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# Electrochemical and biological behaviour of synthesized (2*Z*)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substitutedphenyl)hydrazinecarbothioamide

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**Abstract : The electrochemical behaviour of substituted thiosemicarbazones has been investigated. A series of thiosemicarbazones (2*Z*)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substitutedphenyl)hydrazinecarbothioamide have been prepared. The structure elucidation was done by elemental and spectral analysis. The electro reductions of synthesized compounds have been also studied by cyclic voltammetry at glassy carbon electrode, which indicate the reducible behaviour from its cyclic voltammogram. The nature of electrochemical process of all the synthesized compounds were studied on DME and HMDE using polarography, cyclic voltammetry, and constant-potential coulometry techniques. The data revealed that the electrode process was irreversible and diffusion controlled. The kinetic parameters, i.e. charge transfer coefficient ($\alpha_{na}$), formation constant ($k^{\circ}_{f,h}$) and diffusion coefficient were calculated. The synthesized compounds were also screened for antimicrobial activities.**

**Keywords : Thiosemicarbazones, differential pulse polarography, cyclic voltammetry, antimicrobial activity.**

## Introduction

Thiosemicarbazones have been reported to exhibit antitubercular activity[1,2]. Besides this, 1,3,4-thiadiazoles, 1,3-thiazoles and their derivatives exhibit various biological activities such as antimicrobial[3–5], anti-inflammatory[6–8], anticonvulsant[9,10], antihypertensive[11,12], local anaesthetic[13], anticancer[14,15], hypoglycemic[16], and cytotoxic activities[17–23]. A phenyl thiosemicarbazide bearing methoxy and chloro moieties showed good antimicrobial activity[24,25].

The reduction of aromatic thiosemicarbazones should occur through the cleavage of N=N bond by 4 electrons process. In the present work, a series of (2*E*)-3-(2-hydroxyphenyl)-*N*-(4-phenylsubstituted)triaz-2-ene-1-carbothioamide have been synthesized and the nature of electrochemical process of all the synthesized thiosemicarbazones were studied on DME and HMDE using polarography, cyclic voltammetry, and constant-potential coulometric techniques.

## Experimental

*Materials* : All the chemicals were obtained from Aldrich Chemical Co., Germany. KCl (1.0 mol $L^{-1}$) solution was prepared in distilled water and used as supporting electrolyte. Stock solutions of synthesized (2*E*)-3-(2-hydroxyphenyl)-*N*-(4-substitutedphenyl)triaz-2-ene-1-carbothioamide were prepared in ethanol and dimethyl formamide. A series of Britton-Robinson buffers in the pH range 2.5 to 11.0 were prepared. The readymade precoated TLC silica gel plates from E. Merck, Germany were used for TLC separation. The differential pulse polarographic studies were carried out using 2.0 mL stock solution of depolarizer, 1.0 mL of 1 *M* KCl, and 7.0 mL of appropriate buffer. The influence of several buffers (phosphate, Britton-Robinson and acetate buffer) on the analytical signals was also studied and best reduction peaks were observed in BR buffer. The microbial sensitivity has been assessed by using disc diffusion technique and broth dilution technique.

*Apparatus :*

The differential pulse polarographic (DPP) measurements were carried out using the ELICO CL 362 polarographic analyzer (India). The drop time of 1 s was electronically controlled using a 663 VA stand from the company. The polarograms were recorded using a potential rate of 100 mV $s^{-1}$. A three-electrode system composed of a dropping mercury electrode (DME), saturated calomel electrode (SCE) as reference electrode and platinum wire as an auxiliary electrode were used. The voltammetry experiments were performed using an Autolab type II (Eco-Chemie B.V., Utrecht, The Netherlands) potentiostat-galvanostat with 757 VA computrace software. The utilized electrodes were hanging mercury drop electrode (HMDE) as working electrode, Ag/AgCl as reference electrode and a graphite rod as auxiliary electrode. The electrochemical cell was Metrohm 663 VA stand. Controlled potential electrolysis experiments were performed using an Autolab Potentiostat/Galvanostat PGSTAT Metrohm 663 VA stand as electrochemical cell, fitted with a computer provided with the appropriate GPES 4.2 (General Purpose Electrochemical Software). Coulometric experiments were performed in the potentiostatic mode using Pt foil with large surface area as working electrode and a Pt wire as counter electrode. Elemental analysis was carried out on Carlo Erba 1108 analyser. The IR spectra were recorded on Shimadzu, Japan, model Prestige IR 20, spectrophotometer, $^1$H NMR spectra were recorded on Varian EM-390 MHz NMR spectrometer in DMSO-$d_6$ using TMS as internal reference and chemical shift values were expressed in ppm. The pH of the buffer was checked using a pH meter (Decible DB-1011 digital pH meter) with the help of combined glass calomel electrode.

