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**[Research article]**

# Synthesis and screening of new 2-(2-oxoindoline-3-ylidene)-n-Phenyl hydrazine carbothioamides

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**ABSTRACT**

The purpose of this work to prepare or to synthesize the little compound, 2-(2-oxoindoline-3-ylidene)-N-phenyl hydrazine carbothioamide derivatives by adopting appropriate synthetic routes. Then Purification and characterization of all the new compounds including those of intermediates by recrystallization from appropriate solvents or by chromatographic techniques. And Characterization of the newly synthesized compounds by physical and spectral methods (IR, $^1$HNMR & MASS). Finally evaluation of the new compounds for antimicrobial activities by standard methods available in the literature. It is evident from literature that the presence of the indole nucleus found to have various pharmacological activities like antimicrobial, anti-convulsant, MAO inhibitory, anticancer and psychotropic activities. The very fact that one of indole derivatives (isatins) is potential synthon, for building synthetically a variety of chemical systems known for their broader and pharmacological applications. In view of pharmacological significance of indole derivatives is planned to synthesize some new indole derivatives containing thiosemicarbazides.

**Key Words:** Carbothioamide, Antimicrobial activity, Dimethylsulfoxide.

## EXPERIMENTAL PROCEDURE

### STEP-I

### Synthesis of Methyl aryl carbamodithioate (III)

To a solution of aryl amine (I, 0.02M) in dimethyl sulfoxide (DMSO) (20 ml), carbon disulfide (1.9gm, 0.02M) and 20 molar aqueous sodium hydroxide solution (1.2 ml) were added drop wise simultaneously over 30 min with stirring at room temperature. The mixture was further stirred for 5 hrs. To the reaction mixture dimethyl sulphate (2.5g, 0.02M) was added drop wise with stirring at 0-5$^o$C and stirring was continued for another 3hrs. The reaction mixture was poured in to ice cold water. The solid obtained was filtered, dried in vacuum desiccators and recrystalized from n-hexane to give methyl aryl carbamodithioate.

Melting point of methyl aryl carbamodithioate: 120$^0$C

Mobile phase – Chloroform: Ethyl acetate = 1.2: 0.8

### STEP-II

### Synthesis of 4-aryl-3-thiosemicarbazide (IV)

Methyl aryl carbamodithioate (III, 0.02M) was dissolved in absolute ethanol (50ml) and an excess

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amount of hydrazine hydrate was added drop wise with stirring by keeping the reaction mixture at 5-10$^{o}$C, stirring was further continued for 5hrs at 50$^{0}$C and then the reaction mixture was poured into ice water. The solid obtained was filtered, dried and recrystallized from ethanol (65% yields) to give 4-aryl-3-thiosemicarbazide.

