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Novel Analogues of 2-Pyrazoline: Synthesis, Characterization, and Antimycobacterial Evaluation

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Fourteen new 1-[(N,N-disubstitutedthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives were synthesized by reacting 1-(chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines and appropriate sodium salts of N,N-disubstituted dithiocarbamoic acids in acetone. The structures of the synthesized compounds were confirmed by UV, IR, ^{1}H-NMR, and FAB$^+$-MS spectral data. The compounds were evaluated for in vitro antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv using the BACTEC 460 radiometric system and BACTEC 12B medium. The preliminary results showed that all of the tested compounds were inactive against the test organism.

Key Words: 2-Pyrazolines, Sodium salts of N,N-disubstituted dithiocarbamoic acids, Antimycobacterial activity.

Introduction

Pyrazolines are well known, and important nitrogen-containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis.[1] Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field. They have several prominent effects, such as antimicrobial,[2−5] antimycobacterial[6], anti-inflammatory, analgesic,[7,8] and antidepressant[9,10] activities. 2-Pyrazolines seem to be the most frequently studied pyrazoline type compounds. As a result, a large number of 2-pyrazolines using different synthetic methods for their preparation have been described in the chemistry literature. An especially popular procedure is based on the reaction of α,β-unsaturated aldehydes and ketones with hydrazines.[1−3,6,10−24] However, a series of specially substituted representatives have been prepared rarely. For this reason, the aim of our present study was to synthesize systematically 1-[(N,N-disubstitutedthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-aryl-2-pyrazolines for the study of their structure-activity relationships.

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Experimental

All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck). The necessary chemicals were purchased from Merck and Fluka. Spectroscopic data were recorded with the following instruments; UV: Shimadzu Model 160A UV spectrophotometer, IR: Shimadzu IR-435 spectrophotometer, ^{1}H-NMR: Bruker 250 MHz spectrometer in DMSO-d_6 using TMS as an internal standard, and MS-FAB: VG Quattro Mass spectrometer.

Synthesis of 1-(2-Thienyl)-3-aryl-2-propen-1-ones (3a-g)

A mixture of 2-acetylthiophene (0.04 mol) **(1)**, aromatic aldehyde (0.04 mol) **(2),** and 10% aqueous sodium hydroxide (10 mL) in ethanol (30 mL) was stirred at room temperature for about 3 h. The resulting solid was washed, dried, and crystallized from ethanol.[15]

Synthesis of 5-Aryl-3-(2-thienyl)-2-pyrazolines (4a-g)

A solution of the appropriate thienyl chalcone (0.01 mol) **(3)** and hydrazine hydrate (80%) (0.02 mol) in ethanol (30 mL) was refluxed for 3 h. The reaction mixture was cooled and kept at 0 °C overnight. The resulting solid was crystallized from ethanol.[15]

Synthesis of 1-(Chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines (5a-g)

The 5-Aryl-3-(2-thienyl)-2-pyrazolines **(4)** (0.01 mol) and triethylamine (0.01 mol) were dissolved in dry toluene (30 ml) with constant stirring. Later, the mixture was cooled in an ice bath, and chloroacetylchloride (0.01 mol) was added dropwise with stirring. The reaction mixture thus obtained was further agitated for 1 h at room temperature. The precipitate was filtrated, the solvent was evaporated to dryness under reduced pressure, and the products were recrystallized from ethanol.[25]

Synthesis of Sodium salts of N,N-disubstitued dithiocarbamic acids (6a-b)

Sodium salts of N,N-disubstitued dithiocarbamic acids **(6a-b)** used in the synthesis were prepared according to the methods reported in the literature.[26]

Synthesis of 1-[(N,N-Disubstitutedthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-aryl-2-pyrazolines (7a-n)

A mixture of 1-(chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines (**5**) (0.01 mol) and sodium salts of the appropriate N,N-disubstituted dithiocarbamoic acid (**6**) (0.01 mol) was treated in acetone at room temperature for 2 h. The solvent was evaporated, washed with water, and recrystallized from ethanol[2] (Figure 1).

Some characteristics of the synthesized compounds are shown in Table 1. Analytical and spectral data (UV, IR, ^{1}H-NMR, FAB$^+$-MS) confirmed the structures of the new compounds.

