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# Pyrazole Carbothioamide Analogues: Synthesis, Characterisation and Antifungal Evaluation

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**Abstract :** A simple procedure for the synthesis of pyrazole carbothioamides was developed. Cyclocondensation reaction of chalcones with thiosemicarbazide hydrochloride in the presence of acid in methanol under reflux conditions produced pyrazole carbothioamides in good yields. The synthesized new compounds were characterized by spectral studies and elemental analysis; and were screened *in vitro* for their antifungal susceptibilities against different fungi species.
**Key words :** Antifungal, condensation, inhibition, pyrazoline, spectral.

## Introduction

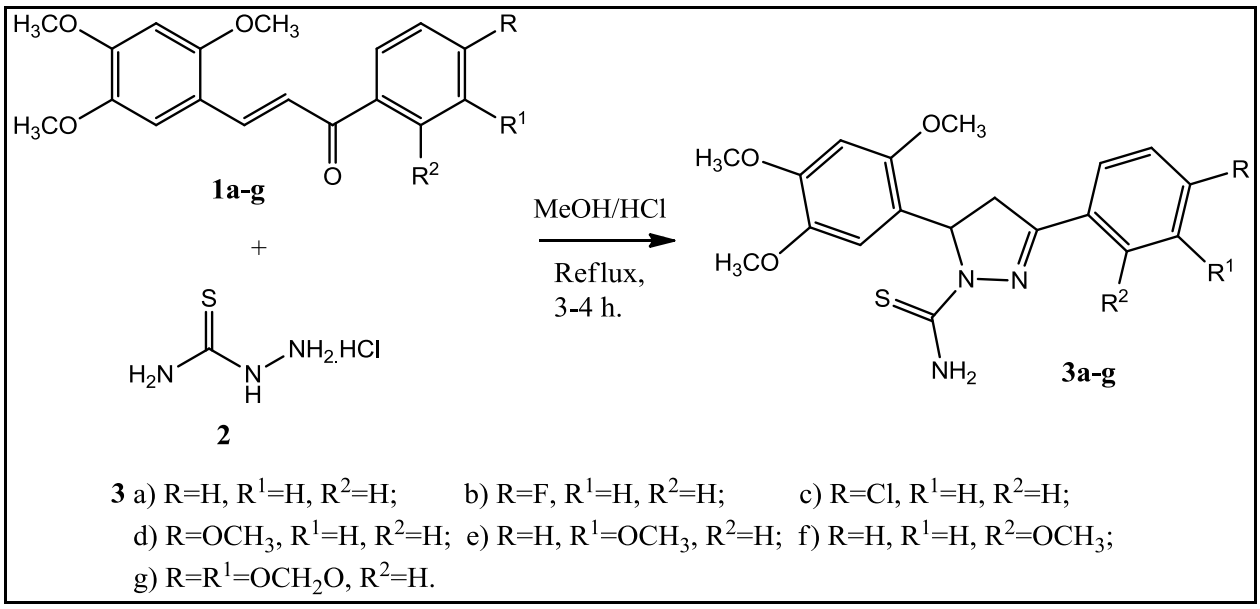
The synthesis of pyrazole and its analogues has been a subject of consistent interest because of the wide range of synthetic and pharmaceutical applications. These classes of simple molecules have been extensively used as building blocks for the construction of various classes of bioactive molecules. Usual synthesis of pyrazoles involves, (i) the reaction of 1, 3-diketones with hydrazines, (ii) the reaction of α, β-unsaturated aldehyde and ketones with hydrazines, 1, 3-dipolar cycloaddition reactions of nitrile imines to alkenes and alkynes.[1] Cyclocondensation of β-thioalkyl-α,β-unsaturated ketones with hydrazines produced pyrazoles with high regioselectivity.[2] Earlier we have reported an efficient synthesis of substituted pyrazole carbothioamides by an acid catalysed reaction of α,β-unsaturated ketones with semicarbazide hydrochloride in good yields.[3]

Pyrazole derivatives have been known to exhibit broad spectrum of biological applications, such as swine kidney diamine oxidase (SKDAO) inhibitors,[4] antioxidant,[5] anti-tubercular,[6] antimicrobial,[7] anticancer,[8] anti-inflammatory[9] activities. In view of broad spectrum of synthetic and biological applications associated with pyrazole derivatives; and in continuation of our work on pyrazole carbothioamides,[10] we herein report the synthesis of series of new pyrazole carbothioamides and the results of their antimicrobial activities.

