

# Design, Synthesis, Spectroscopic Characterization of New Pyrazole Carbothioamides as Antifungal and Antibacterial Candidates

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**Abstract:** In a sustained search for novel *antimicrobial agents* as weaponry in the war against infectious diseases, resulting in improved survivability for both humans and their domestic animals, the present study demonstrates an efficient synthesis of *N,N*-dimethylaminophenyl substituted pyrazole carbothioamide derivatives. The synthesis involves (3+2) cycloaddition of chalcones with hydrazinecarbothioamide hydrochloride in the presence of the amberlyst-15 catalyzed at room temperature. The structures of new compounds were characterized by spectroscopic analysis. Furthermore, the synthesized new compounds 5(a-g) were assessed *in vitro* for their antimicrobial susceptibilities. The results indicate that compounds 5a found potent against tested bacteria species; 5b and 5c show excellent inhibition against the tested fungi and bacteria species. Therefore, these could act as antifungal and antibacterial leads for further investigations.

**Keywords:** annulation; antimicrobial; chalcones; cycloaddition; minimum inhibition.

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## 1. Introduction

The discovery of new antimicrobials with greater efficacy and lesser toxicity is a huge revolutionary task for researchers all over. This is because the subsequent realization immediately ebbed the health triumph that bacterial populations could quickly modify themselves to resist antimicrobials, propagate these resistance traits, and even share resistance genes with other contemporary bacteria within their environment [1]. The compounds with pyrazole core are important pharmacophores in the development of antimicrobials, they alone or combined with other pharmacophores display potent antimicrobial activity [2]. The discovery of antipyrine by Knorr led to wide openings for scientists to work in the field of pyrazoles. A silver-mediated cycloaddition of *N*-isocyanoiminotriphenylphosphorane synthesized the pyrazole derivatives to terminal alkynes under mild reaction conditions [3], one-pot synthesis from ketones, aldehydes, and hydrazines [4], 1,3-dipolar cycloaddition of aldehyde hydrazones to alkenes in the presence of chloramine-T as dehydrogenating agent [5-7], and a phosphine-free [3+2] cycloaddition of dialkyl azodicarboxylates to propargyl amines [8]. The regioselective synthesis of pyrazoles was achieved by ruthenium [RuH<sub>2</sub>(PPh<sub>3</sub>)<sub>3</sub>CO]/Xanthphos catalyzed reaction of 1,3-diols and alkyl hydrazines [9]. An acid-mediated (3+2) annulation reaction of chalcones with hydrazines [10-13], semicarbazide [14-16], and thiosemicarbazide [17-20] produce pyrazole derivatives with high regioselectivity.

Chalcones involve the biosynthesis of flavonoids and isoflavonoids [21] and are useful intermediates in the synthesis of pyrazole derivatives [22, 23]. The pyrazole analogs are found as versatile pharmacophores for their pharmaceutical utilities. The molecules with pyrazole skeleton have reported to; inhibit the growth of DLA cells *in vitro* by committing them towards apoptosis [24], inhibit  $\alpha$ -glucosidase [25], 15-LOX [26], selective BuChE [27], metallo- $\beta$ -lactamase [28], phospholipase A2 [29, 30], and CDK2 [31]. These classes of compounds also known to exhibit anticancer [32, 33], angiogenic [34], anti-inflammatory [35], and antioxidant [36, 37] properties. Extensive studies on the antimicrobial properties of pyrazole derivatives have been reported [37, 38]. Overall, the modifications of the pyrazole core with varied substitutions could enhance the antimicrobial inhibition potentials. In this pretext, aiming to build up more antimicrobial active templates, a series of pyrazole carbothioamides, 5(a-g), were synthesized through a simple approach. After the structural confirmations, the compounds were examined for their *in vitro* antimicrobial susceptibilities.

## 2. Materials and Methods

### 2.1. Synthesis of chalcones, 3(a-g).

To a solution mixture of 4-(dimethylamino)benzaldehyde 1 (10 mmol) and substituted acetophenones 2(a-g) (10 mmol) in methyl alcohol, potassium hydroxide solution (40%, 2 mL) was added. Then the solution mixture was stirred at room temperature for 3-4 h. After completion, the mixture was cooled to room temperature, poured into ice-cold water, and kept overnight in the refrigerator. The solids separated were filtered, washed successively with cold hydrochloric acid (5%) and cold water. The crude solids were recrystallized from methyl alcohol to obtain compounds 3(a-g).

### 2.2. Synthesis of pyrazole carbothioamide derivatives, 5(a-g).

A mixture of chalcones 3(a-g) (5.0 mmol), hydrazinecarbothioamide hydrochloride, 4 (5 mmol), and amberlyst-15 (10%, w/w) in acetonitrile (25 mL) was stirred at room temperature for 1-2 hr. The reactions were checked on thin-layer chromatography (TLC) plates pre-coated with silica gel using solvent system hexane: ethyl acetate (1:4) as eluent. After completion, the solid separated was filtered, washed by diethyl ether (2 x 20 mL), dried, and treated with EtOAc (20 mL). After stirring for 10 min, the mixture was filtered to remove the insoluble catalyst. The filtrate was collected and concentrated under a vacuum. The solid isolated was triturated in diethyl ether, filtered, and dried to obtain the desired products 5(a-g).