***1-[(4-Morpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-phenyl-2-pyrazoline* (7a):** Yield 48%; mp 144 °C; UV [$\lambda_{\max}^{EtOH}$ nm), log ε]: 208.2 (4.90), 316.4 (4.72). - IR [ν, cm^{-1}, KBr]: 1666 (C=O), 1517-1407 (C=N, C=C), 1232 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 3.18 (1H, dd $J_{AM} = 18.00$

Hz, J_{AX} = 4.60 Hz, C_4-H_A of pyrazoline ring), 3.70 (4H, m, morpholine CH_2-O-CH_2), 3.85-4.30, 3.92 (5H, m, morpholine CH_2-N-CH_2 and dd J_{MA} = 18.00 Hz, J_{MX} = 11.70 Hz, C_4-H_M of pyrazoline ring), 4.60 (1H, d J = 16.20 Hz, CO<u>CH</u> geminal proton), 4.65 (1H, d J = 16.18 Hz, CO<u>CH</u> geminal proton), 5.57 (1H, dd J_{MX} = 11.57 Hz, J_{AX} = 4.45 Hz, C_5-H_X of pyrazoline ring), 7.20 (6H, m, phenyl protons and thiophene C_4-H), 7.50 (1H, s, thiophene C_3-H), 7.80 (1H, s, thiophene C_5-H). – MS-FAB$^+$: m/z: 432 [M+1].

***1-[(4-Thiomorpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-phenyl-2-pyrazoline* (7b):** Yield 50%; mp 161 °C; UV [$\lambda_{\max}^{EtOH}$ nm), log ε]: 208.6 (4.94), 317.8 (4.76). - IR [ν, cm^{-1}, KBr]: 1664 (C=O), 1446-1406 (C=N, C=C), 1218 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.60-2.80 (4H, m, thiomorpholine CH_2-S-CH_2), 3.18 (1H, dd J_{AM} = 18.00 Hz, J_{AX} = 4.60 Hz, C_4-H_A of pyrazoline ring), 3.92 (1H, dd J_{MA} = 17.80 Hz, J_{MX} = 11.60 Hz, C_4-H_M of pyrazoline ring), 4.10-4.60 (4H, 2 m, thiomorpholine CH_2-N-CH_2), 4.65 (1H, d J = 16.15 Hz, CO<u>CH</u> geminal proton), 4.70 (1H, d J = 16.13 Hz, CO<u>CH</u> geminal proton), 5.57 (1H, dd J_{MX} = 11.70 Hz, J_{AX} = 4.60 Hz, C_5-H_X of pyrazoline ring), 7.10-7.40 (6H, m, phenyl protons and thiophene C_4-H), 7.45 (1H, s thiophene C_3-H), 7.75 (1H, s, thiophene C_5-H).– MS-FAB$^+$: m/z: 447 [M], 448 [M+1].

***1-[(4-Morpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-methylphenyl)-2-pyrazoline* (7c):** Yield 49%; mp 116-118 °C; UV [$\lambda_{\max}^{EtOH}$ nm), log ε]: 208.8 (5.05), 320.1 (4.61). - IR [ν, cm^{-1}, KBr]: 1658 (C=O), 1588-1410 (C=N, C=C), 1229 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.25 (3H, s, Ar-CH_3), 3.20 (1H, dd J_{AM} = 17.88 Hz, J_{AX} = 4.43 Hz, C_4-H_A of pyrazoline ring), 3.70 (4H, m, morpholine CH_2-O-CH_2), 3.90 (1H, dd J_{MA} = 17.97 Hz, J_{MX} = 11.76 Hz, C_4-H_M of pyrazoline ring), 3.95 (4H, m, morpholine CH_2-N-CH_2), 4.70 (1H, d J = 16.53 Hz, CO<u>CH</u> geminal proton), 4.75 (1H, d J = 16.55 Hz, CO<u>CH</u> geminal proton), 5.55 (1H, dd J_{MX} = 11.57 Hz, J_{AX} = 4.45 Hz, C_5-H_X of pyrazoline ring), 7.20 (5H, m, phenyl protons and thiophene C_4-H), 7.50 (1H, d J = 2.66 Hz, thiophene C_3-H), 7.80 (1H, d J = 4.10 Hz, thiophene C_5-H). – MS-FAB$^+$: m/z: 445 [M], 446 [M+1].