## Materials And Methods

Melting points were determined by an open capillary tube method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates pre-coated with silica gel using solvent system ethyl acetate: dichloromethane (1:4 v/v). The spots were visualized under UV light. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on Agilent-NMR 400 MHz and 100 MHz spectrometer respectively. The solvent $CDCl_3$ with TMS as an internal standard was used to record the spectra. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Mass Lynx SCN781 spectrometer TOF mode. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer. Purification of compounds was done by column chromatography on silica gel (70-230 mesh Merck).

The cyclocondensation reaction of chalcones **1a-g** with thiosemicarbazide hydrochloride, **2** and few drops of concentrated hydrochloric acid in methyl alcohol under reflux conditions produced a series of pyrazole cabothioamides, **3a-g**. The schematic diagram for the synthesis of pyrazole carbothioamides is outlined in **Scheme 1**.




**3** a) R=H, $R^1$=H, $R^2$=H; b) R=F, $R^1$=H, $R^2$=H; c) R=Cl, $R^1$=H, $R^2$=H; d) R=$OCH_3$, $R^1$=H, $R^2$=H; e) R=H, $R^1$=$OCH_3$, $R^2$=H; f) R=H, $R^1$=H, $R^2$=$OCH_3$; g) R=$R^1$=$OCH_2O$, $R^2$=H.

**Scheme 1: Schematic diagram for the synthesis of pyrazole carbothioamides, 3a-g.**

**Synthesis of chalcones:**

The intermediate chalcones, **1a-g** were obtained according to our reported procedure by Claisen-Schmidt condensation of 2,4,5-trimethoxybenzaldehyde and substituted acetophenones in the presence of potassium hydroxide in 95% ethyl alcohol at room temperature.[11,12]

**General procedure for the synthesis of pyrazole carbixanudes, 3a-g:**

To a stirred solution of chalcones, **1a-g** (5 mmol) and thiosemicarbazide hydrochloride, **2** (10 mmol) in methyl alcohol (15 mL), concentrated hydrochloric acid (7-8 drops) were added. The mixture was refluxed for 3-4 h and the progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured in to ice cold water; solid separated was filtered, washed with ice cold water and dried. The products were purified column chromatography using silica gel (60-120 mesh) and ethyl acetate : dichloromethane (1:3 v/v) as eluent.

***3-Phenyl-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide,*** **3a:** Obtained from (*E*)-1-phenyl-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **1a** (5 mmol) and thiosemicabazide hydrochloride, **2** (10 mmol) in 72% yield, m.p. 112–114°C. $^1$H NMR: δ 3.132-3.178 (dd, 1H, *J= 7.2, 15.3Hz*, $C_4$-$H_a$), 3.904-3.968 (dd, 1H, *J= 6.0, 13.2Hz*, $C_4$-$H_b$), 3.834 (s, 6H, $OCH_3$), 3.852 (s, 3H, $OCH_3$), 4.290-4.344 (dd, 1H, *J= 8.9, 14.0Hz*, $C_5$-H), 7.106–7.750 (m, 7H, Ar–H), 8.126 (s, 2H, $NH_2$); $^{13}$C NMR: δ 40.48 (1C, C-4), 55.45 (3C), 62.56 (1C, C-5), 101.80 (1C), 113.87 (1C), 122.34 (1C), 128.36 (2C), 129.05 (2C), 131.20 (1C), 136.62 (1C), 142.10 (1C), 147.20 (1C), 149.86 (1C), 151.32 (1C, C-3), 176.80 (1C, C=S). MS *m/z*: 371 ($M^+$, 100); Anal. calcd. for $C_{19}H_{21}N_3O_3S$ (%): C, 61.44; H, 5.70; N, 11.3; Found: C, 61.40; H, 5.58; N, 11.25.