*Procedure* :

*Synthesis of N-phenylhydrazinecarbothioamide* :

Thiosemicarbazide has been synthesized in two steps.

*Synthesis of phenyl isothiocyanate* :

A 500 ml round bottom flask equipped with a mechanical stirrer and a dropping funnel, was cooled in a freezing mixture. A concentrated aqueous ammonia solution (41 mL) was added into the flask through the dropping funnel, followed by $CS_2$ (24 mL) and the resulting mixture was stirred continuously. Aniline (30 mL), dissolved in ethanol (40 mL) was added through the dropping funnel and stirred for about 20 min, and heavy precipitate of ammonium tolyldithiocarbamate separated out. This salt was transferred into a 5 liter round bottom flask containing 200 mL water. A solution of lead nitrate (90 g in 200 mL water) was added to it and the reaction mixture was continuously stirred till lead sulphide precipitated out. The reaction mixture was subjected to steam distillation. Isothiocyanatobenzene was separated out from the distillate. Diethyl ether was used to extract white shining crystals of isothiocyanatobenzene.

*Synthesis of N-phenylhydrazinecarbothioamide* :

Solution of isothiocyanatobenzene (0.1 *M*) in ethanol was taken in a round bottom flask. A solution of hydrazine hydrate (0.1 *M*) in ethanol was added to it at once, followed by continuous stirring. An exothermic reaction accompanied by vigorous effervescences was observed. The contents of the flask were refluxed on a water bath for 4–5 h, resulting in the formation of a white precipitate of phenyl thiosemicarbazide, which was filtered and washed repeatedly with ethanol. The product was found to be soluble in dioxane, dimethyl sulphoxide and dimethylformamide. Yield : 72%, m.p. 175 °C; IR (KBr) $cm^{-1}$ : 3296 and 3254 (N-H str.), 1277 (C=S str.), 2924 and 2850 (C-H str.).

*Synthesis of (2Z)-2-(4-hydroxy-3-methoxy benzylidene)-N-(4-substitutedphenyl)hydrazinecarbothioamide* :

A solution of phenylhydrazinecarbothioamide (1 mM; in minimum, quantity of dimethyl formamide) was taken in a flask and 4-hydroxy-3-methoxy benzaldehyde (1 mM; in dimethyl formamide) was slowly added with continuous stirring. The contents of the flask were refluxed for 8–10 h. The reaction mixture was cooled in an ice-bath and a drop of sulphuric acid was added. On addition of requisite amount of distilled water, the product (2*Z*)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substituted-phenyl)hydrazinecarbothioamide separated out as a light brown solid. It was repeatedly washed with distilled wa-

ter and finally with ethanol. The product was found to be soluble in dimethyl sulfoxide and dimethyl formamide. Yield : 70%, m.p. 189 °C; IR (KBr) $cm^{-1}$ : 3296 and 3254 (N-H str.), 1277 (C=S str.), 2924 and 2850 (C-H str.). The other thiosemicarbazone[26–30] derivatives were synthesized by the aforementioned procedure.

**Results and discussion**

The route followed for the synthesis of (2*Z*)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substitutedphenyl)-hydrazinecarbothioamide in the laboratory was based on the condensation of aldehyde and primary aromatic amines (Scheme 1). The products were obtained in 80–85% yields.