Alternatively, a solution mixture of chalcones 3(a-g) (5 mmol) and hydrazinecarbothioamide hydrochloride, 4 (5.0 mmol) in acetic acid (30%) was refluxed on a water bath for 2-3 h. After the completion, the mixture was filtered, and the filtrate was poured into crushed ice. The separated solids were filtered and washed successively with 5% NaHCO<sub>3</sub> and water. The crude solids were recrystallized from methyl alcohol to get 5(a-g).

### 2.3. Antimicrobial activity.

The antimicrobial activities of the compounds 5(a-g) were determined as minimum inhibitory concentrations (MIC) by the serial dilution method [40, 41]. The antibacterial tests were conducted against bacterial pathogens like *Escherichia coli* (MTCC 1687), *Bacillus subtilis* (MTCC 441), and *Staphylococcus aureus* (MTCC 737), and antifungal activity against

*Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans* (MTCC 227) stains. The antibiotics ciprofloxacin and nystatin were used as a positive control against bacterial and fungal species, respectively, and dimethyl sulfoxide was used as solvent control. The experiments were conducted in triplicate; the results were taken as a mean  $\pm$  variance (SD).

### 3. Results and Discussion

#### 3.1. Analytical data.

##### 3.1.1. 3-(4-(Dimethylamino)phenyl)-1-phenylprop-2-en-1-one, 3.

Obtained from 4-(dimethylamino)benzaldehyde, 1 (1.49g, 10 mmol) and acetophenone, 2a (1.20g, 10 mmol) in 72% yield, m.p. 102-104 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.010 (s, 6H,  $\text{CH}_3$ ), 6.721 (d, 1H,  $J=16.6\text{MHz}$ , =CH), 6.998-7.250 (m, 6H, Ar-H), 7.465-7.534 (m, 3H, Ar-H), 8.245 (d, 1H,  $J=15.0\text{MHz}$ , CH=);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ,  $\delta$  ppm): 40.2 (2C,  $\text{N}(\text{CH}_3)_2$ ), 111.0 (2C), 121.4 (1C), 124.6 (1C), 128.1 (1C), 128.3 (1C), 128.5 (1C), 129.3 (1C), 129.7 (2C), 134.3 (1C), 136.0 (1C), 143.4 (1C), 153.0 (1C), 188.3 (1C, C=O); MS  $m/z$ : 251.13 (M+, 100); Anal. Calcd. (found) for  $\text{C}_{17}\text{H}_{17}\text{NO}$  (%): C, 81.24 (81.17); H, 6.82 (6.78); N, 5.57 (5.49).

##### 3.1.2. 3-(4-(Dimethylamino)phenyl)-1-(4-fluorophenyl)prop-2-en-1-one, 3b.

Obtained from 4-(dimethylamino)benzaldehyde, 1 (1.49g, 10 mmol) and 1-(4-fluorophenyl)ethan-1-one, 2b (1.38g, 10 mmol) in 68% yield, m.p. 94-95 °C;  $^1\text{H}$  NMR ( $\text{DMSO-d}_6$ ,  $\delta$  ppm): 3.005 (s, 6H,  $\text{CH}_3$ ), 6.898 (d, 1H,  $J=17.2\text{MHz}$ , =CH), 7.103-7.303 (m, 4H, Ar-H), 7.378-7.596 (m, 4H, Ar-H), 8.355 (d, 1H,  $J=15.2\text{MHz}$ , CH=);  $^{13}\text{C}$  NMR ( $\text{DMSO-d}_6$ ,  $\delta$  ppm): 39.5 (2C,  $\text{N}(\text{CH}_3)_2$ ), 111.6 (2C), 114.8 (2C), 124.4 (1C), 128.9 (2C), 129.0 (1C), 129.6 (1C), 136.0 (1C), 143.1 (1C), 143.4 (1C), 153.2 (1C), 167.1 (1C), 187.2 (1C, C=O); MS ( $m/z$ ): 269.1 (M+, 100); Anal. Calcd. (found) for  $\text{C}_{17}\text{H}_{16}\text{FNO}$  (%): C, 75.82 (75.74); H, 5.99 (5.94); N, 5.20 (5.15).

