Design, Synthesis, and Anti-mycobacterial Evaluation of New 3,5-Disubstituted-pyrazole-1-carbothioamides

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Abstract: *Two series of new 3,5-disubstituted-pyrazole-1-carbothioamides (**4a-f** and **5a-e**) were designed and synthesized through condensation reaction between chalcones and thiosemicarbazides under alkaline condition via cyclocondensation reaction. The structures have been elucidated by Fourier-transform infrared (FTIR), High-resolution mass spectrometry (HRMS), one- and two-dimensional nuclear magnetic resonance (NMR) analyses. These compounds were assayed for* in vitro *anti-tuberculosis activity against* Mycobacterium tuberculosis *H37Ra using the Tetrazolium microplate assay (TEMA) method. As a result, six compounds (i.e., **4a**, **4d**, **4f**, **5a**, **5c**, and **5d**) showed a weak activity with minimum inhibition concentration (MIC) between 650–530 μM, and other compounds showed no inhibition against MTB. In addition, all tested compounds also did not show any cidal effects for minimum bactericidal concentration (MBC), even at the highest test concentration.*

Keywords: *3,5-disubstituted-pyrazole-1-carbothioamide; pyrazolines;* Mycobacterium tuberculosis*; antitubercular agents; anti-mycobacterial*

■ INTRODUCTION

Tuberculosis (TB) is one of the global major health problems, in which these microbial infections could potentially cause the death of human beings. Although its incidence has been diminished significantly in the industrially more developed countries, it remains out of control in many developing nations [1]. It was estimated that 1.3 million lives among human immunodeficiency virus (HIV)-negative people died of TB in 2016, which surpassed the number of deaths caused by HIV. In addition, about 374,000 death cases of TB were coinfected with HIV [2]. In 2006, Corbett et al. reported that the emergence of anti-TB resistance and HIV are vital contributors to death by TB [3]. Recently, several drugs in the market were used for TB treatment, including isoniazid (INH), rifampicin, pyrazinamide, and delamanid, all of which possess a nitrogen atom in their ring structure [4]. The problem is commonly known that *Mycobacterium tuberculosis* (MTB) has developed resistance to any of these drugs [5]. Oxygen and nitrogen-containing heterocyclic moieties have been reported as active compounds against herpes simplex virus-1 (HSV), and HIV thus exhibited a potential diversity of biological activities [6-9]. In particular, electron-rich nitrogen heterocycles play a vital role in various pharmacological activities [10]. Pyrazoline was first discovered by Knorr in the year 1883 [11-12]. Pyrazolines are dihydropyrazole, heterocycles having an endocyclic double bond with two adjacent nitrogen atoms at positions 1- and 2- within the five-membered ring, are the most studied group of compounds in the azole family [13]. Pyrazoline derivatives are reported as new potent compounds possessing biological activities;

anticancer [14], antimalarial [15], antitumor [16], anti-cardiovascular [17], monoamine oxidase (MAO) inhibitors [18-19], antiamoebic [20], and anticonvulsant activities [21-23]. Meanwhile, a previous study reported that a new set of pyrazoline derivatives were synthesized and exhibited significant anti-mycobacterial activity [24]. This information has prompted us to synthesize a new pyrazoline derivative with possible anti-TB activity against MTB H37Ra. These derivatives may reduce the duration of the TB therapy and consequently minimize the adverse effects of the medication.

■ EXPERIMENTAL SECTION

Materials

Chemicals were purchased from Sigma-Aldrich Co., Acros Organics, QReC, and Merck Chemical Co. All chemicals and solvents were of reagent grade and were used without further purification. Column chromatography was performed using Merck silica gel (40–63 μm).

Instrumentation

The reactions were monitored on Merck pre-coated aluminum Thin-layer chromatography (TLC) plates 60 F254, and the products were visualized under a UV radiation lamp (λ_{max} = 254 nm). Melting points were determined on open capillary tubes. Infrared (FTIR-ATR) spectra were recorded on a Perkin-Elmer RX1 spectrometer. Bruker Advance 500 MHz FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) equipped with ultra-shield were recorded the NMR analyses of ^{1}H (500 MHz) and ^{13}C (125 MHz) using $CDCl_3$ and CD_3COCD_3 as the solvents and tetramethylsilane (TMS) as internal standard. Chemical shifts were reported in part per million (δ-scale), and the coupling constants *J* are reported in Hertz (Hz). The HRMS mass spectra were obtained from the Waters Xevo QTOF MS system.

Procedure

General procedure for preparation of chalcone derivatives

All the synthesized chalcone derivatives (**3a**-**f**) were prepared through Claisen-Schmidt condensation. A mixture of acetophenone (**1**) (1.17 mL, 0.01 mol) and benzaldehydes (**2a**-**f**) (0.015 mol) was dissolved in methanol (10 mL) and refluxed in the presence of piperidine for 72 h [25-26]. After filtration, the solid was washed, recrystallized, and dried to give the desired chalcone derivatives (**3a**-**f**). The spectroscopic data were compared with the literature (Refer supporting information) [27-30].

General procedure for the preparation of 3,5-disubstituted-pyrazole-1-carbothioamide (4a-f and 5a-e)

The reaction scheme was depicted in Scheme 1. In a round-bottomed flask, chalcone derivatives (**3a**-**f**) (1 mmol) and 4-methyl-3-thiosemicarbazide (0.1576 g, 1.5 mmol)/4-ethyl-3-thiosemicarbazide (0.1788 g, 1.5 mmol) were dissolved in ethanol (6 mL). The reaction mixture was then heated and stirred. Sodium hydroxide (0.12 g, 3 mmol) was then slowly added to the reaction mixture and continued refluxed under vigorous stirring for 3–8 h at 100 °C. Upon completing, the mixture was allowed to cool and stirred at room temperature overnight. On the next day, crushed ice was added into the reaction mixture to precipitate the final products. The precipitate was filtered and dried at room temperature. Purification by column chromatography using silica gel (*n*-hexane:ethyl acetate) or recrystallization with ethanol to afford compounds **4a**-**f** and **5a**-**e**.