***1-[(4-Thiomorpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-methylphenyl)-2-pyrazoline* (7d):** Yield 51%; mp 91-93 °C; UV [$\lambda_{\max}^{EtOH}$ nm), log ε]: 208.6 (4.94), 318.2 (4.67). - IR [ν, cm^{-1}, KBr]: 1662 (C=O), 1514-1409 (C=N, C=C), 1215 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.20 (3H, s, Ar-CH_3), 2.70 (4H, m, thiomorpholine CH_2-S-CH_2), 3.15 (1H, dd J_{AM} = 18.10 Hz, J_{AX} = 4.50 Hz, C_4-H_A of pyrazoline ring), 3.90 (1H, dd J_{MA} = 17.92 Hz, J_{MX} = 11.59 Hz, C_4-H_M of pyrazoline ring), 4.20 (4H, m, thiomorpholine CH_2-N-CH_2), 4.70 (1H, d J = 16.13 Hz, CO<u>CH</u> geminal proton), 4.75 (1H, d J = 16.10 Hz, CO<u>CH</u> geminal proton), 5.50 (1H, dd J_{MX} = 11.63 Hz, J_{AX} = 4.46 Hz, C_5-H_X of pyrazoline ring), 7.15 (5H, m, phenyl protons and thiophene C_4-H), 7.45 (1H, d J = 2.81 Hz, thiophene C_3-H), 7.75 (1H, d J = 4.32 Hz, thiophene C_5-H). – MS-FAB$^+$: m/z: 461 [M], 462 [M+1].

***1-[(4-Morpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-dimethylaminophenyl)-2-pyrazoline* (7e):** Yield 69%; mp 86°C; UV [$\lambda_{\max}^{EtOH}$ nm), log ε]: 207.6 (5.15), 317.8 (4.78). - IR [ν, cm^{-1}, KBr]: 1660 (C=O), 1614-1410 (C=N, C=C), 1230 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.88 (6H, s, $N(CH_3)_2$), 3.18 (1H, dd J_{AM} = 17.83 Hz, J_{AX} = 4.44 Hz, C_4-H_A of pyrazoline ring), 3.60 (4H, m, morpholine CH_2-O-CH_2), 3.80 (1H, dd J_{MA} = 17.85 Hz, J_{MX} = 11.50 Hz, C_4-H_M of pyrazoline ring), 3.90 (4H, m, morpholine CH_2-N-CH_2), 4.60 (1H, d J = 16.20 Hz, CO<u>CH</u> geminal proton), 4.65 (1H, d J = 16.10 Hz, CO<u>CH</u> geminal proton), 5.52 (1H, dd J_{MX} = 11.55 Hz, J_{AX} = 4.47 Hz, C_5-H_X of pyrazoline ring), 6.80 (2H, dJ = 8.58 Hz, phenyl $C_{2,6}$-H), 7.17 (2H, dJ = 8.59 Hz, phenyl $C_{3,5}$-H and 1H, dJ = 4.18 Hz, thiophene C_4-H), 7.40 (1H, dJ = 2.75 Hz, thiophene C_3-H), 7.75 (1H, dJ = 4.48 Hz, thiophene C_5-H). – MS-FAB$^+$: m/z: 475 [M+1].

***1-[(4-Thiomorpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-dimethylaminophenyl)-2-pyrazoline* (7f):** Yield 71%; mp 87 °C; UV [λ_{max}^{EtOH} nm), log ε]: 207.4 (4.87), 316.6 (4.59). - IR [ν, cm^{-1}, KBr]: 1660 (C=O), 1614-1402 (C=N, C=C), 1215 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.75 (4H, m, thiomorpholine CH_2-S-CH_2), 2.90 (6H, s, $N(CH_3)_2$), 3.20 (1H, dd J_{AM} = 17.87 Hz, J_{AX} = 4.31 Hz, C_4-H_A of pyrazoline ring), 3.90 (1H, dd J_{MA} = 17.93 Hz, J_{MX} = 11.51 Hz, C_4-H_M of pyrazoline ring), 4.30 (4H, m, thiomorpholine CH_2-N-CH_2), 4.70 (1H, d J = 16.06 Hz, CO<u>CH</u> geminal proton), 4.75 (1H, d J = 16.05 Hz, CO<u>CH</u> geminal proton), 5.50 (1H, dd J_{MX} = 11.42 Hz, J_{AX} = 4.14 Hz, C_5-H_X of pyrazoline ring), 6.65 (2H, d J = 8.72 Hz, phenyl $C_{2,6}$-H), 7.10 (2H, d J = 8.70 Hz, phenyl $C_{3,5}$-H), 7.20 (1H, t J = 4.35, thiophene C_4-H), 7.55 (1H, d J = 2.81 Hz, thiophene C_3-H), 7.80 (1H, d J = 4.98 Hz, thiophene C_5-H). – MS-FAB$^+$: m/z: 490 [M], 491 [M+1].

