTB H 37Rv (ATCC 27294) strain.

CONCLUSION: In the present work, it has been reported a convenient method by condensing different Isatins *(Va-g)* with N-(Benzoxazol-2-yl) hydrazine carbothioamide in a four-step process **Scheme 1** for the synthesis of a novel series of fourteen N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides (*VIa-n).* The antimicrobial screening and structural elucidation of the synthesized molecules encourage further investigation to develop potent antimicrobial compounds to treat deadly diseases like tuberculosis and leprosy. From the above results, it can be concluded that the benzoxazole moieties condensed with various Isatins can be a potent source for the antimicrobial agents. Further research is necessary to explore the mechanism involved in the antitubercular activity.

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