Spectral data for synthesized compounds

5-(4-methylphenyl)-*N*-methyl-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (4a). Yellowish crystals; yield: 0.0845 g (26%); mp: 180–184 °C; FTIR (ATR) ν_{max} cm^{-1}: 3361 (N–H), 3042 (C_{sp^2}–H), 2958 (C_{sp^3}–H), 1516, 1427 (aromatic C=C), 1403 (C=S); ^{1}H-NMR (δ/ppm, 500 MHz, $CDCl_3$): δ 2.32 (3H, s, 9-C<u>H</u>$_3$), 3.18 (1H, dd, *J* = 4.0 Hz and 17.5 Hz, 4α-C<u>H</u>), 3.22 (3H, s, 8-C<u>H</u>$_3$), 3.81 (1H, dd, *J* = 11.5 Hz and 17.5 Hz, 4β-C<u>H</u>), 6.07 (1H, dd, *J* = 3.5 Hz and 11.5 Hz, 5-C<u>H</u>), 7.14 (4H, m, H-2′, H-3′, H-5′ and H-6′), 7.45 (3H, m, H-3″, H-4″ and H-5″), 7.74 (2H, dd, *J* = 1.5 Hz and 7.5 Hz, H-2″ and H-6″); ^{13}C-NMR (δ/ppm, 125 MHz, $CDCl_3$): δ 21.1 (9-<u>C</u>H$_3$), 31.4 (8-<u>C</u>H$_3$), 42.6 (4-<u>C</u>H$_2$), 63.3 (5-<u>C</u>H), 125.4 (C-2″ and C-6″), 126.8 (C-2′ and C-6′), 128.8 (C-3″ and C-5″), 129.5 (C-3′ and C-5′), 130.7 (C-4″), 131.1 (C-1″), 137.1 (C-4′), 139.6 (C-1′), 154.4 (3-<u>C</u>=N), 177.0

(6-C=S); HRMS: 310.1380 $[M+H]^+$ {calcd. 309.1378 for $C_{18}H_{20}N_3S$}.

5-(4-methoxyphenyl)-*N*-methyl-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (4b). Yellowish crystals; yield: 0.1528 g (47%); mp: 152–155 °C; FTIR (ATR) ν_{max} cm^{-1}: 3371 (N–H), 3066 (C_{sp}^2–H), 2997 (C_{sp}^3–H), 1614 (C=N), 1510, 1428 (aromatic C=C), 1402 (C=S), 1246 (C–O); ^{1}H-NMR (δ/ppm, 500 MHz, $CDCl_3$): δ 3.19 (4H, m, 4α-CH and 8-CH_3), 3.79 (4H, m, 4β-CH and 9-OCH_3), 6.05 (1H, dd, *J* = 3.5 Hz and 11.5 Hz, 5-CH), 6.86 (2H, d, *J* 9.0 Hz, H-3′ and H-5′), 7.18 (2H, d, *J* = 8.5 Hz, H-2′ and H-6′), 7.45 (3H, m, H-3″, H-4″ and H-5″), 7.75 (2H, dd, *J* = 1.5 Hz and 7.5 Hz, H-2″ and H-6″); ^{13}C-NMR (δ/ppm, 125 MHz, $CDCl_3$): δ 31.4 (8-CH_3), 42.5 (4-CH_2), 55.2 (9-OCH_3), 63.0 (5-CH), 114.2 (C-3′ and C-5′), 126.8 (C-2′ and C-6′), 126.8 (C-2″ and C-6″), 128.8 (C-3″ and C-5″), 130.7 (C-4″), 131.1 (C-1″), 134.7 (C-1′), 154.5 (3-C=N), 158.9 (C-4′), 177.0 (6-C=S); HRMS: 326.1326 $[M+H]^+$ {calcd. 326.1327 for $C_{18}H_{20}N_3OS$}.

5-(4-chlorophenyl)-4,5-dihydro-*N*-methyl-3-phenyl-1*H*-pyrazole-1-carbothioamide (4c). Yellow powder; yield: 0.1924 g (53%); mp: 184–188 °C; FTIR (ATR) ν_{max} cm^{-1}: 3358 (N–H), 3041 (C_{sp}^2–H), 1594 (C=N), 1522, 1429 (aromatic C=C), 1402 (C=S); ^{1}H-NMR (δ/ppm, 500 MHz, $CDCl_3$): δ 3.15 (1H, dd, *J* 4.0 Hz and 17.5 Hz, 4α-CH), 3.21 (3H, d, *J* = 4.5 Hz, 8-CH_3), 3.83 (1H, dd, *J* 11.5 Hz and 18.0 Hz, 4β-CH), 6.06 (1H, dd, *J* = 4.0 Hz and 11.5 Hz, 5-CH), 7.18 (2H, d, *J* 8.5 Hz, H-2′ and H-6′), 7.30 (2H, m, H-3′ and H-5′), 7.46 (3H, m, H-3″, H-4″ and H-5″), 7.74 (2H, dd, *J* = 1.0 Hz and 8.0 Hz, H-2″ and H-6″); ^{13}C-NMR (δ/ppm, 125 MHz, $CDCl_3$): δ 31.4 (8-CH_3), 42.4 (4-CH_2), 62.9 (5-CH), 126.8 (C-2″ and C-6″), 127.0 (C-2′ and C-6′), 128.9 (C-3″ and C-5″), 129.0 (C-3′ and C-5′), 130.8 (C-4″), 130.8 (C-1″), 133.2 (C-4′), 141.0 (C-1′), 154.3 (3-C=N), 177.1 (6-C=S); HRMS: 352.0646 $[M+Na]^+$ {calcd. 352.0651 for $C_{17}H_{16}ClN_3SNa$}.