***1-[(4-Morpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-hydroxyphenyl)-2-pyrazoline* (7g):** Yield 56%; mp 203 °C; UV [λ_{max}^{EtOH} nm), log ε]: 208.6 (5.50), 318.8 (5.36). - IR [ν, cm^{-1}, KBr]: 3232 (O-H), 1660 (C=O), 1614-1417 (C=N, C=C), 1269 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 3.15 (1H, dd J_{AM} = 17.98 Hz, J_{AX} = 4.26 Hz, C_4-H_A of pyrazoline ring), 3.65 (4H, m, morpholine CH_2-O-CH_2), 3.75 (1H, dd J_{MA} = 17.95 Hz, J_{MX} = 11.60 Hz, C_4-H_M of pyrazoline ring), 4.00 (4H, m, morpholine CH_2-N-CH_2), 4.60 (1H, d J = 16.12 Hz, CO<u>CH</u> geminal proton), 4.65 (1H, d J = 16.14 Hz, CO<u>CH</u> geminal proton), 5.40 (1H, dd J_{MX} = 11.56 Hz, J_{AX} = 4.28 Hz, C_5-H_X of pyrazoline ring), 6.64 (2H, d J = 8.42 Hz, phenyl $C_{2,6}$-H), 6.68 (2H, d J = 8.44 Hz, phenyl $C_{3,5}$-H), 7.12 (1H, t J = 3.70, thiophene C_4-H), 7.43 (1H, d J = 2.83 Hz, thiophene C_3-H), 7.78 (1H, d J = 4.86 Hz, thiophene C_5-H), 9.40 (1H, s, Ar-OH). – MS-FAB$^+$: m/z: 448 [M+1].

***1-[(4-Thiomorpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-hydroxyphenyl)-2-pyrazoline* (7h):** Yield 58%; mp 208 °C; UV [λ_{max}^{EtOH} nm), log ε]: 207.6 (5.03), 318.2 (4.98). - IR [ν, cm^{-1}, KBr]: 3222 (O-H), 1643 (C=O), 1614-1419 (C=N, C=C), 1278 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.70 (4H, m, thiomorpholine CH_2-S-CH_2), 3.10 (1H, dd J_{AM} = 18.09 Hz, J_{AX} = 4.28 Hz, C_4-H_A of pyrazoline ring), 3.80 (1H, dd J_{MA} = 17.83 Hz, J_{MX} = 11.62 Hz, C_4-H_M of pyrazoline ring), 4.40 (4H, m, thiomorpholine CH_2-N-CH_2), 4.60 (1H, d J = 16.15 Hz, CO<u>CH</u> geminal proton), 4.65 (1H, d J = 16.17 Hz, CO<u>CH</u> geminal proton), 5.45 (1H, dd J_{MX} = 11.56 Hz, J_{AX} = 4.28 Hz, C_5-H_X of pyrazoline ring), 6.60 (2H, d J = 8.46 Hz, phenyl $C_{2,6}$-H), 7.00 (2H, d J = 8.48 Hz, phenyl $C_{3,5}$-H), 7.10 (1H, t J = 3.82, thiophene C_4-H), 7.45 (1H, d J = 3.11 Hz, thiophene C_3-H), 7.75 (1H, d J = 4.96 Hz, thiophene C_5-H), 9.35 (1H, s, Ar-OH). – MS-FAB$^+$: m/z: 464 [M+1].