##### 3.1.3. 1-(4-Chlorophenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one, 3c.

Obtained from 4-(dimethylamino)benzaldehyde, 1 (1.49g, 10 mmol) and 1-(4-chlorophenyl)ethan-1-one, 2c (1.54g, 10 mmol) in 77% yield, m.p. 135-136 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.010 (s, 6H,  $\text{CH}_3$ ), 6.956 (d, 1H,  $J=16.1\text{MHz}$ , =CH), 7.012-7.233 (m, 4H, Ar-H), 7.560-7.788 (m, 4H, Ar-H), 8.205 (d, 1H,  $J=17.8\text{MHz}$ , CH=);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ,  $\delta$  ppm): 40.2 (2C,  $\text{N}(\text{CH}_3)_2$ ), 112.8 (2C), 121.2 (1C), 124.8 (1C), 127.2 (2C), 129.1 (2C), 130.0 (2C), 135.8 (1C), 140.2 (1C), 144.5 (1C), 149.6 (1C), 188.1 (1C, C=O); MS ( $m/z$ ): 287.2 (M+2, 32), 285.1 (M+, 100); Anal. Calcd. (found) for  $\text{C}_{17}\text{H}_{16}\text{ClNO}$  (%): C, 71.45 (75.36); H, 5.64 (5.61); N, 4.90 (4.87).

##### 3.1.4. 3-(4-(Dimethylamino)phenyl)-1-(p-tolyl)prop-2-en-1-one, 4c.

The analytical data of the compound were in good agreement with the previous report [39].

##### 3.1.5. 3-(4-(Dimethylamino)phenyl)-1-(3-methoxyphenyl)prop-2-en-1-one, 3e.

Obtained from 4-(dimethylamino)benzaldehyde, 1 (1.49g, 10 mmol) and 1-(3-methoxyphenyl)ethan-1-one, 2e (1.50g, 10 mmol) in 61% yield, m.p. 119-121 °C;  $^1\text{H}$  NMR

(CDCl<sub>3</sub>, δ ppm): 3.022 (s, 6H, CH<sub>3</sub>), 3.810 (s, 3H, OCH<sub>3</sub>), 6.992 (d, 1H, J= 15.8 MHz, =CH), 7.009 (d, 2H, J= 11.3 MHz, Ar-H), 7.426-7.690 (m, 6H, Ar-H), 8.211 (d, 1H, J=13.5MHz, CH=); <sup>13</sup>C NMR (CDCl<sub>3</sub>, δ ppm): 41.2 (2C, N(CH<sub>3</sub>)<sub>2</sub>), 55.4 (1C, OCH<sub>3</sub>) 111.9 (2C), 114.5 (1C), 115.2 (1C), 120.1 (1C), 121.9 (1C), 125.2 (1C), 128.9 (2C), 130.0 (1C), 133.2 (1C), 144.6 (1C), 160.0 (1C), 148.8 (1C), 190.2 (1C, C=O); MS (m/z): 281.1 (M<sup>+</sup>, 100); Anal. Calcd. (found) for C<sub>18</sub>H<sub>19</sub>NO<sub>2</sub> (%): C, 76.84 (76.74); H, 6.81 (6.77); N, 4.98 (4.94).

3.1.6. 3-(4-(Dimethylamino)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one, 3f.

Obtained from 4-(dimethylamino)benzaldehyde, 1 (1.49g, 10 mmol) and 1-(4-methoxyphenyl)ethan-1-one, 2f (1.50g, 10 mmol) as semisolid in 66% yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, δ ppm): 3.010 (s, 6H, CH<sub>3</sub>), 3.840 (s, 3H, OCH<sub>3</sub>), 6.922 (d, 1H, J= 14.6MHz, =CH), 7.022-7.540 (m, 6H, Ar-H), 7.745-7.782 (m, 2H, Ar-H), 8.223 (d, 1H, J=14.2MHz, CH=); <sup>13</sup>C NMR (CDCl<sub>3</sub>, δ ppm): 41.6 (2C, N(CH<sub>3</sub>)<sub>2</sub>), 55.2 (1C, OCH<sub>3</sub>) 111.8 (2C), 114.0 (2C), 121.4 (1C), 124.3 (1C), 128.7 (2C), 129.3 (2C), 131.4 (2C), 144.0 (1C), 150.0 (1C), 165.1 (1C), 189.5 (1C, C=O); Anal. Calcd. (found) for C<sub>18</sub>H<sub>19</sub>NO<sub>2</sub> (%): C, 76.84 (76.76); H, 6.81 (6.78); N, 4.98 (4.95).

3.1.7. 1-(3,4-Dimethoxyphenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one, 3g.