*Differential pulse polarography* :

A single reduction wave was observed for all the synthesized compounds (i to iii) at dropping mercury electrode in the pH range 2.5 to 10.0 assigning to the reduction of -CH=N- group (Fig. 1). The peak potential shifted towards more negative potential with the rise in pH for all the compounds indicating the participation of protons in electrode process (Fig. 2). On increasing the concentration of (2*E*)-3-(2-hydroxyphenyl)-*N*-(4-substituted-phenyl)triaz-2-ene-1-carbothioamide $-E_{1/2}$ shifted towards more negative potential. This behavior clearly indicated towards irreversible nature of the electrode process, which was further confirmed by logarithmic analysis i.e. the slope of the plot of [$-E_{d.e.}$ vs log ($i/i_d - i$) – 0.546 log $t$] was greater than 59.2/$n$ mV.

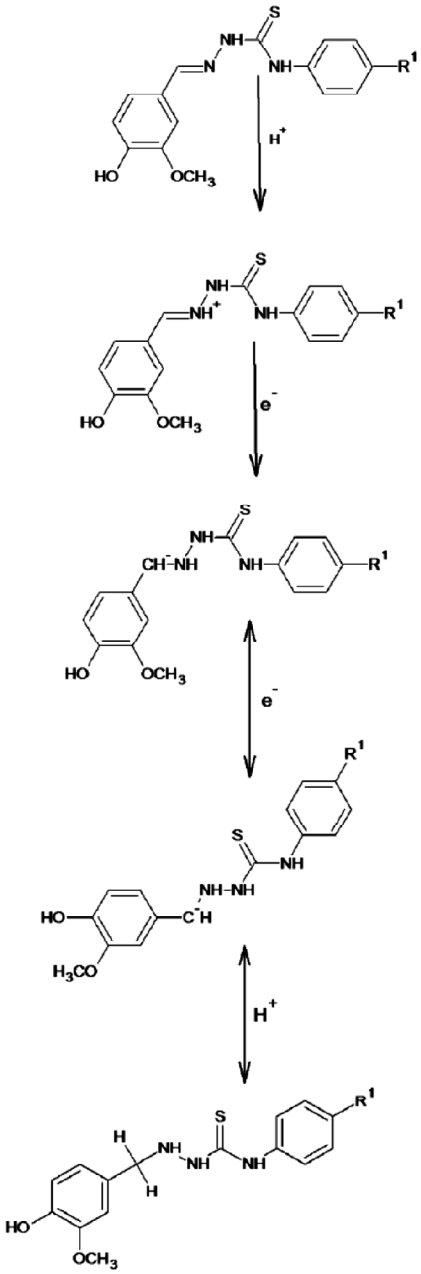
*Effect of concentration* :

For all the synthesized (2*Z*)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substitutedphenyl)hydrazine-carbothioamide the cathodic peak current increased linearly with concentration. The plot of $i_d$ vs concentration (Fig. 3) was a straight line which also supports the diffusion controlled nature of the electrode process.

Logarithmic analysis of the current vs peak potential indicated towards the irreversible nature of the electrode process. The value of the slope of the plots of $-E_{d.e.}$ vs [log ($i/i_d - i$) –0.546 log $t$] (Table 1 and Fig. 4) exceeded appreciably from 0.059/$n$ mV, confirming the irreversible nature of the electrode reaction.

*Cyclic voltammetry* :

Cyclic voltammograms of (2*Z*)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substitutedphenyl)hydrazine-carbothioamide ($10^{-3}$ *M*) in BR buffer exhibited a single cathodic peak in the potential range –0.20 to –1.50 V, assignable to the reduction of –CH=N- group in the pH range 2.5 to 11.5 (Fig. 5). A small shoulder before the peak is due to the adsorption phenomenon. In this pH range no anodic peak could be realized indicating the