5-(2,4-dichlorophenyl)-4,5-dihydro-*N*-methyl-3-phenyl-1*H*-pyrazole-1-carbothioamide (4d). Yellowish crystals; yield: 0.3051 g (84%); mp: 166–170 °C; FTIR (ATR) ν_{max} cm^{-1}: 3365 (N–H), 3064 (C_{sp}^2–H), 2934 (C_{sp}^3–H), 1592 (C=N), 1520, 1470 (aromatic C=C), 1399 (C=S); ^{1}H-NMR (δ/ppm, 500 MHz, CD_3COCD_3): δ 3.12 (3H, d, *J* 5.0 Hz, 8-CH_3), 3.17 (1H, dd, *J* = 4.5 Hz and 18.5 Hz, 4α-CH), 4.07 (1H, dd, *J* = 12.0 Hz and 18.0 Hz, 4β-CH), 6.27 (1H, dd, *J* = 4.5 Hz and 12.0 Hz, 5-CH), 7.07 (1H, d, *J* = 8.5 Hz, H-6′), 7.31 (1H, dd, *J* = 2.0 Hz and 8.0 Hz, H-5′), 7.46 (3H, m, H-3″, H-4″ and H-5″), 7.52 (1H, d, *J* = 2.5 Hz, H-3′), 7.87 (2H, dd, *J* = 1.5 Hz and 8.0 Hz, H-2″ and H-6″), 8.38 (1H, s, 7-NH); ^{13}C-NMR (δ/ppm, 125 MHz, CD_3COCD_3): δ 30.9 (8-CH_3), 40.5 (4-CH_2), 61.1 (5-CH), 126.9 (C-2″ and C-6″), 127.4 (C-5′ and C-6′), 128.7 (C-3″ and C-5″), 129.1 (C-3′), 130.5 (C-4″), 131.3 (C-1″), 132.0 (C-2′), 136.4 (C-4′), 139.5 (C-1′), 154.3 (3-C=N), 177.4 (6-C=S); HRMS: 364.0445 $[M+H]^+$ {calcd. 364.0442 for $C_{17}H_{16}Cl_2N_3S$}.

5-(2-chlorophenyl)-4,5-dihydro-*N*-methyl-3-phenyl-1*H*-pyrazole-1-carbothioamide (4e). Yellowish crystals; yield: 0.0400 g (12%); mp: 242–245 °C; FTIR (ATR) ν_{max} cm^{-1}: 3366 (N–H), 3040 (C_{sp}^2–H), 2937 (C_{sp}^3–H), 1572 (C=N), 1523, 1471 (aromatic C=C), 1400 (C=S); ^{1}H-NMR (δ/ppm, 500 MHz, $CDCl_3$): δ 3.12 (3H, d, *J* = 5.0 Hz, 8-CH_3), 3.17 (1H, dd, *J* = 4.5 Hz and 18.5 Hz, 4α-CH), 4.07 (1H, dd, *J* = 12.0 Hz and 18.0 Hz,4β-CH), 6.27 (1H, dd, *J* = 4.5 Hz and 12.0 Hz, 5-CH), 7.07 (1H, m, H-5′), 7.22 (2H, dd, *J* = 3.5 Hz and 7.5 Hz, H-3′ and H-6′), 7.43 (4H, m, H-4′, H-3″, H-4″ and H-5″), 7.75 (2H, dd, *J* = 1.5 Hz and 8.0 Hz, H-2″ and H-6″); ^{13}C-NMR (δ/ppm, 125 MHz, $CDCl_3$): δ 31.0 (8-CH_3), 41.3 (4-CH_2), 61.3 (5-CH), 126.8 (C-2″ and C-6″), 127.2 (C-6′), 128.6 (C-3′), 128.8 (C-5′, C-3″ and C-5″), 130.0 (C-4′), 130.8 (C-4″), 130.9 (C-2′), 131.3 (C-1″), 139.3 (C-1′), 154.7 (3-C=N), 176.1 (6-C=S); HRMS: 330.0816 $[M+H]^+$ {calcd. 330.0832 for $C_{17}H_{17}ClN_3S$}.

5-(2-methoxyphenyl)-4,5-dihydro-*N*-methyl-3-phenyl-1*H*-pyrazole-1-carbothioamide (4f). Light brown crystals; yield: 0.0549 g (17%); mp: 172–175 °C; FTIR (ATR) ν_{max} cm^{-1}: 3380 (N–H), 3048 (C_{sp}^2–H), 2835 (C_{sp}^3–H), 1600 (C=N), 1518, 1432 (aromatic C=C), 1401 (C=S), 1237 (C-O); ^{1}H-NMR (δ/ppm, 500 MHz, CD_3COCD_3): δ 3.06 (1H, dd, *J* = 3.5 Hz and 18.0 Hz, 4α-CH), 3.14 (3H, d, *J* = 4.5 Hz, 8-CH_3), 3.90 (3H, t, *J* = 7.5 Hz, 4β-CH and 9-OCH_3), 6.26 (1H, dd, *J* = 3.5 Hz and 11.5 Hz, 5-CH), 6.84 (1H, t, *J* = 7.5 Hz, H-5′), 6.95 (1H, d, *J* = 7.5 Hz, H-3′), 7.01 (1H, d, *J* = 8.5 Hz, H-6′), 7.22 (1H, m, H-4′), 7.44 (3H, m, H-3″, H-4″ and H-5″), 7.84 (2H, dd, *J* = 1.5 Hz and 8.0 Hz, H -2″ and H-6″), 8.26

(1H, s, 7-NH); ^{13}C-NMR (δ/ppm, 125 MHz, CD_3COCD_3): δ 30.8 (8-CH_3), 41.0 (4-CH_2), 55.1(9-OCH_3), 59.5 (5-CH), 111.0 (C-6′), 120.1 (C-5′), 125.7 (C-3′), 126.8 (C-2″ and C-6″), 128.1 (C-4′), 128.6 (C-3″ and C-5″), 130.2 (C-4″), 130.5 (C-1″), 131.7 (C-1′), 154.8 (3-C=N), 156.1 (C-2′), 176.1 (6-C=S); HRMS: 326.1325 $[M+H]^+$ {calcd. 326.1327 for $C_{18}H_{20}N_3OS$}.