***3-(4-Fluorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide,*** **3b:** Obtained from (*E*)-1-(4-fluorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **1b** (5 mmol) and thiosemicarbazide hydrochloride, **2** (10 mmol) in 74% yield. $^1$H NMR: δ 3.140-3.186 (dd, 1H, *J= 7.0, 12.1Hz*, $C_4$-$H_a$), 3.913-3.959 (dd, 1H, *J= 6.7, 13.8Hz*, $C_4$-$H_b$), 3.840 (s, 6H, $OCH_3$), 3.856 (s, 3H, $OCH_3$), 4.310-4.346 (dd, 1H, *J= 8.1, 14.7Hz*, $C_5$-H), 7.116–7.758 (m, 6H, Ar–H), 8.130 (s, 2H, $NH_2$); $^{13}$C NMR: δ 40.78 (1C, C-4), 55.50 (3C), 62.70 (1C, C-5), 101.98 (1C), 113.60 (1C), 116.30 (2C), 122.32 (1C), 129.15 (2C), 131.66 (1C), 136.60 (1C),

142.55 (1C), 147.70 (1C), 151.77 (1C, C-3), 163.80 (1C), 177.12 (1C, C=S). MS *m/z*: 389 ($M^+$, 100); Anal. calcd. for $C_{19}H_{20}FN_3O_3S$ (%): C, 58.60; H, 5.18; N, 10.79; Found: C, 58.46; H, 5.08; N, 10.66.

***3-(4-Chlorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide,*** **3c:** Obtained from (E)-1-(4-chlorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **1c** (5 mmol) and thiosemicarbazide hydrochloride, **2** (10 mmol) in 80% yield; $^1$H NMR: δ 3.144-3.180 (dd, 1H, *J= 9.0, 17.0Hz*, $C_4$-$H_a$), 3.924-3.969 (dd, 1H, *J= 7.3, 13.8Hz*, $C_4$-$H_b$), 3.836 (s, 6H, $OCH_3$), 3.855 (s, 3H, $OCH_3$), 4.298-4.340 (dd, 1H, *J= 8.2, 14.4Hz*, $C_5$-H), 7.146–7.778 (m, 6H, Ar–H), 8.158 (s, 2H, $NH_2$); $^{13}$C NMR: δ 41.28 (1C, C-4), 55.46 (3C), 62.45 (1C, C-5), 101.85 (1C), 113.62 (1C), 122.30 (1C), 127.77 (2C), 128.60 (2C), 133.34 (1C), 137.44 (1C), 139.74 (1C), 141.50 (1C), 148.22 (1C), 151.71 (1C, C-3), 177.10 (1C, C=S). MS *m/z*: 405 ($M^+$, $^{37}$Cl, 34), 403 ($M^+$, $^{35}$Cl, 100); Anal. calcd. for $C_{19}H_{20}ClN_3O_3S$ (%): C, 56.22; H, 4.97; N, 10.35; Found: C, 56.10; H, 4.88; N, 10.25.