R′ = -$CH_3$, -$OCH_3$, -Cl

**Scheme 1**

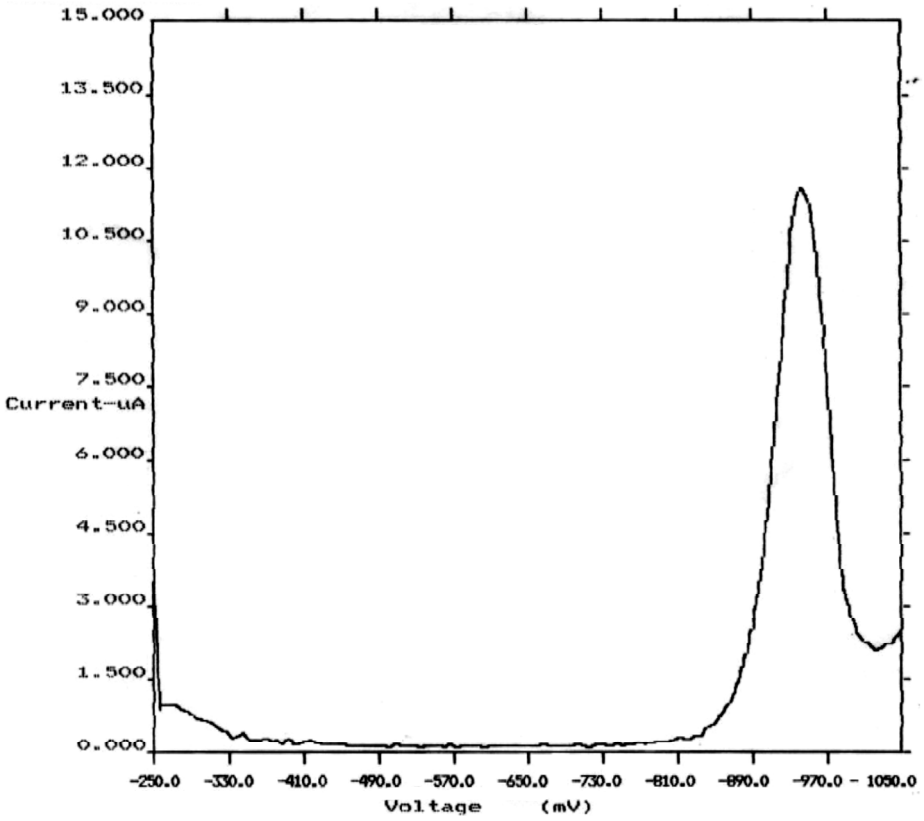



**Fig. 1.** A typical differential pulse polarogram of (2*Z*)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substitutedphenyl)hydrazinecarbothioamide at conc. $0.5 \times 10^{-3}$ *M*, pulse amplitude 100 mV $s^{-1}$ and pH 2.5.

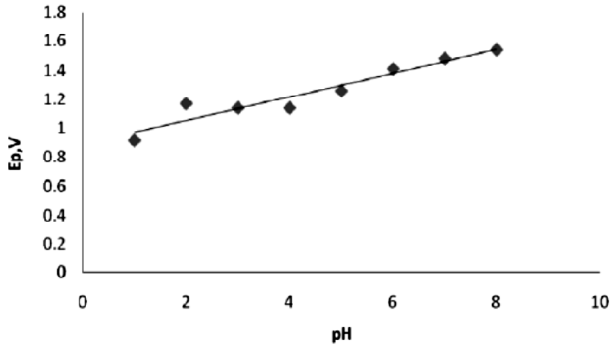



**Fig. 2.** Plot of peak potential vs pH for 2-methoxy-4-[(*Z*)-{2-[(4-methylphenyl)carbamothioyl]hydrazinylidene}methyl]phenyl acetate at different pH, conc. $0.5 \times 10^{-3}$ *M* and pulse amplitude 100 mV $s^{-1}$.

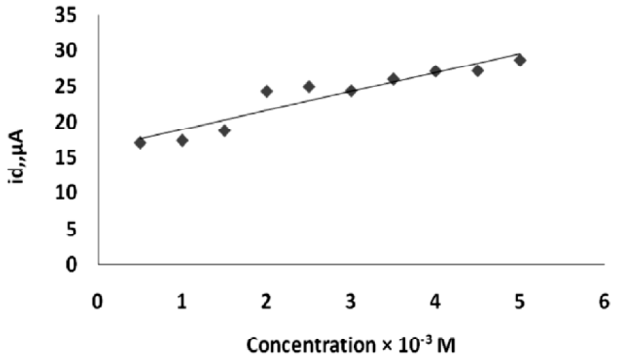



**Fig. 3.** Graph between peak current vs different concentrations of (2*Z*)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-methoxyphenyl)hydrazine carbothioamide at pH 2.5.

irreversible nature of the electrode process.