***1-[(4-Morpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-methoxyphenyl)-2-pyrazoline* (7i):** Yield 57%; mp 84 °C; UV [λ_{max}^{EtOH} nm), log ε]: 207.8 (4.89), 319.6 (4.49). - IR [ν, cm^{-1}, KBr]: 1662 (C=O), 1610-1408 (C=N, C=C), 1267 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 3.15 (1H, dd J_{AM} = 18.00 Hz, J_{AX} = 4.43 Hz, C_4-H_A of pyrazoline ring), 3.60 (4H, m, morpholine CH_2-O-CH_2), 3.70 (3H, s, Ar-OCH_3), 3.85 (1H, dd J_{MA} = 17.91 Hz, J_{MX} = 11.63 Hz, C_4-H_M of pyrazoline ring), 3.90 (4H, m, morpholine CH_2-N-CH_2), 4.65 (1H, d J = 16.11 Hz, CO<u>CH</u> geminal proton), 4.70 (1H, d J = 16.10 Hz, CO<u>CH</u> geminal proton), 5.50 (1H, dd J_{MX} = 11.56 Hz, J_{AX} = 4.46 Hz, C_5-H_X of pyrazoline ring), 6.85 (2H, d J = 8.66 Hz, phenyl $C_{2,6}$-H), 7.15 (2H, d J = 8.69 Hz, phenyl $C_{3,5}$-H and 1H, d J = 4.22, thiophene C_4-H), 7.45 (1H, d J = 3.49 Hz, thiophene C_3-H), 7.75 (1H, d J = 5.04 Hz, thiophene C_5-H). – MS-FAB$^+$: m/z: 461 [M], 462 [M+1].

***1-[(4-Thiomorpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-methoxyphenyl)-2-pyrazoline* (7j):** Yield 59%; mp 168 °C; UV [λ_{max}^{EtOH} nm), log ε]: 208.4 (4.96), 319.5 (4.67). - IR [ν, cm^{-1}, KBr]: 1659 (C=O), 1611-1410 (C=N, C=C), 1226 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.70

(4H, m, thiomorpholine CH_2-S-CH_2), 3.15 (1H, dd J_{AM} = 17.84 Hz, J_{AX} = 4.45 Hz, C_4-H_A of pyrazoline ring), 3.30 (3H, s, Ar-OCH_3), 3.90 (1H, dd J_{MA} = 17.90 Hz, J_{MX} = 11.66 Hz, C_4-H_M of pyrazoline ring), 4.25 (4H, m, thiomorpholine CH_2-N-CH_2), 4.70 (1H, d J = 16.13 Hz, CO<u>CH</u> geminal proton), 4.75 (1H, d J = 16.14 Hz, CO<u>CH</u> geminal proton), 5.50 (1H, dd J_{MX} = 11.59 Hz, J_{AX} = 4.37 Hz, C_5-H_X of pyrazoline ring), 6.85 (2H, d J = 8.69 Hz, phenyl $C_{2,6}$-H), 7.15 (2H, d J = 8.61 Hz, phenyl $C_{3,5}$-H and 1H, d J = 4.38 Hz, thiophene C_4-H), 7.45 (1H, d J = 2.68 Hz, thiophene C_3-H), 7.75 (1H, d J = 4.21 Hz, thiophene C_5-H). – MS-FAB$^+$: m/z: 477 [M], 478 [M+1].

***1-[(4-Morpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-fluorophenyl)-2-pyrazoline* (7k):** Yield 53%; mp 119 °C; UV [λ_{max}^{EtOH} nm), log ε]: 208.8 (4.80), 318.5 (4.57). - IR [ν, cm^{-1}, KBr]: 1664 (C=O), 1602-1409 (C=N, C=C), 1228 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 3.20 (1H, dd J_{AM} = 18.00 Hz, J_{AX} = 4.54 Hz, C_4-H_A of pyrazoline ring), 3.65 (4H, m, morpholine CH_2-O-CH_2), 3.85 (1H, dd J_{MA} = 17.95 Hz, J_{MX} = 11.59 Hz, C_4-H_M of pyrazoline ring), 3.92 (4H, m, morpholine CH_2-N-CH_2), 4.65 (1H, d J = 16.15 Hz, CO<u>CH</u> geminal proton), 4.70 (1H, d J = 16.17 Hz, CO<u>CH</u> geminal proton), 5.57 (1H, dd J_{MX} = 11.65 Hz, J_{AX} = 4.57 Hz, C_5-H_X of pyrazoline ring), 7.12 - 7.27 (5H, m, phenyl protons and thiophene C_4-H), 7.47 (1H, dJ = 2.70 Hz, thiophene C_3-H), 7.75 (1H, dJ = 4.16 Hz, thiophene C_5-H). – MS-FAB$^+$: m/z: 450 [M+1]**.**