***3-(4-Methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide,*** **3d:** Obtained from (E)-1-(4-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **1d** (5 mmol) and thiosemicarbazide hydrochloride, **2** (10 mmol) in 85% yield; $^1$H NMR: δ 3.162-3.190 (dd, 1H, *J= 7.7, 15.0Hz*, $C_4$-$H_a$), 3.932-3.981 (dd, 1H, *J= 8.1, 15.0Hz*, $C_4$-$H_b$), 3.833 (s, 6H, $OCH_3$), 3.858 (s, 6H, $OCH_3$), 4.310-4.348 (dd, 1H, *J= 7.9Hz, 16.0Hz*, $C_5$-H), 7.140–7.786 (m, 6H, Ar–H), 8.150 (s, 2H, $NH_2$); $^{13}$C NMR: δ 40.66 (1C, C-4), 55.54 (4C), 62.77 (1C, C-5), 101.60 (1C), 113.65 (1C), 115.10 (2C), 123.54 (1C), 126.30 (1C), 128.33 (2C), 139.42 (1C), 147.50 (1C), 148.82 (1C), 151.98 (1C, C-3), 162.56 (1C), 177.60 (1C, C=S). MS *m/z*: 401 ($M^+$, 100); Anal. calcd. for $C_{20}H_{23}N_3O_4S$ (%): C, 59.83; H, 5.77; N, 10.47; Found: C, 59.72; H, 5.70; N, 10.37.

***3-(3-Methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide,*** **3e:** Obtained from (E)-1-(3-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **1e** (5 mmol) and thiosemicarbazide hydrochloride, **2** (10 mmol) in 70% yield; $^1$H NMR: δ 3.160-3.196 (dd, 1H, *J= 7.9, 15.5Hz*, $C_4$-$H_a$), 3.931-3.978 (dd, 1H, *J= 7.1, 13.4Hz*, $C_4$-$H_b$), 3.838 (s, 6H, $OCH_3$), 3.855 (s, 6H, $OCH_3$), 4.308-4.352 (dd, 1H, *J= 7.3, 13.1Hz*, $C_5$-H), 7.1220–7.776 (m, 6H, Ar–H), 8.201 (s, 2H, $NH_2$); $^{13}$C NMR: δ 40.51 (1C, C-4), 55.56 (4C), 62.66 (1C, C-5), 101.67 (1C), 113.12 (1C), 115.33 (2C), 123.23 (1C), 126.41 (1C), 128.30 (2C), 139.58 (1C), 147.59 (1C), 148.88 (1C), 151.90 (1C, C-3), 162.47 (1C), 177.80 (1C, C=S). MS *m/z*: 401 ($M^+$, 100); Anal. calcd. for $C_{20}H_{23}N_3O_4S$ (%): C, 59.83; H, 5.77; N, 10.47; Found: C, 59.68; H, 5.65; N, 10.33.

***3-(2-Methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide,*** **3f:** Obtained from (E)-1-(2-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **1f** (5 mmol) and thiosemicarbazide hydrochloride, **2** (10 mmol) in 78% yield; $^1$H NMR: δ 3.164-3.192 (dd, 1H, *J= 7.9, 14.0Hz*, $C_4$-$H_a$), 3.945-3.990 (dd, 1H, *J= 8.0, 14.1Hz*, $C_4$-$H_b$), 3.840 (s, 6H, $OCH_3$), 3.860 (s, 6H, $OCH_3$), 4.326-4.360 (dd, 1H, *J= 6.9, 13.7Hz*, $C_5$-H), 7.155–7.797 (m, 6H, Ar–H), 8.188 (s, 2H, $NH_2$); $^{13}$C NMR: δ 40.74 (1C, C-4), 55.46 (4C), 62.66 (1C, C-5), 101.63 (1C), 113.46 (1C), 115.15 (2C), 123.50 (1C), 126.23 (1C), 128.31 (2C), 139.78 (1C), 147.83 (1C), 148.85 (1C), 151.93 (1C, C-3), 162.51 (1C), 177.47 (1C, C=S). MS *m/z*: 401 ($M^+$, 100); Anal. calcd. for $C_{20}H_{23}N_3O_4S$ (%): C, 59.83; H, 5.77; N, 10.47; Found: C, 59.74; H, 5.66; N, 10.37.