*Effect of scan rate* :

The plot of $i_d$ (µA) vs $\upsilon^{1/2}$ ($\upsilon$ = scan rate) was found to be straight line passing through the origin, indicating the diffusion-controlled nature of the electrode process. The current function ($i_{pc}/\upsilon^{1/2}$) for -CH=N- group depends on the scan rates. The cathodic peak potentials were found to shift towards more negative potential with increasing scan rate, as expected for irreversible electron transfer (Fig. 6).

**Table 1.** Logarithmic analysis of (2Z)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substitutedphenyl)hydrazinecarbothioamide at pulse amplitude 100 mV $s^{-1}$

| Conc. (mL) | pH | –*R* | $-E_d$ (V) | $i_d$ (μA) | log $\{i/i_d - i\}$ – 0.546 log *t* |
|---|---|---|---|---|---|
| 0.5 | 2.5 | $-CH_3$ | 0.91 | 8.7 | 0.296 |
| | | | | 9.02 | |
| | | | 0.92 | 10.6 | 0.344 |
| | | | 0.94 | 11.2 | 0.627 |
| | | | 0.95 | 12.4 | 0.770 |
| | | | 0.97 | 13.1 | 1.117 |
| 1.5 | 3.9 | $-OCH_3$ | 0.95 | 3.06 | –0.53 |
| | | | 0.97 | 4.79 | –0.25 |
| | | | 0.98 | 6.53 | –0.22 |
| | | | 1.01 | 8.27 | 0.22 |
| | | | 1.04 | 10 | 0.46 |
| | | | 1.1 | 11.75 | 0.84 |
| | | | 1.11 | 13.4 | 1.12 |
| 2.0 | 2.5 | -Cl | 0.89 | 6.8 | –0.1 |
| | | | 0.91 | 8.51 | 0.09 |
| | | | 0.93 | 10.23 | 0.29 |
| | | | 0.95 | 11.9 | 0.53 |
| | | | 0.97 | 13.6 | 0.89 |
| | | | 0.99 | 15.34 | 1.18 |

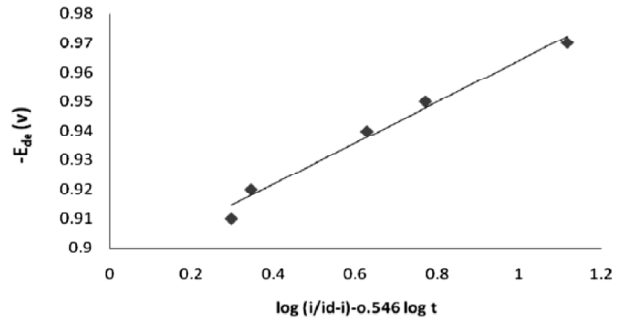



**Fig. 4.** Plots of $-E_{d.e}$ vs log $(i/i_d - i) - 0.546$ log *t* of (2Z)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-methylphenyl)hydrazinecarbothioamide at pulse amplitude 100 mV $s^{-1}$, conc. $0.5 \times 10^{-3}$ *M* and pH 2.5.

*Controlled potential coulometry* :

Controlled potential coulometry was employed for determining the number of electrons (*n*) transferred in the electrode process. Number of electrons *n*, were calculated from the charge consumed by the desired concentration of (2Z)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-methoxyphenyl)hydrazinecarbothioamide. For this purpose 2 mL of $10^{-3}$ *M* solution of the electroactive species was placed in the cell and electrolysis was carried out. During electrolysis, the solution was continuously stirred and purged with nitrogen. Number of electrons *n* was calculated using the equation $Q = n\,F\,N$, where *Q* is the charge in coulombs, *F* is the Faraday constant, and *N* is the number of moles of substrate.