5-(4-methylphenyl)-4,5-dihydro-*N*-ethyl-3-phenyl-1*H*-pyrazole-1-carbothioamide (5a). Yellowish oil; yield: 0.1875 g (58%); FTIR (ATR) ν_{max} cm^{-1}: 3375 (N–H), 3022 (C_{sp}^2–H), 2970 (C_{sp}^3–H), 1571 (C=N), 1514, 1448 (aromatic C=C), 1407 (C=S); ^{1}H-NMR (δ/ppm, 500 MHz, CD_3COCD_3): δ 1.20 (3H, t, *J* = 7.0 Hz, 9-CH_3), 2.26 (3H, s, 10-CH_3), 3.14 (1H, dd, *J* = 4.0 Hz and 18 Hz, 4α-CH), 3.66 (2H, m, 8-CH_2), 3.92 (1H, dd, *J* = 11.5 Hz and 17.5 Hz, 4β-CH), 6.01 (1H, dd, *J* = 4.0 Hz and 11.5 Hz, 5-CH), 7.09 (4H, m, H-2′, H-3′, H-5′, and H-6′), 7.44 (3H, m, H-3″, H-4″ and H-5″), 7.85 (2H, d, *J* = 9.5 Hz, H-2″ and H-6″), 8.21 (1H, s, 7-NH); ^{13}C-NMR (δ/ppm, 125 MHz, CD_3COCD_3): δ 14.1 (9-CH_3), 20.2 (10-CH_3), 38.9 (8-CH_2), 42.0 (4-CH_2), 63.3 (5-CH), 125.5 (C-2″ and C-6″), 126.8 (C-2′, and C-6′), 128.7 (C-3″ and C-5″), 129.0 (C-3′ and C-5′), 130.3 (C-4″), 131.6 (C-1″), 136.3 (C-4′), 140.6 (C-1′), 154.1 (3-C=N), 176.4 (6-C=S); HRMS: 324.1436 $[M+H]^+$ {calcd. 324.1534 for $C_{19}H_{22}N_3S$}.

5-(4-methoxyphenyl)-4,5-dihydro-*N*-ethyl-3-phenyl-1*H*-pyrazole-1-carbothioamide (5b). Yellow crystals; yield: 0.1900 g (56%); mp: 145–148 °C; FTIR (ATR) ν_{max} cm^{-1}: 3324 (N–H), 3050 (C_{sp}^2–H), 2979 (C_{sp}^3–H), 1610 (C=N), 1525, 1445 (aromatic C=C), 1383 (C=S), 1245 (C–O); ^{1}H-NMR (δ/ppm, 500 MHz, $CDCl_3$): δ 1.31 (3H, t, *J* = 7.5 Hz, 9-CH_3), 3.17 (1H, dd, *J* = 3.5 Hz and 18.0 Hz, 4α-CH), 3.76 (6H, m, 4β-CH, 10-OCH_3 and 8-CH_2), 6.06 (1H, dd, *J* = 3.5 Hz and 11.5 Hz, 5-CH), 6.86 (2H, d, *J* = 8.5 Hz, H-3″ and H-5), 7.17 (2H, d, *J* = 8.5 Hz, H-2′ and H-6′), 7.45 (3H, m, H-3″, H-4″ and H-5″), 7.75 (2H, dd, *J* = 2.0 Hz and 7.5 Hz, H-2″ and H-6″); ^{13}C-NMR (δ/ppm, 125 MHz, $CDCl_3$): δ 14.6 (9-CH_3), 39.5 (8-CH_2), 42.5 (4-CH_2), 55.2 (10-OCH_3), 62.9 (5-CH), 114.2 (C-3′ and C-5′), 126.8 (C-2′, C-6′, C-2″ and C-6″), 128.8 (C-3″ and C-5″), 130.7 (C-4″), 131.1 (C-1″), 134.7 (C-1′), 154.4 (3-C=N), 158.8 (C-4′), 175.9 (6-C=S); HRMS: 340.1490 $[M+H]^+$ {calcd. 340.1484 for $C_{19}H_{22}N_3OS$}.

5-(4-chlorophenyl)-4,5-dihydro-*N*-ethyl-3-phenyl-1*H*-pyrazole-1-carbothioamide (5c). Yellowish needle; yield: 0.1874 g (55%); mp: 173–175 °C (lit. 164–166 °C) [31]; FTIR (ATR) ν_{max} cm^{-1}: 3375 (N–H), 3062 (C_{sp}^2–H), 2927 (C_{sp}^2–H), 1596 (C=N), 1518, 1445 (aromatic C=C), 1381 (C=S); ^{1}H-NMR (δ/ppm, 500 MHz, $CDCl_3$): δ 1.31 (3H, t, *J* = 7.5 Hz, 9-CH_3), 3.14 (1H, dd, *J* = 3.5 Hz and 18.0 Hz, 4α-CH), 3.72 (2H, m, 8-CH_2), 3.82 (1H, dd, *J* = 11.5 Hz and 17.5 Hz, 4β-CH), 6.07 (1H, dd, *J* = 4.0 Hz and 11.5 Hz, 5-CH), 7.18 (2H, d, *J* = 8.5 Hz, H-2′ and H-6′), 7.30 (2H, t, *J* = 8.5 Hz, H-3′ and H-5′), 7.46 (3H, m, H-3″, H-4″ and H-5″), 7.74 (2H, dd, *J* = 2.0 Hz and 8.0 Hz, H-2″ and H-6″); ^{13}C-NMR (δ/ppm, 125 MHz, $CDCl_3$): δ 14.5 (9-CH_3), 39.6 (8-CH_2), 42.3 (4-CH_2), 62.8 (5-CH), 126.8 (C-2″ and C-6″), 127.0 (C-2′ and C-6′), 128.9 (C-3″ and C-5″), 129.0 (C-3′ and C-5′), 130.8 (C-1″ and C-4″), 132.2 (C-4′), 142.1 (C-1′), 154.2 (3-C=N), 176.0 (6-C=S); HRMS: 344.1048 $[M+H]^+$ {calcd. 344.0988 for $C_{18}H_{19}ClN_3S$}.