***3-(Benzo[d][1,3]dioxol-5-yl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide,*** **3g:** Obtained from (E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **1g** (5 mmol) and thiosemicabazide hydrochloride, **2** (10 mmol) in 83% yield; $^1$H NMR: δ 3.144-3.180 (dd, 1H, *J= 6.8, 13.3Hz*, $C_4$-$H_a$), 3.914-3.952 (dd, 1H, *J= 6.6, 13.5Hz*, $C_4$-$H_b$), 3.842 (s, 6H, $OCH_3$), 3.859 (s, 3H, $OCH_3$), 4.302-4.349 (dd, 1H, *J= 8.1, 14.8Hz*, $C_5$-H), 6.229 (s, 2H, $OCH_2O$), 7.123–7.785 (m, 5H, Ar–H), 8.156 (s, 2H, $NH_2$); $^{13}$C NMR: δ 40.40 (1C, C-4), 55.44 (3C), 62.72 (1C, C-5), 101.32 (1C), 102.74 (1C), 110.30 (1C), 113.20 (1C), 114.09 (1C), 120.33 (1C), 122.21 (1C), 126.36 (1C), 130.80 (1C), 141.40 (1C), 148.16 (1C), 148.14 (1C), 149.55 (1C), 152.70 (1C), 177.10 (1C, C=S). MS *m/z*: 415 ($M^+$, 100); Anal. calcd. for $C_{20}H_{21}N_3O_5S$ (%): C, 57.82; H, 5.09; N, 10.11; Found: C, 57.72; H, 4.98; N, 10.02.

## Result and Discussion

Structure proof of synthesized compounds, **3a-g** were provided by $^1$H NMR, $^{13}$C NMR, Mass spectral studies and elemental analysis. The structural assignments were made by NMR analysis by considering 3-(4-chlorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3c** as the representative compound amongst the series. In $^1$H NMR spectra, two methylene protons designated as $C_4$-$H_a$ and $C_4$-$H_b$ of the newly formed pyrazole ring are of diastereotopic nature. The $C_4$-$H_a$, $C_4$-$H_b$ and $C_5$-H protons appeared as a

doublet of doublets. The doublet of doublet for $C_4$-$H_a$ appeared at δ 3.144-3.180 (*J=9.0, 17.0Hz*) ppm; doublet of doublet for $C_4$-$H_b$ appeared in the region δ 3.924-3.969 (*J=7.3, 13.8Hz*) ppm; and that of $C_5$-H in the region δ 4.298-4.340 (*J=8.2, 14.4Hz*) ppm. Among $C_4$-$H_a$, $C_4$-$H_b$ and $C_5$-H protons, $C_3$-H is the most deshielded due to its close proximity to benzene ring and electronegative nitrogen. $C_5$-H couples not only with $C_4$-$H_a$ but also with $C_4$-$H_b$ and appears as doublet of doublet instead of a triplet. A signal appeared as singlet for two protons at δ 8.158 ppm was assigned to $NH_2$ protons. A collection of signal observed singlet for six and three protons each at δ 3.836 and δ 3.855 ppm; a multiplet for six protons in the region δ 7.146–7.778 ppm were assigned to $OCH_3$ and aromatic protons respectively.

In $^{13}$C NMR spectrum, compound **5c** showed a signal at δ 41.28, 62.45 and 151.71 ppm due to C-4, C-5 amd C-3 carbons of the newly formed pyrazole ring. A signal appeared for three carbons at δ 55.46 ppm was assigned to three $OCH_3$ carbons. A signal for thiocarbonyl carbon of thioamide function appeared at δ 177.10 ppm. An array of signals one carbon each appeared at δ 101.85, 113.62, 122.30, 133.34, 137.44, 139.74, 141.50, 148.22 ppm and for two carbons each at δ 127.77, 128.60 ppm were ambiguously assigned to aromatic carbons. Compound **5c** showed molecular mass peak at m/z 405 with a relative abundance of 34% corresponding to corresponding to its molecular mass and $^{37}$Cl isotope, and a base peak at m/z 403 corresponds to $^{35}$Cl isotope. Further, satisfactorily elemental analysis data obtained for the compound were in good agreement with theoretically calculated values. Similar and consistent pattern signals were observed in the $^1$H NMR, $^{13}$C NMR and Mass spectra of the synthesized series of compounds **3a-g**, which strongly supports the structure proof for the synthesized compounds.