Obtained from 4-(dimethylamino)benzaldehyde, 1 (1.49g, 10 mmol) and 1-(3,4-dimethoxyphenyl)ethan-1-one, 2g (1.80g, 10 mmol) in 68% yield, m.p. 102-104 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, δ ppm): 3.036 (s, 6H, CH<sub>3</sub>), 3.845 (s, 6H, OCH<sub>3</sub>), 6.975 (d, 1H, J= 18.2MHz, =CH), 6.990-7.212 (m, 4H, Ar-H), 7.734-7.847 (m, 3H, Ar-H), 8.312 (d, 1H, J=16.1MHz, CH=); <sup>13</sup>C NMR (CDCl<sub>3</sub>, δ ppm): 41.1 (2C, N(CH<sub>3</sub>)<sub>2</sub>), 55.4 (2C, OCH<sub>3</sub>) 108.4 (1C), 112.5 (2C), 113.3 (1C), 120.8 (1C), 122.9 (1C), 123.4 (1C), 124.7 (1C), 129.5 (2C), 144.8 (1C), 149.6 (1C), 150.7 (1C), 154.1 (1C), 190.1 (1C, C=O); MS (m/z): 311.1 (M<sup>+</sup>, 100); Anal. Calcd. (found) for C<sub>19</sub>H<sub>21</sub>NO<sub>3</sub> (%): C, 73.29 (73.22); H, 6.80 (6.76); N, 4.50 (4.46).

3.1.8. 5-(4-(Dimethylamino)phenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide, 5a.

Obtained from 3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one, 3a (1.75g, 5 mmol) and hydrazinecarbothioamide hydrochloride, 4 (0.64g, 5 mmol); <sup>1</sup>H NMR (CDCl<sub>3</sub>, δ ppm): 3.005 (s, 6H, CH<sub>3</sub>), 3.655 (dd, 1H, J=9.6, 17.2Hz, 4-H<sub>a</sub>), 3.965 (dd, 1H, J=19.4, 6.6Hz, 4-H<sub>b</sub>), 4.304 (dd, 1H, J=11.8, 7.0Hz, C<sub>5</sub>-H), 6.874-7.008 (m, 4H, Ar-H), 7.435-7.691 (m, 5H, Ar-H), 8.345 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, δ ppm): 41.8 (2C, N(CH<sub>3</sub>)<sub>2</sub>), 43.8 (1C, C-4), 69.9 (1C, C-5), 113.1 (2C), 128.1 (2C), 128.6 (2C), 129.4 (2C), 131.6 (1C), 132.6 (1C), 137.0 (1C), 149.0 (1C), 152.7 (1C, C-3), 175.1 (1C, C=S); MS (m/z): 324.1 (M<sup>+</sup>, 100); Anal. Calcd. (found) for C<sub>18</sub>H<sub>20</sub>N<sub>4</sub>S (%): C, 66.64 (66.53); H, 6.21 (6.17); N, 17.27 (17.22).

3.1.9. 5-(4-(Dimethylamino)phenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, 5b.

Obtained from 3-(4-(dimethylamino)phenyl)-1-(4-fluorophenyl)prop-2-en-1-one, 3b (1.35g, 5 mmol) and hydrazinecarbothioamide hydrochloride, 4 (0.64g, 5 mmol); <sup>1</sup>H NMR (CDCl<sub>3</sub>, δ ppm): 2.990 (s, 6H, CH<sub>3</sub>), 3.614 (dd, 1H, J= 10.5, 17.4MHz, 4-H<sub>a</sub>), 3.910 (dd, 1H, J= 19.2, 7.6MHz, 4-H<sub>b</sub>), 4.215 (dd, 1H, J= 12.0, 5.7MHz, 5-H), 6.820-7.015 (m, 4H, Ar-H), 7.432-7.658 (m, 4H, Ar-H), 8.323 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, δ ppm): 40.1 (2C,

$N(CH_3)_2$ , 41.2 (1C, C-4), 69.2 (1C, C-5), 112.6 (2C), 114.5 (2C), 129.2 (2C), 129.5 (2C), 131.7 (1C), 132.6 (1C), 148.1 (1C), 151.3 (1C, C-3), 160.1 (1C), 174.4 (1C, C=S); MS (m/z): 342.1 (M<sup>+</sup>, 100); Anal. Calcd. (found) for C<sub>18</sub>H<sub>19</sub>FN<sub>4</sub>S (%): C, 63.14 (63.01); H, 5.59 (5.56); N, 16.36 (16.31).

3.1.10. 3-(4-Chlorophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, 5c.

Obtained from 1-(4-chlorophenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one, 3c (1.43g, 5 mmol) and hydrazinecarbothioamide hydrochloride, 4 (0.64g, 5 mmol); <sup>1</sup>H NMR (CDCl<sub>3</sub>, δ ppm): 2.988 (s, 6H, CH<sub>3</sub>), 3.642 (dd, 1H, J= 6.1, 16.3MHz, 4-H<sub>a</sub>), 3.948 (dd, 1H, J= 18.0, 6.0MHz, 4-H<sub>b</sub>), 4.246 (dd, 1H, J= 13.6, 5.6MHz, C<sub>5</sub>-H), 6.888-7.070 (m, 4H, Ar-H), 7.408-7.655 (m, 4H, Ar-H), 8.290 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, δ ppm): 39.8 (2C, N(CH<sub>3</sub>)<sub>2</sub>), 42.6 (1C, C-4), 68.4 (1C, C-5), 110.7 (2C), 128.7 (2C), 129.8 (2C), 130.1 (2C), 131.3 (1C), 133.8 (1C), 135.4 (1C), 149.3 (1C), 153.7 (1C, C-3), 176.1 (1C, C=S); MS (m/z): 360.0 (M+2, 32); 358.1 (M<sup>+</sup>, 100); Anal. Calcd. (found) for C<sub>18</sub>H<sub>19</sub>ClN<sub>4</sub>S (%): C, 60.24 (60.13); H, 5.34 (5.30); N, 15.61 (15.56).