5-(2,4-dichlorophenyl)-4,5-dihydro-*N*-ethyl-3-phenyl-1*H*-pyrazole-1-carbothioamide (5d). Yellowish needle; yield: 0.1577 g (42%); mp: 136–139 °C; FTIR (ATR) ν_{max} cm^{-1}: 3364 (N–H), 3066 (C_{sp}^2–H), 2931 (C_{sp}^3–H), 1588 (C=N), 1518, 1446 (aromatic C=C), 1382 (C=S); ^{1}H–NMR (δ/ppm, 500 MHz, CD_3COCD_3): δ 1.23 (3H, t, *J* = 7.0 Hz, 9-CH_3), 3.16 (1H, dd, *J* = 4.0 Hz and 18.0 Hz, 4α-CH), 3.68 (2H, m, 8-CH_2), 4.06 (1H, dd, *J* = 12.0 Hz and 18.0 Hz, 4β-CH), 6.28 (1H, dd, *J* = 4.5 Hz and 12.0 Hz, 5-CH), 7.06 (1H, d, *J* = 8.5 Hz, H-6′), 7.31 (1H, dd, *J* = 2.0 Hz and 8.5 Hz, H-5′), 7.46 (3H, m, H-3″, H-4″ and H-5″), 7.52 (1H, d, *J* = 2.0 Hz, H-3′), 7.87 (2H, dd, *J* = 1.5 Hz and 8.0 Hz, H-2″ and H-6″), 8.38 (1H, s, 7-NH); ^{13}C-NMR (δ/ppm, 125 MHz, CD_3COCD_3): δ 14.0 (9-CH_3), 39.1 (8-CH_2), 40.5 (4-CH_2), 61.0 (5-CH), 126.9 (C-2″ and C-6″), 127.4 (C-6′), 128.7 (C-5′, C-3″ and C-5″), 129.1 (C-3′), 130.5 (C-4″), 131.3 (C-1″), 131.9 (C-2′), 132.7 (C-4′), 139.5 (C-1′), 154.4 (3-C=N), 176.3 (6-C=S); HRMS: 378.0565 $[M+H]^+$ {calcd. 378.0598 for $C_{18}H_{18}Cl_2N_3S$}.

5-(2-chlorophenyl)-4,5-dihydro-*N*-ethyl-3-phenyl-1*H*-pyrazole-1-carbothioamide (5e). Yellowish crystals; yield: 0.1521 g (44%); mp: 176–179 °C; FTIR (ATR) ν_{max} cm^{-1}: 3359 (N–H), 3055 (C_{sp}^2–H), 2967 (C_{sp}^3–

H), 1600 (C=N), 1520, 1445 (aromatic C=C), 1379 (C=S); ^{1}H-NMR (δ/ppm, 500 MHz, $CDCl_3$): δ 1.34 (3H, t, *J* = 7.5 Hz, 9-C<u>H</u>$_3$), 3.10 (1H, dd, *J* = 4.0 Hz and 17.5 Hz, 4α-C<u>H</u>), 3.76 (2H, m, 8-C<u>H</u>$_2$), 3.91 (1H, dd, *J* = 11.5 Hz and 18.0 Hz, 4β-C<u>H</u>), 6.40 (1H, dd, *J* = 4.0 Hz and 12.0 Hz, 5-C<u>H</u>), 7.07 (1H, m, H-5′), 7.22 (2H, dd, *J* = 3.5 Hz and 7.5 Hz, H-3′ and H-6′), 7.43 (4H, m, H-4′, H-3″, H-4″ and H-5″), 7.75 (2H, dd, *J* = 1.5 Hz and 8.0 Hz, H-2″ and H-6″); ^{13}C-NMR (δ/ppm, 125 MHz, $CDCl_3$): δ 14.6 (9-<u>C</u>H$_3$), 39.6 (8-<u>C</u>H$_2$), 41.3 (4-<u>C</u>H$_2$), 61.3 (5-<u>C</u>H), 126.8 (C-2″ and C-6″), 127.2 (C-6′), 128.6 (C-3′), 128.8 (C-5′, C-3″ and C-5″), 130.0 (C-4′), 130.8 (C-4″), 130.9 (C-2′), 131.3 (C-1″), 139.3 (C-1′), 154.7 (3-<u>C</u>=N), 176.1 (6-<u>C</u>=S); HRMS: 344.0909 $[M+H]^+$ {calcd. 344.0988 for $C_{18}H_{19}ClN_3S$}.

Screening of anti-tuberculosis activity against M. tuberculosis *H37Ra*

The Tetrazolium microplate assay (TEMA) method was performed to evaluate the anti-mycobacterial activity of the derivatives as described by Amilah et al. [32] with minor modification. The assay was carried out in 96-well plates in duplicate and at least three times independently. The derivatives were dissolved in DMSO and serially diluted to the desired concentration in complete Middlebrook 7H9 media to reduce the DMSO concentration below 1%. A log phase MTB ATCC 25177 was added and incubated at 37 °C and 5% CO_2 for 5 days. On the fifth day, 50 µL of tetrazolium reagent mixture was added into all wells and re-incubated for 24 h. The MIC is defined as the lowest drug concentration that prevented the color change from yellow to purple. A small volume of the culture from the 96-well plate was transferred into Middlebrook 7H10 agar media, and the plates were incubated at 37 °C and 5% CO_2 for 28 days. The MBC is defined as the lowest concentration of compound that did not show any bacterial colonies.