***1-[(4-Thiomorpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-fluorophenyl)-2-pyrazoline* (7l):** Yield 56%; mp 171-172 °C; UV [λ_{max}^{EtOH} nm), log ε]: 208.4 (5.08), 318.6 (4.84). - IR [ν, cm^{-1}, KBr]: 1662 (C=O), 1602-1413 (C=N, C=C), 1228 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.70 (4H, m, thiomorpholine CH_2-S-CH_2), 3.20 (1H, dd J_{AM} = 17.99 Hz, J_{AX} = 4.68 Hz, C_4-H_A of pyrazoline ring), 3.95 (1H, dd J_{MA} = 17.98 Hz, J_{MX} = 11.69 Hz, C_4-H_M of pyrazoline ring), 4.25 (4H, m, thiomorpholine CH_2-N-CH_2), 4.65 (1H, d J = 16.21 Hz, CO<u>CH</u> geminal proton), 4.70 (1H, d J = 16.20 Hz, CO<u>CH</u> geminal proton), 5.60 (1H, dd J_{MX} = 11.56 Hz, J_{AX} = 4.48 Hz, C_5-H_X of pyrazoline ring), 7.10-7.25 (5H, m, phenyl protons and thiophene C_4-H), 7.45 (1H, d J = 2.75 Hz, thiophene C_3-H), 7.75 (1H, d J = 4.99 Hz, thiophene C_5-H). – MS-FAB$^+$: m/z: 466 [M+1].

***1-[(4-Morpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-chlorophenyl)-2-pyrazoline* (7m):** Yield 66%; mp 90-92 °C; UV [λ_{max}^{EtOH} nm), log ε]: 208.6 (4.94), 320.1 (4.68). - IR [ν, cm^{-1}, KBr]: 1662 (C=O), 1593-1408 (C=N, C=C), 1230 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 3.25 (1H, dd J_{AM} = 18.02 Hz, J_{AX} = 4.72 Hz, C_4-H_A of pyrazoline ring), 3.75 (4H, m, morpholine CH_2-O-CH_2), 3.85 (1H, dd J_{MA} = 17.97 Hz, J_{MX} = 11.78 Hz, C_4-H_M of pyrazoline ring), 3.95 (4H, m, morpholine CH_2-N-CH_2), 4.72 (1H, d J = 16.17 Hz, CO<u>CH</u> geminal proton), 4.77 (1H, d J = 16.19 Hz, CO<u>CH</u> geminal proton), 5.60 (1H, dd J_{MX} = 11.75 Hz, J_{AX} = 4.70 Hz, C_5-H_X of pyrazoline ring), 7.25 (1H, tJ = 4.28 Hz, thiophene C_4-H), 7.35 (2H, dJ = 8.58 Hz, phenyl $C_{2,6}$-H), 7.45 (2H, dJ = 8.55 Hz, phenyl $C_{3,5}$-H), 7.48 (1H, dJ = 3.53 Hz, thiophene C_3-H), 7.75 (1H, dJ = 4.87 Hz, thiophene C_5-H).– MS-FAB$^+$: m/z: 466 [M], 467 [M+1], 468 [M+2]**.**

***1-[(4-Thiomorpholinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-chlorophenyl)-2-pyrazoline* (7n):** Yield 67%; mp 166 °C; UV [λ_{max}^{EtOH} nm), log ε]: 212.8 (4.87), 318.5 (4.62). - IR [ν, cm^{-1}, KBr]: 1662 (C=O), 1593-1411 (C=N, C=C), 1228 (C=S). - ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.80 (4H, m, thiomorpholine CH_2-S-CH_2), 3.30 (1H, dd J_{AM} = 17.97 Hz, J_{AX} = 4.70 Hz, C_4-H_A of pyrazoline ring), 4.00 (1H, dd J_{MA} = 17.97 Hz, J_{MX} = 11.83 Hz, C_4-H_M of pyrazoline ring), 4.30 (4H, m, thiomorpholine CH_2-N-CH_2), 4.65 (1H, d J = 16.20 Hz, CO<u>CH</u> geminal proton), 4.70 (1H, d J = 16.21 Hz, CO<u>CH</u> geminal proton), 5.65 (1H, dd J_{MX} = 11.73 Hz, J_{AX} = 4.63 Hz, C_5-H_X of pyrazoline ring), 7.20 (1H, t J =4.33 Hz, thiophene C_4-H), 7.30 (2H, d J = 8.49 Hz, phenyl $C_{2,6}$-H), 7.40 (2H, d J = 8.48 Hz, phenyl $C_{3,5}$-H), 7.55

(1H, d J = 2.71 Hz, thiophene C_3-H), 7.80 (1H, d J = 4.24 Hz, thiophene C_5-H). – MS-FAB$^+$: m/z: 482 [M], 483 [M+1], 484 [M+2].