Melting point of 4-aryl-3-thiosemicarbazide: 138-140$^{0}$C

Mobile phase – Chloroform: Ethyl acetate = 1.2: 0.8

**STEP-III**

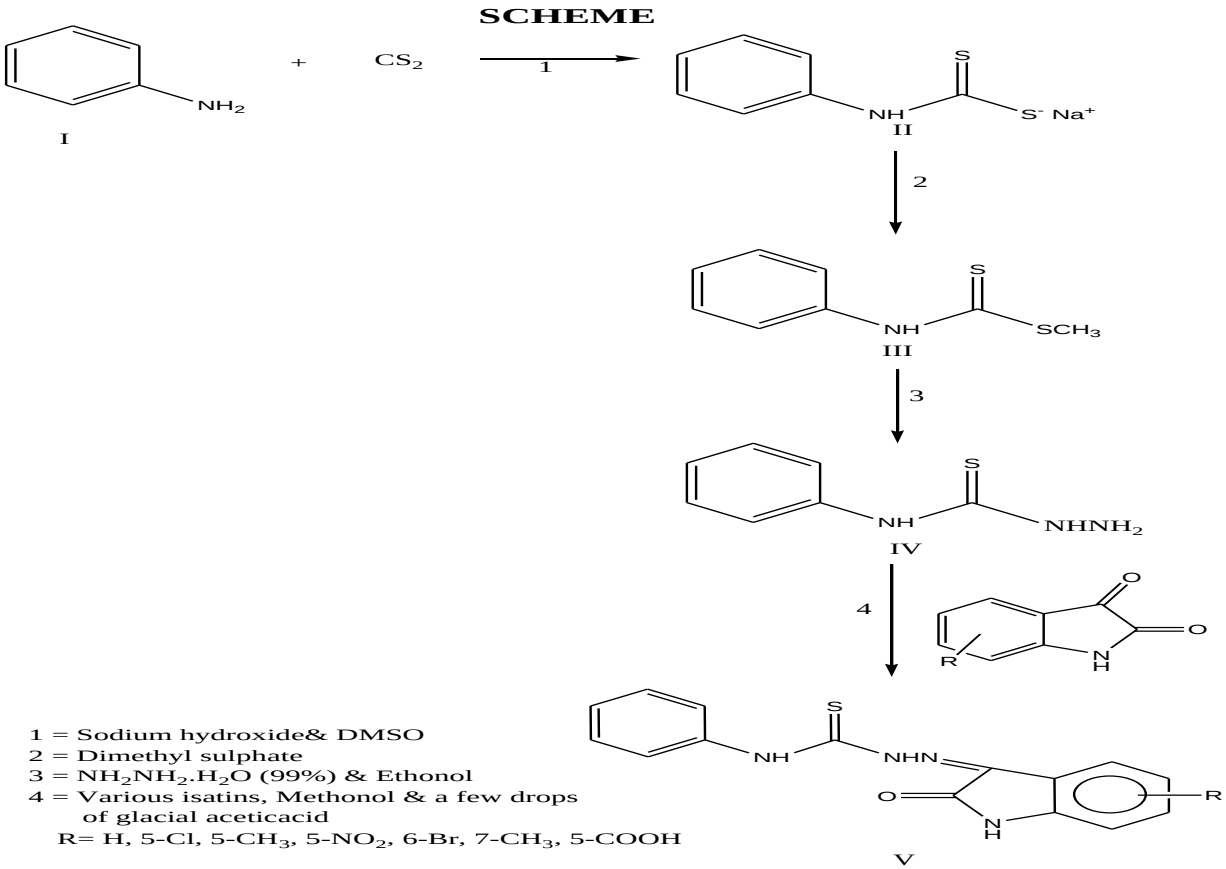
**Synthesis of substituted 2-(2-oxoindoline-3-ylidene)-N-phenyl hydrazine carbothioamide (V)**

Equimolar quantities of 4-aryl-3-thiosemicarbazide (IV) and different substituted isatins (R=H) dissolved in ethanol and few drops of glacial acetic acid was added to the mixture and refluxed for 3-4hrs. after cooling red solid was formed, filtered and recrystallized with ethanol or methanol (75 % yield) . The purity of the compound was checked by TLC. The compound has been characterized by physical and spectral data.

Melting point - 195-198$^{0}$C

Mobile phase- Chloroform: Ethyl acetate = 1.5: 0.5

**SYNTHESIS AND CHARECTERIZATION OF 2-(2-OXOINDOLINE-3-YLIDENE)-N-PHENYL HYDRAZINE CARBOTHIOAMIDES**



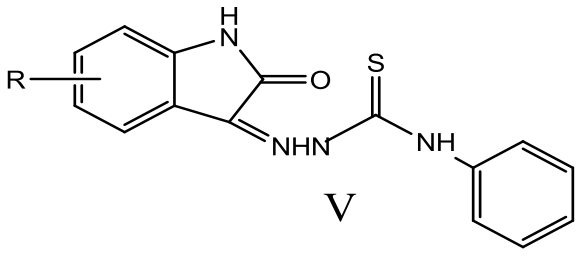
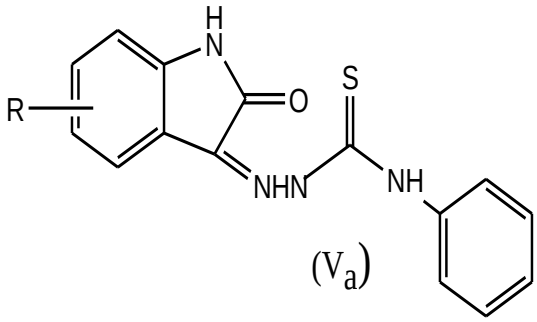