Molecular docking studies

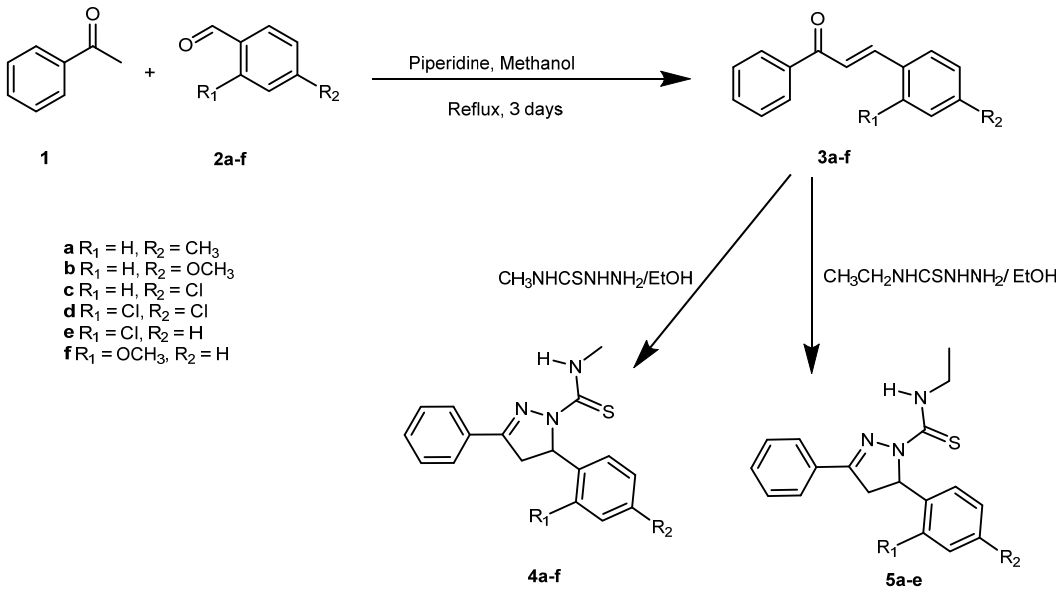
Molecular docking studies were performed using AutoDock v. 4.2.2 to identify appropriate binding modes and the conformation of the ligand molecule. The crystal structure of dihydrofolate reductase complexed with novel 7-aryl-2,4-diaminoquinazolines (PDB code: 3SRQ) was retrieved from the RCSB protein data bank in PDB format [33]. The structures of all the ligands were drawn using ChemDraw Ultra 13.0 and converted into 3D structures using Hyperchem Pro 8.0 software (www.hyper.com). Autodock Tools (ADT) version 1.5.6 (www.autodock.scrips.edu) was used for molecular docking. The active site was considered as a rigid molecule, while the ligands were treated as being flexible. Using default parameters, grid-based docking studies were carried out, and docking was performed on all compounds using standard ligand 7-aryl-2,4-diaminoquinazolines. The best binding conformation was selected from the docking log (dlg) file for each ligand, and further interaction analysis was performed using PyMol and Discovery Studio Visualizer 4.0.

■ RESULTS AND DISCUSSION

Chemistry

The synthesis of 3,5-disubstituted-4,5-dihydro-*N*-alkyl-1*H*-pyrazole-1-carbothioamides (**4a**-**f** and **5a**-**e**) was achieved by condensation reaction between chalcones (**3a**-**f**) with 4-methyl-3-thiosemicarbazide/4-ethyl-3-thiosemicarbazide under the alkaline condition (Scheme 1). Chalcone derivatives (**3a**-**f**) were synthesized through Claisen-Schmidt condensation. Compound **4a** was taken as an example to describe the obtained data. Compound **4a** was obtained yellowish crystals with a melting point 180–184 °C. The HRMS spectrum of **4a** revealed molecular ion peak $[M+H]^+$ at *m/z* 310.1380, which is consistent with the molecular formula $C_{18}H_{20}N_3S$ (calcd. 310.1378). The important absorption bands in the FTIR spectrum were observed at 3361 cm^{-1} (N-H stretching), 1590 cm^{-1} (C=N stretching), 1516 cm^{-1} and 1427 cm^{-1} (C=C stretching of the aromatic), and 1403 cm^{-1} (C=S stretching). The absence of C=O and C=C bands, as well as the appearance of new C=N and C=S bands in the IR spectra, suggested the complete formation of the compound **4a** via the cyclocondensation reaction between chalcone **3a** and 4-methyl-3-thiosemicarbazide (Scheme 1).