3.1.11. 5-(4-(Dimethylamino)phenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, 5d.

Obtained from 3-(4-(dimethylamino)phenyl)-1-(p-tolyl)prop-2-en-1-one, 3d (1.33g, 5 mmol) and hydrazinecarbothioamide hydrochloride, 4 (0.64g, 5 mmol); <sup>1</sup>H NMR (CDCl<sub>3</sub>, δ ppm): 2.382 (s, 3H, CH<sub>3</sub>), 2.995 (s, 6H, CH<sub>3</sub>), 3.685 (dd, 1H, J= 7.8, 18.5MHz, 4-H<sub>a</sub>), 3.980 (dd, 1H, J= 17.7, 5.5MHz, 4-H<sub>b</sub>), 4.270 (dd, 1H, J= 11.3, 6.6MHz, C<sub>5</sub>-H), 6.902-7.060 (m, 4H, Ar-H), 7.322-7.515 (m, 4H, Ar-H), 8.182 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, δ ppm): 20.9 (1C, CH<sub>3</sub>), 40.4 (2C, N(CH<sub>3</sub>)<sub>2</sub>), 42.6 (1C, C-4), 69.4 (1C, C-5), 110.8 (2C), 127.6 (2C), 128.5 (2C), 129.1 (2C), 131.4 (1C), 134.1 (1C), 139.9 (1C), 147.7 (1C), 152.0 (1C, C-3), 177.5 (1C, C=S); MS (m/z): 338.2 (M<sup>+</sup>, 100); Anal. Calcd. (found) for C<sub>19</sub>H<sub>22</sub>N<sub>4</sub>S (%): C, 67.42 (67.35); H, 6.55 (6.51); N, 16.55 (16.50).

3.1.12. 5-(4-(Dimethylamino)phenyl)-3-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, 5e.

Obtained from 3-(4-(dimethylamino)phenyl)-1-(3-methoxyphenyl)prop-2-en-1-one, 3e (1.40g, 5 mmol) and hydrazinecarbothioamide hydrochloride, 4 (0.64g, 5 mmol); <sup>1</sup>H NMR (CDCl<sub>3</sub>, δ ppm): 3.012 (s, 6H, CH<sub>3</sub>), 3.655 (dd, 1H, J= 10.6, 17.5MHz, 4-H<sub>a</sub>), 3.835 (s, 3H, OCH<sub>3</sub>), 3.960 (dd, 1H, J= 18.2, 7.6MHz, 4-H<sub>b</sub>), 4.330 (dd, 1H, J= 11.9, 6.9MHz, 5-H), 6.978-7.109 (m, 4H, Ar-H), 7.538-7.812 (m, 4H, Ar-H), 8.216 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, δ ppm): 39.0 (2C, N(CH<sub>3</sub>)<sub>2</sub>), 40.9 (1C, C-4), 55.3 (1C, OCH<sub>3</sub>), 67.7 (1C, C-5), 112.2 (2C), 113.9 (1C), 116.3 (1C), 120.1 (1C), 129.5 (1C), 129.5 (2C), 132.3 (1C), 134.7 (1C), 149.0 (1C), 152.7 (1C, C-3), 159.5 (1C), 175.1 (1C, C=S); MS (m/z): 354.2 (M<sup>+</sup>, 100); Anal. Calcd. (found) for C<sub>19</sub>H<sub>22</sub>N<sub>4</sub>OS (%): C, 64.38 (64.30); H, 6.26 (6.22); N, 15.81 (15.76).

3.1.13. 5-(4-(Dimethylamino)phenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, 5f.