**TABLE – I Physical data of 2-(2-oxoindoline-3-ylidene)-N-phenyl hydrazine carbothioamides (Va – g)**

| S. No | Compound | Substituent's (R) | Molecular Formula | Melting Point($^0$C) | Molecular Weight | % Yield |
|---|---|---|---|---|---|---|
| 1 | $V_a$ | H | $C_{15}H_{12}N_4OS$ | 195-197 | 296 | 75 |
| 2 | $V_b$ | 5-Cl | $C_{15}H_{11}ClN_4OS$ | 216-217 | 366 | 70 |
| 3 | $V_c$ | 5-$CH_3$ | $C_{16}H_{14}N_4OS$ | 200-202 | 310 | 65 |
| 4 | $V_d$ | 5-$NO_2$ | $C_{15}H_{11}N_5O_3S$ | 210-212 | 341 | 70 |
| 5 | $V_e$ | 6-Br | $C_{15}H_{11}BrN_4OS$ | 198-200 | 374 | 60 |
| 6 | $V_f$ | 7-$CH_3$ | $C_{16}H_{14}N_4OS$ | 207-208 | 310 | 72 |
| 7 | $V_g$ | 5-COOH | $C16H_{12}N_4O_3S$ | 196-198 | 340 | 60 |

## SPECTRAL DATA

**Spectral data of 2-(2-oxoindoline-3-ylidene)-N-phenyl hydrazine carbothioamides ($V_a$):**




($V_a$)

(R= H)
Molecular formula - $C_{15}H_{12}N_4OS$
Molecular weight - 296.3
Melting point - 195-198$^0$C
Percentage yield - 75%
TLC solvent system – Chloroform: Ethyl acetate = 1.5: 0.5

**IR (KBr) Cm$^{-1}$:** 3369.36 (N-H str.), 1622.58 (C=O str.), 1537.36 (C=N str.), 1161.60 (C=S str.).

**$^1$H-NMR spectra** – 1H NMR Spectrum (DMSO) of compound V (R=H) showed characteristic peaks (δppm) at: 10.5 (1H, NH, lactam); 9.2 (S, 1H, Ar-NH); 8.2 (s, 1H, C=NH); 6.6-7.2 (m, 9H, Ar-H).

**MASS spectra** – (m /z) the mass spectrum of the compound V(R=H) showed its molecular ion ($M^{+1}$) peak at m/z 297.

## BIOLOGICAL ACTIVITY

In view of varied biological and pharmacological importance of different isatins, it has been prompted us to evaluate the new series 2-(2-oxoindoline-3-ylidene)-N-phenyl hydrazine carbothioamides for antimicrobial activities.

## ANTIBACTERIAL ACTIVITY

### Materials and Methods

Four bacterial test organisms such as *Bacillus subtilis* (MTCC441), *staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 722), and *proteus vulgaris* (MTCC 109) were selected.

Cultures of test organisms were maintained on nutrient agar slants and were sub cultured in petridishes prior to testing. The media used was nutrient agar, nutrient procured from HiMedia laboratories, Mumbai. Stock solutions of the synthesized compounds in the different concentrations, viz., 100 µg/ml, 500 µg/ml, 300 µg/ml, 50 µg/ml using dimethylsulfoxide (DMSO) as solvent for antimicrobial activity. The antibacterial activity of little compounds was assayed against four different strains of bacteria by agar diffusion method.

## CULTURED MEDIUM

Nutrient broth was used for the preparation of inoculums of the bacteria and the nutrient agar used for the screening method. Composition of medium,

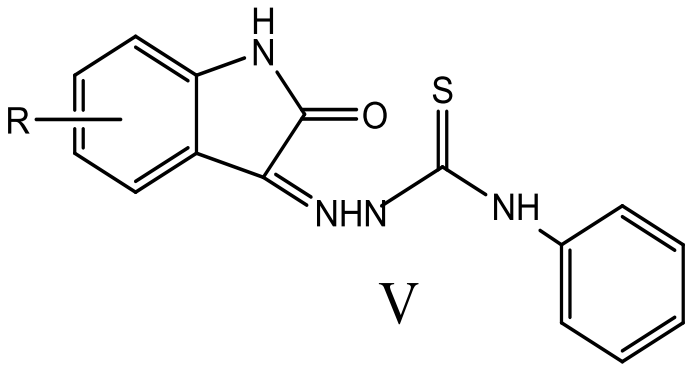
**Nutrient agar:**

| | |
|---|---|
| Peptone | 5.0gm |
| Sodium chloride | 5.0gm |
| Beef extract | 1.5gm |
| Yeast extracts | 1.5gm |
| Agar | 1.5gm |
| Distilled water | 1000ml |
| $P^H$ | 7.4±0.2 |

The test organism was subculture using nutrient agar medium. The tubes contain sterilized medium were inoculated with respective bacterial strain. After incubation at 37±1$^o$ C for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacterial medium in oculum was prepared by transferring a loop full of stock culture to nutrient broth. The flasks were incubated at 37±1 $^0$C for 48 hours before the experimentation.