The ^{1}H-NMR spectrum of compound **4a** showed two singlet signals at the region of δ_H 2.32 and 3.22 ppm indicating the presence of two methyl protons, 9-C<u>H</u>$_3$ and 8-C<u>H</u>$_3$, respectively. Two geminal protons, H-4α and H-4β of the methylene group, were resonated as



Scheme 1. Synthesis of chalcone and pyrazoline derivatives

doublet of doublets at δ_H 3.18 and 3.81 ppm, respectively. The appearance of these two signals could be attributed to the non-equivalent nature of the two germinal protons with *J* coupling constant of $J_{4\alpha4\beta}$ = 17.5 Hz, $J_{4\alpha5}$ = 4.0 Hz, and $J_{4\beta5}$ = 11.5 Hz. Meanwhile, the vicinal proton (H-5) also appeared as a doublet of doublets at a slightly downfield region, δ_H 6.07 ppm, due to the vicinal coupling with the two neighboring germinal protons of methylene group at position-4 of the pyrazoline ring. In compound **4a**, some aromatic protons such as H-3″, H-4″, and H-5″ were observed at the same chemical shift, δ_H 7.45 ppm, due to the overlapping of peaks in a similar environment. The absence of one board signal in the thioamide (7-N-H) of compound **4a** due to the deuterium proton will exchange with the proton of the synthesized compound by intermolecular proton transfer [34]. The ^{13}C-NMR spectrum of carbothioamide compound **4a** was showed fourteen carbons signals. One carbon signal each at δ_C 42.6 and 63.3 ppm suggested the presence of the pyrazoline ring carbons, which are assigned to 4-CH_2 and 5-CH, respectively. Also, a signal at δ_C 154.4 ppm was attributed to 3-C=N in the pyrazoline ring, which is a carbon attached to electronegative nitrogen by a double bond also to a benzene ring. Besides, a signal at δ_C 177.0 ppm was indicated to 6-C=S. The 2D-NMR correlation of ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC spectra were used to

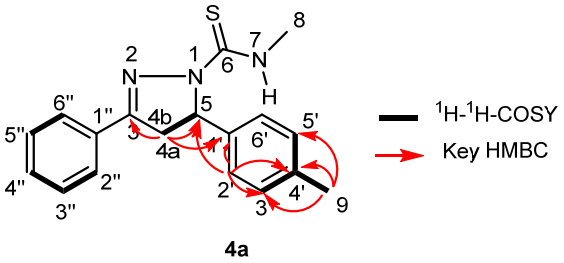


Fig 1. 2D-COSY and 2D-HMBC correlations of compound **4a**

assign the aromatic signals, especially in the case of the pyrazoline ring. The analysis of ^{1}H–^{1}H COSY the cross peak of 9-CH_3 with H-3′-H-5′, 4α-CH with 5-CH-4β-CH, and H-2″-H-6″ with H-3″-H-4″-H-5″ (Fig. 1). The ^{1}H–^{13}C HMBC spectrum of **4a** shows the cross-correlations of 4α-CH with carbons 5-CH, C-1′, and 3-C=N, and H-2′ with 5-CH, C-3′, C-4′ and C-1′ (Fig. 1). Additionally, the cross-correlation of 9-CH_3 with C-3′, C-5′, and C-4′. According to the above spectra data analysis, compound **4a** was identified as a new 2-pyrazoline compound named 5-(4-methylphenyl)-4,5–dihydro-*N*-methyl-3-phenyl-1*H*-pyrazole-1-carbothioamide.

Anti-tuberculosis Activity

Eleven synthesized pyrazoline derivatives **4a**-**f** and **5a**-**e** were screened for anti-TB activity against MTB

H37Ra using the TEMA method. The INH was used as a positive control. The following Table 1 depicts the results of the anti-TB activity of these compounds based on their MIC and MBC values. Among the eleven tested compounds, six exhibited promising activity against MTB H37Ra with MIC values in the range 530 to 647 μM. Compounds **4a**, **4d**, **4f**, **5a**, **5c** and **5d** showed weak activity with MICs of 647 μM (200 μg/mL), 551 μM (200 μg/mL), 615 μM (200 μg/mL), 619 μM (200 μg/mL), 583 μM (200 μg/mL) and 530 μM (200 μg/mL), respectively. Meanwhile, other compounds **4b**, **4c**, **4e**, **5b**, and **5e** showed no inhibition against MTB H37Ra. The six compounds (**4a**, **4d**, **4f**, **5a**, **5c**, and **5d**) were further evaluated for their bactericidal activity against MTB H37Ra. As the results for MBC, all tested compounds did not show any cidal effects against MTB H37Ra, even at the highest test concentration of 200 μg/mL. The *in vitro* anti-TB evaluation revealed that the compounds containing *para* methoxy substituted (**4b** and **5b**) in both series showed no inhibition against MTB H37Ra even at the highest tested concentration of 200 μg/mL. This is not surprising as the previous studies reported that (OCH_3) group substitution at phenyl ring in pyrazoline analog worsens the anti-TB activity [35].

Molecular Docking Analysis

To gain further support regarding the antibacterial effect of the most promising pyrazole derivative **5d** against TB, a molecular docking study was carried out on the dihydrofolate reductase (DHFR) enzyme (PDB ID: 3SRQ) using the AutoDock program [33]. AutoDock 4.2.2 with a Lamarckian genetic algorithm-implemented program suite was employed to identify appropriate binding modes and the conformations of the ligand molecules. DHFR catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate, which is essential for the synthesis of purines, some amino acids, and thymidine required for bacterial growth and proliferation. Thus, DHFR represents an attractive antifolate drug target that produces antibacterial action by selectively disrupting the folate pathway. The docking protocol was validated by redocking the co-crystallized ligand 7-aryl-2,4-diaminoquinazoline at the active site (RMSD 0.10). The results of docking studies clearly showed that all compounds fit nicely into the active site and form van der Waals, alkyl, π- π, and π-alkyl interactions with the active site residues. The binding free energy of compounds **5d** was found to be -8.2 kcal/mol, indicating

Table 1. *In vitro* anti-tuberculosis activities of pyrazoline derivatives **4a**-**e** and **5a**-**f** against *Mycobacterium tuberculosis* H37Ra

Compounds	MIC		MBC	
	(μg/mL)	(μM)	(μg/mL)	(μM)
4a	200	647	> 200	> 647 (NC)
4b	> 200	> 615	> 200	> 615
4c	> 200	> 608	> 200	> 608
4d	200	551	> 200	> 551 (NC)
4e	> 200	> 608	> 200	> 608
4f	200	615	> 200	> 615 (NC)
5a	200	619	> 200	> 619 (NC)
5b	> 200	> 590	> 200	> 590 (NC)
5c	200	583	> 200	> 583 (NC)
5d	200	530	> 200	> 530 (NC)
5e	> 200	> 583	> 200	> 583
Isoniazid (Control)	0.625	5	0.625	5