**Antifungal activity**

Antimicrobial studies of synthesized compounds **3a-g** were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method.[13] The compounds were screened for their antimicrobial activities against fungi species *Candila albicans*, *Aspergillus nigar* and *Aspergillus flavus.* The experiments were carried out in triplicate; the results were taken as a mean of three determinations. The antibiotic Nystatin was used as a positive control. The results of MIC's were summarised in **Figure 1**.

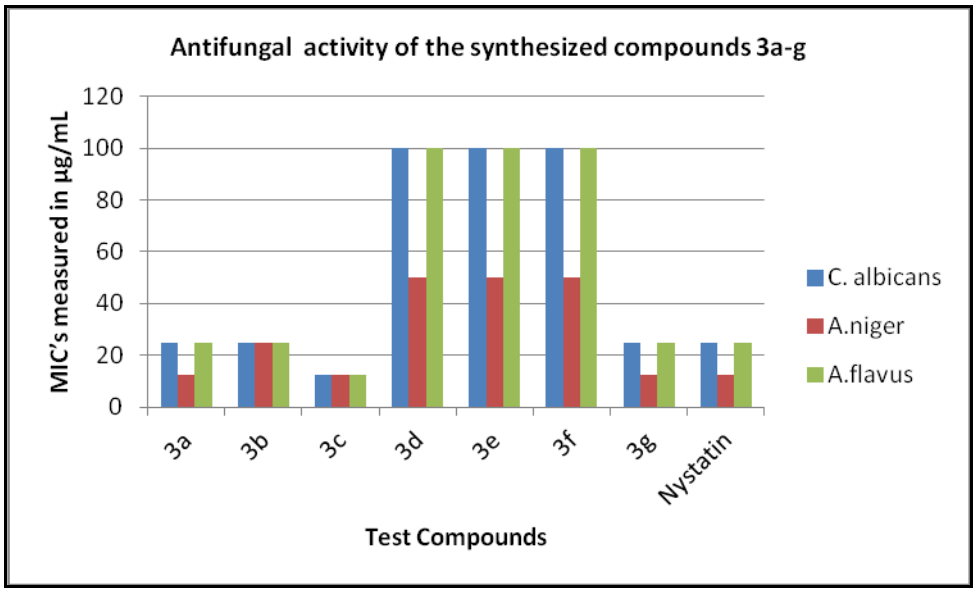



**Figure 1: Minimum inhibitory concentrations (in µg/mL) of the synthesized compounds 3a-g against fungal stains; results are expressed as mean of three determinations (n=3).**

The synthesized pyrazole carbothioamides **3a-g** exerted a wide range of *in vitro* antifungal activities against the tested fungi species. Preliminary investigation results of the study reveals that, amongst the series, compound **3c** having chloro substitution in the aromatic ring exhibited excellent inhibition against all the organisms. Compounds, **3a**, **3b** and **3g** having unsubstitution, fluoro and methylenedioxy substitutions showed promising antifungal susceptibilities, where as the remaining compounds amongst the series showed moderate inhibition potential against all the tested organisms.

## Conclusions

The simple procedure for the synthesis of pyrazole carbothioamides and their potential antifungal activities validates the significance of the study. Amongst synthesized series, 3-phenyl-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3a**, 3-(4-fluorophenyl)-5-(2,4,5-trimethoxy phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3b**, 3-(4-chlororophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3c** and 3-(benzo[d][1,3]dioxol-5-yl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3g** act as a potential antifungal agents against the tested microorganisms.

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