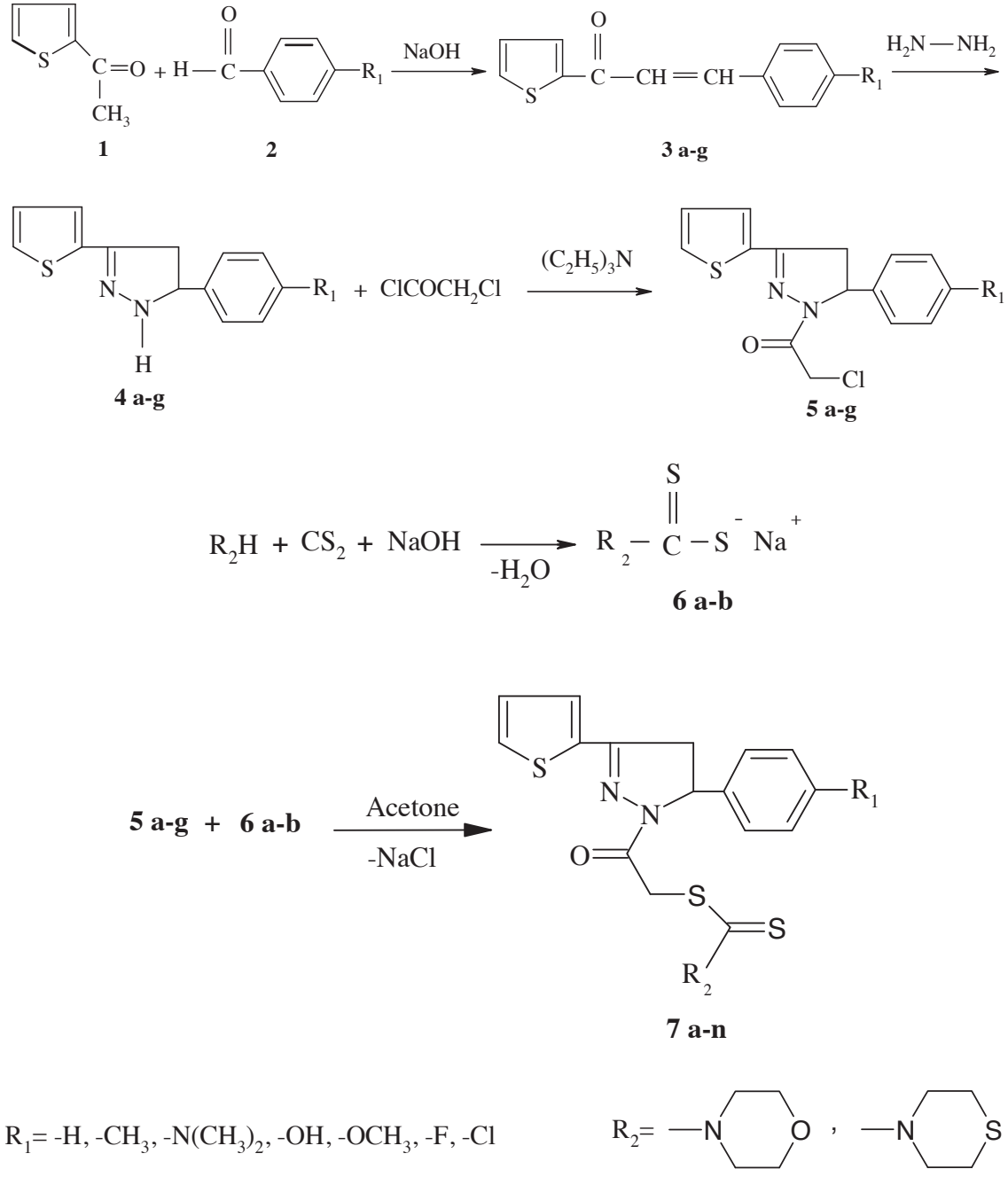

Figure 1. The reaction scheme for the complete syntheses of compounds.

Microbiology

In-vitro evaluation of antimycobacterial activity against Mycobacterium tuberculosis H_{37}Rv

Antituberculotic activities of the compounds were tested at the center of Tuberculosis Antimicrobial Acquisiton & Coordinating Facility (TAACF). Compounds were tested for in-vitro antituberculosis activity

against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 μg/ml, in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence are tested in the BACTEC 460 Radiometric System.[27] Compounds effecting <90% inhibition in the primary screen (i.e., MIC > 6.25 μg/ml) are not generally evaluated further.