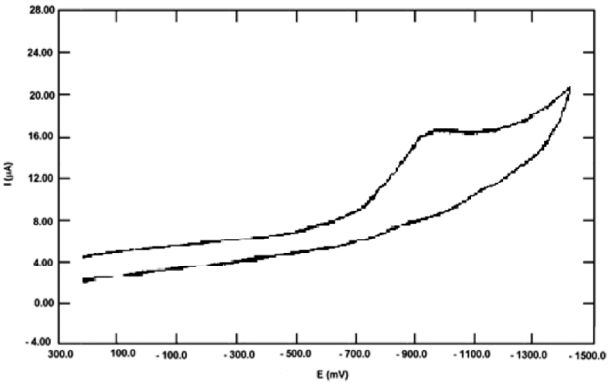



**Fig. 5.** Cyclic voltammetry of (2Z)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substitutedphenyl)hydrazinecarbothioamide.

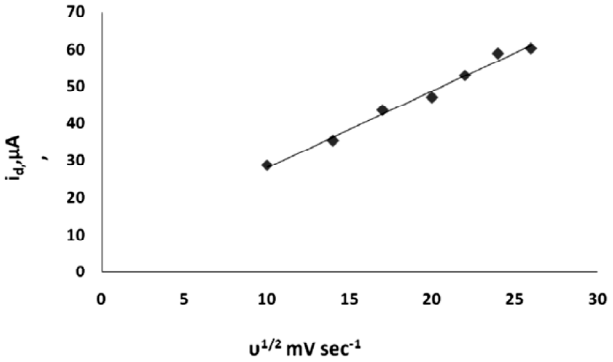



**Fig. 6.** Plot of $i_d$ vs scan rate of (2Z)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-methoxyphenyl)hydrazinecarbothioamide at pH 2.5.

*Reaction mechanism* :

On the basis of differential pulse polarography, CV and coulometry a mechanism has been postulated for the reduction of (2Z)-2-(4-hydroxy-3-methoxybenzylidene)-*N*-(4-substitutedphenyl)hydrazinecarbothioamides. General sequences for addition of proton and electron viz. $H^+$, $e^-$, $H^+$, $e^-$ and $H^+$, $e^-$, $e^-$, $H^+$, the later is found to be more probable for these compounds.

**Table 2.** Antimicrobial study of synthesized compounds

| Sr. No. | Compounds | Diameter of zone of inhibition in mm | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *A. niger* | | *C. albicans* | | *S. aureus* | | *B. antricis* | |
| | | 200 µg/mL | 400 µg/mL | 200 µg/mL | 400 µg/mL | 200 µg/mL | 400 µg/mL | 200 µg/mL | 400 µg/mL |
| 1. | (i) | 8.3 | 12.3 | 9.5 | 12.5 | 9.8 | 13.0 | 8.3 | 12.3 |
| 2. | (ii) | 8.6 | 12.7 | 9.4 | 12.5 | 9.8 | 13.3 | 8.6 | 12.7 |
| 3. | (iii) | 8.8 | 12.9 | 9.8 | 12.8 | 9.8 | 13.4 | 8.8 | 12.9 |

*Antimicrobial profile of thiosemicarbazones* :

It is clearly evident from Table 2, in the thiosemicarbazone analogs; the most potent activity has been shown by compound, bearing two methxoy groups against the tested pathogens in the order of *A. niger*, *C. albicans*, *S. aureus*, *B. antricis*. Simultaneously, compound with chloro, methoxy and methyl groups present in aldehydic portion with chloro substitution in thiosemicarbazide also showed appreciable zone of inhibition against the tested pathogens. It has further been found that withdrawal of the above bio-labile groups from the parent compound reduces the activity against the tested pathogens. Antibudenial efficacy of synthesized compound was also found comparable to the standard drugs for *S. aureus*. Among the tested compounds, methoxy and chloro group in the thiosemicarbazone series showed highest activity in the order : *C. albicans* > *S. aureus* > *B. antricis* > *A. niger* at 800 µg/disc concentration. The compounds have also assured antifungal activity in *C. albicans* and *S. aureus* than the standard drug fluconazole.

It was observed that the thiosemicarbazone series was more active. All compounds showed better activity in comparison to the reference drug.

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