Obtained from 3-(4-(dimethylamino)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one, 3f (1.41g, 5 mmol) and hydrazinecarbothioamide hydrochloride, 4 (0.64g, 5 mmol); <sup>1</sup>H NMR  
<https://nanobioletters.com/>



(CDCl<sub>3</sub>,  $\delta$  ppm): 3.006 (s, 6H, CH<sub>3</sub>), 3.661 (dd, 1H, J= 10.2, 17.4MHz, 4-H<sub>a</sub>), 3.826 (s, 3H, OCH<sub>3</sub>), 3.955 (dd, 1H, J= 18.0, 7.3MHz, 4-H<sub>b</sub>), 4.322 (dd, 1H, J= 11.7, 6.8MHz, 5-H), 6.965-7.129 (m, 6H, Ar-H), 7.821-7.904 (m, 2H, Ar-H), 8.209 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>,  $\delta$  ppm): 40.9 (2C, N(CH<sub>3</sub>)<sub>2</sub>), 42.1 (1C, C-4), 55.5 (1C, OCH<sub>3</sub>), 69.7 (1C, C-5), 112.8 (2C), 115.5 (2C), 127.9 (1C), 128.6 (2C), 129.3 (2C), 130.8 (1C), 147.6 (1C), 149.2 (1C), 151.9 (1C, C-3), 176.7 (1C, C=S); MS (m/z): 354.1 (M<sup>+</sup>, 100); Anal. Calcd. (found) for C<sub>19</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub>S (%): C, 64.38 (64.30); H, 6.26 (6.21); N, 15.81 (15.76).

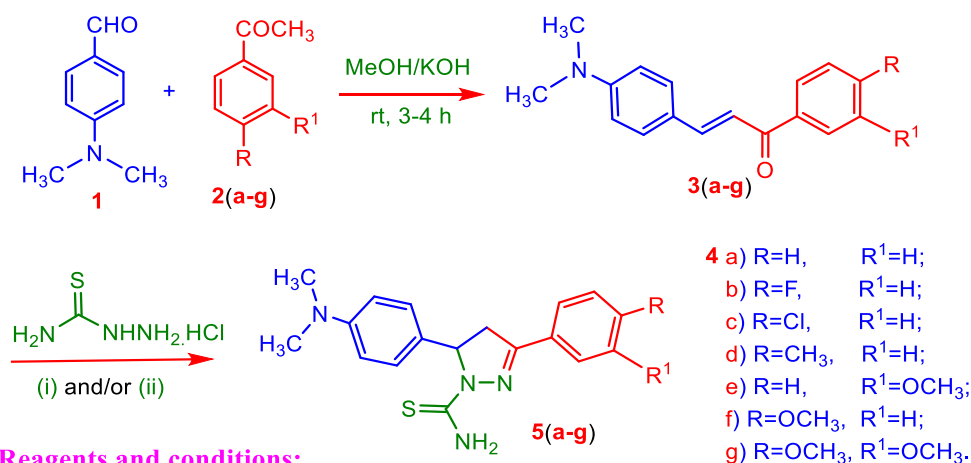
3.1.14. 3-(3,4-Dimethoxyphenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, 5g.

Obtained from 1-(3,4-dimethoxyphenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one, 3g (1.56g, 5 mmol) and hydrazinecarbothioamide hydrochloride, 4 (0.64g, 5 mmol); <sup>1</sup>H NMR (CDCl<sub>3</sub>,  $\delta$  ppm): 3.101 (s, 6H, CH<sub>3</sub>), 3.671 (dd, 1H, J= 8.3, 16.9MHz, 4-H<sub>a</sub>), 3.840 (s, 6H, OCH<sub>3</sub>), 3.928 (dd, 1H, J= 16.0, 6.3MHz, 4-H<sub>b</sub>), 4.314 (dd, 1H, J= 12.2, 5.9MHz, 5-H), 6.870-7.078 (m, 5H, Ar-H), 7.412-7.536 (m, 2H, Ar-H), 8.305 (s, 2H, NH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>,  $\delta$  ppm): 39.8 (2C, N(CH<sub>3</sub>)<sub>2</sub>), 42.0 (1C, C-4), 55.4 (2C, OCH<sub>3</sub>), 68.9 (1C, C-5), 109.1 (1C), 112.8 (2C), 114.4 (1C), 121.9 (1C), 127.0 (1C), 128.8 (2C), 132.6 (1C), 148.3 (1C), 147.7 (1C), 149.4 (1C), 153.0 (1C, C-3), 175.3 (1C, C=S); MS (m/z): 384.0 (M<sup>+</sup>, 100); Anal. Calcd. (found) for C<sub>20</sub>H<sub>24</sub>N<sub>4</sub>O<sub>2</sub>S (%): C, 62.48 (62.41); H, 6.29 (6.25); N, 14.57 (14.52).

### 3.2. Chemistry.

Initially, the intermediate 1-aryl-3-(4-(dimethylamino)phenyl)prop-2-en-1-ones, 3(a-g), were prepared through the base catalyzed Claisen-Schmidt condensation of 4-(dimethylamino)benzaldehyde 1, and substituted acetophenones 2(a-g). Then, amberlyst-15 catalyzed (3 + 2) annulation reaction of 3(a-g) with hydrazinecarbothioamide hydrochloride, 4 in acetonitrile at room temperature produced pyrazole carbothioamide derivatives 5(a-g) in good yields. The reaction was also performed under conventional reflux conditions in acetic acid medium (Fig. 1) (Table 1). The amberlyst-15 catalyst, recovered using the solvent ethyl acetate, was efficient for four consecutive similar experiments [42]. The structures of synthesized new compounds were confirmed by spectroscopic analysis.