Solution of test compounds was prepared by dissolving 10mg each in dimethylsulfoxide (DMSO, 10ml). A reference standard for gram positive and gram-negative bacteria was made by dissolving accurately weighed quantities of ampicilin in DMSO (10µg/ml).

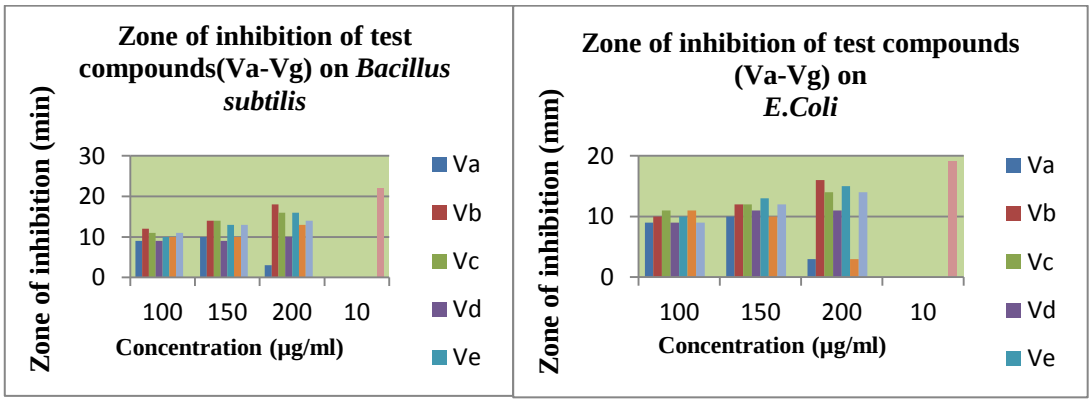
The nutrient agar was sterilized by autoclaving at 121$^o$C (15lb/sq.inch) for fifteen minutes. Petri plates, tubes and flasks plugged in cotton were sterilized in hot-air oven at 160$^o$C for an hour. Into each sterilized petriplate (10cm diameter), about 27ml of molten nutrient agar medium inoculated with the respective strain of bacteria (50µl of in oculum into each plate) was transferred aseptically. The plates were left at room temperature solidification. In each plate, three discs of 6mm diameter were made with a sterile borer. These solutions at concentrations (200µgm/ml, 150µg/ml and 100µg/ml) were added to respective disc aseptically and labeled accordingly. The plates were kept and undisturbed for one hour at room temperature to allow the diffusion of the solution properly in the nutrient agar medium. After incubation of the plates at 37±1$^o$C for 24 hours, the diameter of zone inhibition surrounding each of discs was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1ml of DMSO to observe the solvent effects and the results represented in table-II.




V

**TABLE –II Antibacterial activity of 2-(2-oxoindoline-3-ylidene)-N-phenyl hydrazine carbo -thioamides (Va-Vg)**

| S. No. | Compound | Substituents (R) | Concentration in µg/ml | Zone of inhibition (mm) | |
|---|---|---|---|---|---|
| | | | | *B. subtilis* | *E. Coli* |
| 1 | $V_a$ | H | 100 | 9 | 9 |
| | | | 150 | 10 | 10 |
| | | | 200 | 3 | 3 |
| 2 | $V_b$ | 5-Cl | 100 | 12 | 10 |
| | | | 150 | 14 | 12 |
| | | | 200 | 18 | 16 |
| 3 | $V_c$ | 5-$CH_3$ | 100 | 11 | 11 |
| | | | 150 | 14 | 12 |
| | | | 200 | 16 | 14 |
| 4 | $V_d$ | 5-$NO_2$ | 100 | 9 | 9 |
| | | | 150 | 9 | 11 |
| | | | 200 | 10 | 11 |
| 5 | $V_e$ | 6-Br | 100 | 10 | 10 |
| | | | 150 | 13 | 13 |
| | | | 200 | 16 | 15 |
| 6 | $V_f$ | 7-$CH_3$ | 100 | 10 | 11 |
| | | | 150 | 10 | 10 |
| | | | 200 | 13 | 13 |
| 7 | $V_g$ | 5-COOH | 100 | 11 | 9 |
| | | | 150 | 13 | 12 |
| | | | 200 | 14 | 14 |
| 8 | Standard | | 10 | 22 | 19 |




## ANTIFUNGAL ACTIVITY

For the antifungal screening of synthesized compounds, *Candida albicans* and *Aspergillus Niger* were used.