Results are mean values of duplicate and independently thrice. NC = No cidal effect at high concentration

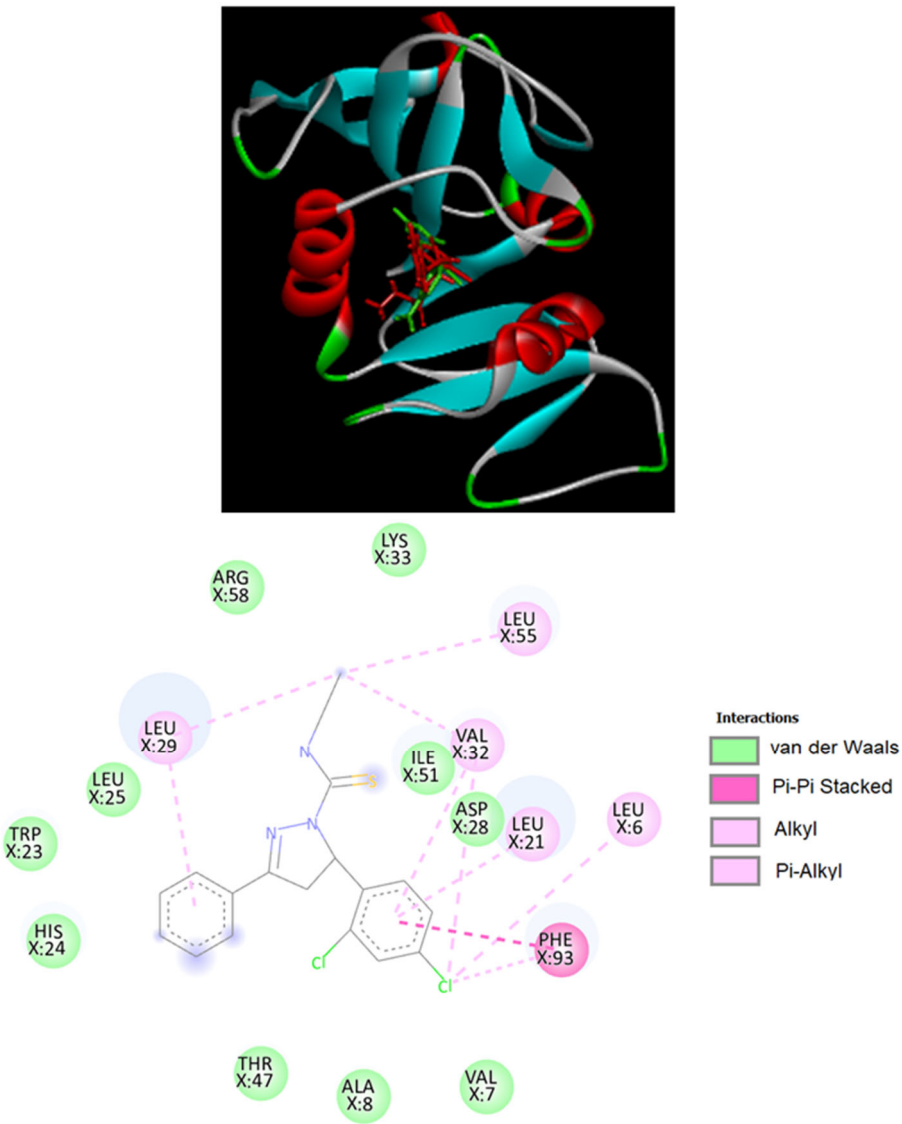


Fig 2. Overlay of the co-crystallized ligand 1-[3-(2,4-diamino-6-methylquinazolin-7-yl)phenyl]ethanone (green) with the compounds **5d** (red) at the same active site of DHFR and 2D binding interactions of **5d**

sufficient affinity between the oxadiazole analog and the enzyme. The 3D and 2D docked conformations of the most active ligand **5d** bound to the active site of DHFR are shown in Fig. 2. Lipophilicity also appears to play a crucial role in the antibacterial activity of **5d**, as the phenyl ring positioned itself into the lipophilic pocket of the binding site and formed π-π interactions with the Phe93 residues. Based on the above study, it can be proved that pyrazole derivatives possess potential DHFR inhibitory activity.

■ CONCLUSION

In the present study, eleven compounds from a series of 3,5-disubstituted-4,5-dihydro-*N*-alkyl-1*H*-pyrazole-1-carbothioamides (**4a**-**f** and **5a**-**e**) have been successfully synthesized via Claisen-Schmidt condensation reaction. All compounds were elucidated using FTIR, 1D- & 2D-NMR, and HRMS, and also tested for their anti-tuberculosis activity by *in vitro* study against *Mycobacterium tuberculosis* H37Ra. Our

preliminary data indicate that the assessed, newly synthesized compounds showed a partial inhibitory effect against *Mycobacterium tuberculosis* H37Ra at a 200 µg/mL concentration.

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■ AUTHOR CONTRIBUTIONS

Conceptualization, HO, TP, and MNA; methodology, KTW, HO, TP, and MNA; software, MZH; validation, HO, TP, and MNA; formal analysis, KTW, MZH, MSAG, and US; investigation, KTW, MSAG, and US; resources, HO, TP, and MNA; data curation, KTW, and MSAG; writing-original draft preparation, KTW, MSAG, MZH, TP, and MNA; writing-review and editing, HO, MNA, US, TP, and MZH; visualization, KTW, and MNA; supervision, HO, TP, and MNA; project administration, HO, and MNA; funding acquisition, HO, and MNA; All authors have read and agreed to the published version of the manuscript.

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