The <sup>1</sup>H NMR spectra of compounds 3(a-g) recorded on 400 MHz Agilent-NMR spectrometer show a singlet in the range of  $\delta$  3.005-3.036 ppm for six N(CH<sub>3</sub>)<sub>2</sub> protons, two doublets in the range of  $\delta$  6.721-6.992 (J= 14.6-18.2MHz) ppm for =CH, and  $\delta$  8.205-8.355 (13.5-17.8MHz) ppm for CH= protons. The constant coupling values in the range of J= 14.6-18.2 MHz and J=13.5-17.8MHz of alkenyl protons indicate the trans configuration around the C=C bond. The compounds 5(a-g) show three doublet of doublets in the range  $\delta$  3.614-3.685 (J= 6.3-10.5, 16.3-18.5MHz), 3.910-3.980 (J= 16.0-19.4, 5.5-7.6MHz), and 4.215-4.322 (J= 11.3-13.6, 5.6-7.0MHz) for 4-H<sub>a</sub>, 4-H<sub>b</sub>, and 5-H, protons respectively. Singlets in the range of  $\delta$  2.988-3.101 ppm for six N(CH<sub>3</sub>)<sub>2</sub>, and  $\delta$  8.182-8.345 ppm for two NH<sub>2</sub> protons. Furthermore, compounds 3(a-g) and 5(a-g) show the signals due to substituent and aromatic protons in the respective absorption region. The absence of signals due to alkenyl protons of 3(a-g) and ABX type coupling of methylene protons of pyrazole ring confirm (3+2) annulation.



**Reagents and conditions:**

(i) Amberlyst-15 (10%, w/w)/acetonitrile, rt, 1-2 h.; (ii) Acetic acid (30%), reflux, 2-3 h.

**Figure 1.** Synthesis of pyrazole carbothioamide derivatives, 4(a-g).

**Table 1.** Reaction time and yields of amberlyst-15 mediated and conventional synthesis.

Entry	Amberlyst-15 mediated method		Conventional method		Melting Point (°C)
	Time (Min)	Yield (%)	Time (Min)	Yield (%)	
4a	95	84	155	82	121-123
4b	90	81	140	76	133-135
4c	60	85	120	81	146-147
4d	115	70	175	69	115-117
4e	120	66	180	60	96-98
4f	75	78	140	71	108-110
4g	100	74	170	70	155-157

The <sup>13</sup>C NMR spectra of compounds 3(a-g) recorded on 100 MHz Agilent-NMR spectrometer show the carbon resonance signals in the range of δ 39.5-41.6 ppm, 121.2-124.4 ppm, 143.1-144.8 ppm, and δ 187.2-190.2 ppm, for two N(CH<sub>3</sub>)<sub>2</sub>, alkenyl α-, alkenyl β-, and carbonyl carbons, respectively. The compounds 5(a-g) show carbon resonance signals in the range of δ 40.9-43.8 ppm, 67.7-69.9 ppm, 151.3-153.7 ppm for C-4, C-5, and C-3 carbons of newly formed pyrazole ring. The C=S carbons were absorbed in the range of δ 174.4-177.5 ppm, while the signals that appear in the range of δ 39.8-41.8 ppm were unambiguously assigned to N(CH<sub>3</sub>)<sub>2</sub> carbons. The compounds 3(a-g) and 5(a-g) show the substituent and aromatic carbon signals in the respective absorption region. The mass spectra of compounds 3(a-g) and 5(a-g) recorded on ESI/APCI-Hybrid Quadrupole, Synapt G2 HDMS ACQUITY UPLC model spectrometer show molecular ion base peaks corresponding to their molecular masses. However, the compounds 3c and 5c show an M+2 peak at m/z 360 with a relative abundance of 32% due to <sup>37</sup>Cl isotopic mass. All the compounds show satisfactory elemental analyses obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer compared with theoretical values.

3.3. Antimicrobial activity.

3.3.1. Antifungal activity.

The results of antifungal activities of the synthesized compounds 5(a-g) measured as minimal inhibitory concentrations were given in Table 2.

**Table 2.** Minimum inhibitory concentrations (µg/mL) of pyrazole derivatives 5(a-g) against fungi species.

Compound	<i>A. niger</i>	<i>A. flavus</i>	<i>C. albicans</i>
5a	25.0 ± 0.75	25.0 ± 0.65	70.0 ± 0.50
5b	20.0 ± 0.55	15.0 ± 0.60	60.0 ± 0.90

Compound	<i>A. niger</i>	<i>A. flavus</i>	<i>C. albicans</i>
<b>5c</b>	15.0 ± 0.80	10.0 ± 0.65	65.0 ± 1.05
<b>5d</b>	25.0 ± 1.00	25.0 ± 0.95	60.0 ± 1.05
<b>5e</b>	45.0 ± 0.75	50.0 ± 1.20	75.0 ± 1.25
<b>5f</b>	30.0 ± 1.00	30.0 ± 1.15	80.0 ± 1.20
<b>5g</b>	40.0 ± 0.75	50.0 ± 0.80	90.0 ± 1.10
<b>Nystatin</b>	25.0 ± 0.75	25.0 ± 0.25	20.0 ± 0.50

Values are mean ± SD of three replicates; nystatin - positive control.