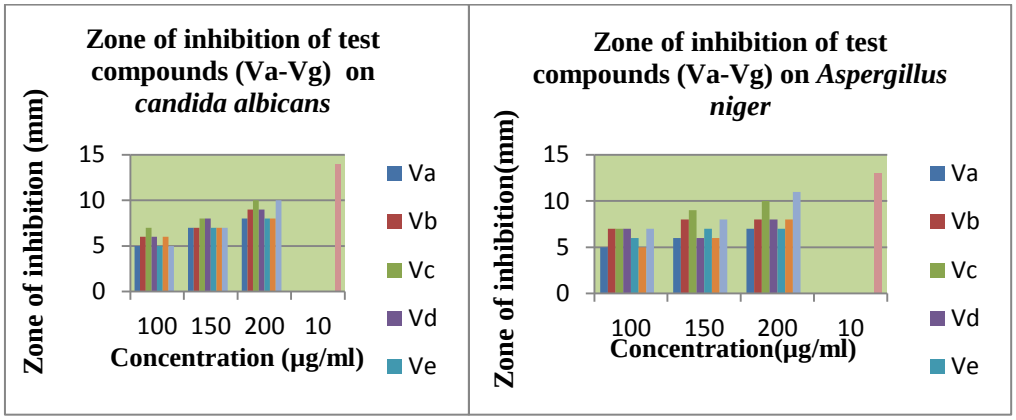
### Sabourad dextrose agar medium

| | |
|---|---|
| Mycological Peptone | 10gm |
| Dextrose | 14gm |
| Agar | 17gm |
| Distilled water | 1000ml |
| $P^H$ | 7.4±0.2 |

The test organisms were subculture using SDA medium. The tubes containing sterilized medium were inoculated with test fungi and after inoculation at 25°C for 48 hours, were stored at 4°C in refrigerator. The in oculum was prepared by taking a loop full of stock culture to about 5ml of sabourad dextrose broth in a test Tube. The tubes were incubated at 25°C for 48 hours before use. The solution of test compound was prepared by a similar procedure described under the antibacterial activity. A reference standard solution of Clotrimazole (10µg/ml) was prepared by dissolving

10mg of clotrimazole in 10ml of dimethylsulfoxide.

The SDA medium was sterilized by autoclaving at 121°C (15 lb/sq. inches) for fifteen minutes. The Petri plates were sterilized in hot air woven at 160°C for an hour. Into each sterilized Petri plate about 27ml of molten SDA medium was added and incubated at 30°C for two days. After two days of incubation, the medium free of contamination was spreader with 50µl of 48 hours culturing. After solidification of the cups of 6mm diameter were made in each plate with sterile borer. Accurately 50µl of 200µg/ml, 150µg/ml and 100µg/ml of test solutions were transferred to the respective petri plates aseptically and labeled accordingly. The reference standard 50µl was also added to the discs in each plate. The plates were kept in refrigerator for one hour to allow the solution to diffuse properly into the SDA medium. Then, the plates were incubated at 25°C for 8 hours at inverted position. The diameter of zone of inhibition was read with the help of an antibiotic zone reader. The experiment was performed in triplicate and the results were represented in table-III.




## DISCUSSION & CONCLUSIONS

The purity of the synthesized compounds was checked by performing TLC and melting points. All the final synthesized compounds were purified by recrystallization using appropriate solvents. All the compounds characterized by IR, $^1$H NMR and Mass spectral data. All the synthesized compounds were tested for *invitro* antibacterial and antifungal activity by agar diffusion method and the values were presented in table-II & III. The zone of inhibition values of the synthesized compounds against *Bacillus subtilis* (Gram positive bacteria) and *Escherichia coli* (Two Gram-Negative bacteria) were presented in table-II. Ampicillin was used for the reference for inhibitory activity against bacteria. All the compounds exhibited mild to moderate activity against bacteria, compound $IV_b$ (R=5-Cl) and $IV_c$ (R=5-$CH_3$) were found to be most active against both Gram (+ve) and Gram (-ve) organisms among all the test compounds. The antifungal activity of the compounds was studied against *candiada albicans* and *Aspergillus Niger*. Ketaconazole was used for the reference for inhibitory activity against fungi. All the compounds showed mild to moderate activity. Compound IVc (R=5-$CH_3$) and $IV_d$(R=$NO_2$) were found to be the most active antifungal agents among all the tested compounds. The synthesized compounds exhibited mild to moderate activity against bacteria and fungi. It has been felt necessary from the results of the present antimicrobial investigations that there is a need for further advanced studies, at least on the few of the test compounds which are found to be superior.

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