The synthesized compounds displayed varying effects on the tested fungi species. All the compounds show poorer inhibition against *C. albicans* with MIC values in the range of 60.0-90.00 µg/mL concentrations comparable with the standard nystatin (MIC-20.0 µg/mL), however, they show moderate to good inhibition against *A. niger*, and *A. flavus*. The compounds 3-(4-chlorophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5c**, and 5-(4-(dimethylamino)phenyl)-3-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5b** show excellent inhibition against *A. niger* (15, and 20 µg/mL), and *A. flavus* (20, and 15 µg/mL); the compounds 5-(4-(dimethylamino)phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5a**, 5-(4-(dimethylamino)phenyl)-3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5d** and 5-(4-(dimethylamino)phenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5f** found good against *A. niger* (25, 25, and 30 µg/mL), and *A. flavus* (25, 25, and 30 µg/mL) concentrations. The compounds 5-(4-(dimethylamino)phenyl)-3-(3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5e** and 3-(3,4-dimethoxyphenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, **5g** have moderate activities against *A. niger* (45, and 40 µg/mL), and *A. flavus* (50, and 50 µg/mL) comparable with the standard nystatin (MIC-25.0 µg/mL). The preliminary assay results indicate that the compounds **5c** and **5b** of the series could be lead molecules against *A. niger* and *A. flavus* organisms.

### 3.3.2. Antibacterial activity.

The results of the compounds 5(a-g) antibacterial activities measured as minimal inhibitory concentrations were summarized in Table 3.

**Table 3.** Minimum inhibitory concentrations (µg/mL) of pyrazole derivatives 5(a–g) against bacteria species.

Compound	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
<b>5a</b>	15.0 ± 0.55	15.0 ± 0.70	20.0 ± 0.60
<b>5b</b>	15.0 ± 0.80	15.0 ± 0.55	25.0 ± 0.75
<b>5c</b>	10.0 ± 0.65	10.0 ± 0.60	15.0 ± 0.80
<b>5d</b>	25.0 ± 0.85	30.0 ± 1.10	30.0 ± 1.05
<b>5e</b>	> 100	> 100	> 100
<b>5f</b>	30.0 ± 1.20	25.0 ± 0.70	35.0 ± 1.10
<b>5g</b>	> 100	> 100	> 100
<b>Ciprofloxacin</b>	20.0 ± 0.50	20.0 ± 0.75	30.0 ± 1.00

Values are mean ± SD of three replicates; ciprofloxacin - positive control

The preliminary assessment results show that 5(a–g) compounds exert varying effects on the tested bacteria species. Amongst the series, the compounds **5a**, **5b**, and **5c** displayed excellent inhibition with lesser MIC values against *S. aureus* (15, 15, and 10 µg/mL), *E. coli* (15, 15, and 10 µg/mL), and *B. subtilis* (20, 25, and 15 µg/mL) comparable with the standard ciprofloxacin (20, 20, and 30 µg/mL). The compounds **5d** and **5e** show good activities by inhibiting spore germination of *S. aureus* (25, and 30 µg/mL), *E. coli* (30 and 25 µg/mL), and *B. subtilis* (30, and 35 µg/mL), respectively. However, it was observed that the compounds **5e** and **5g** having a methoxy substitution in the phenyl ring were found inactive even at 100 µg/mL



concentrations. From the results, it was found that the compounds 5a, 5b, and 5c of the series might be future lead bacterial agents.

#### 4. Conclusions

To sum up, the current work demonstrates the synthesis of series of pyrazole carbothioamide derivatives through (3+2) annulation reaction of chalcones with hydrazinecarbothioamide hydrochloride catalyzed by an amberlyst-15; the method is reliable and efficient for the synthesis of pyrazoles. The *in vitro* antifungal and antibacterial activity assay results indicate the noticeable activity of the synthesized new compounds. The compounds 5a, 5b, and 5c displayed excellent inhibition with least MIC values against the tested *S. aureus* (15, 15, and 10 µg/mL), *E. coli* (15, 15, and 10 µg/mL), *B. subtilis* (20, 25, and 15 µg/mL); 5b, and 5c against *A. niger* (15, and 20 µg/mL), and *A. flavus* (20, and 15 µg/mL), the results of which were comparable with the standards employed. Therefore, these pyrazole carbothioamides are posited to be the lead candidates for developing new antimicrobial drugs.

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#### Conflicts of Interest

The authors declare no conflict of interest.

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