Table 1. Experimental data for compounds **7a-n**.

Comp.	R_1	R_2	Molecular Formula	MW	Mp (°C)	Yield (%)
7 a	H	Morpholine	$C_{20}H_{21}N_3O_2S_3$	431.6	144	48
7 b	H	Thiomorpholine	$C_{20}H_{21}N_3OS_4$	447.6	161	50
7 c	CH_3	Morpholine	$C_{21}H_{23}N_3O_2S_3$	445.6	116-118	49
7 d	CH_3	Thiomorpholine	$C_{21}H_{23}N_3OS_4$	461.6	91-93	51
7 e	$N(CH_3)_2$	Morpholine	$C_{22}H_{26}N_4O_2S_3$	474.6	86	69
7 f	$N(CH_3)_2$	Thiomorpholine	$C_{22}H_{26}N_4OS_4$	490.7	87	71
7 g	OH	Morpholine	$C_{20}H_{21}N_3O_3S_3$	447.6	203	56
7 h	OH	Thiomorpholine	$C_{20}H_{21}N_3O_2S_4$	463.6	208	58
7 i	OCH_3	Morpholine	$C_{21}H_{23}N_3O_3S_3$	461.6	84	57
7 j	OCH_3	Thiomorpholine	$C_{21}H_{23}N_3O_2S_4$	477.6	168	59
7 k	F	Morpholine	$C_{20}H_{20}FN_3O_2S_3$	449.5	119	53
7 l	F	Thiomorpholine	$C_{20}H_{20}FN_3OS_4$	465.6	171-172	56
7 m	Cl	Morpholine	$C_{20}H_{20}ClN_3O_2S_3$	466.0	90-92	66
7 n	Cl	Thiomorpholine	$C_{20}H_{20}ClN_3OS_4$	482.1	166	67

BACTEC Radiometric Method of Susceptibility Testing

Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 more, or suspension of organism isolated earlier on conventional medium. The culture was well mixed with a syringe and 0.1 ml of a positive BACTEC culture was added to each of the vials containing the test drugs. The drug vials contained rifampicin (0.25 μg/ml). A control vial was inoculated with a 1:100 microdilution of the culture. A suspension equivalent to a Mc Farland No.1 standard was prepared in the same manner as a BACTEC positive vial, when growth from a solid medium was used. Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37 °C and tested daily with a BACTEC instrument. When the GI in the control read at least 30, the increase in GI (ΔGI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret the results:

ΔGI control > ΔGI drug = Susceptible

ΔGI control < ΔGI drug = Resistant

If a clear susceptibility pattern (the difference of ΔGI of control and the drug bottle) was not seen at the time the control ΔGI is 30, the vials were read for 1 or 2 additional days to establish a definite pattern of ΔGI differences.

Table 2. Antimycobacterial activity of the compounds.

Comp.	**7d**	**7g**	**7h**	**7i**	**7j**	**7k**	**7l**	**7m**	**7n**	Rifampicin
MIC (μg/ml)	> 6.25	> 6.25	> 6.25	> 6.25	> 6.25	> 6.25	> 6.25	> 6.25	> 6.25	0.25
% inhibition	6	2	6	24	39	8	0	0	35	98

Results and Discussion

In this study, the chalcones (1-(2-Thienyl)-3-aryl-2-propen-1-ones) **(3a-g)** were synthesized by literature methods as described[15] and treated with hydrazine hydrate (80%) to obtain 5-Aryl-3-(2-thienyl)-2-pyrazolines **(4a-g)** (Figure 2).

This reaction was probably involved in the intermediate formation of hydrozones and subsequent addition of N-H on the olephinic bond of the propenone moiety. Condensation of chalcones with hydrazine hydrate can lead to 2 different pyrazolines **(4a-g** or **4'a-g)**, as shown in (Figure 2). According to the currently accepted mechanism[14] the formation of (**4a-g),** instead of the regioisomer **(4'a-g),** is favored via hydrazone formation. Under these reaction conditions, the product stereochemistry is determined by the stereochemistry of the step ***4a-g*** **→4a-g** where a stereoselective enamine-imine tautomerism may take place[28] giving rise to the preferred direction of the proton on C-4 *trans* to the phenyl group at C-5 while 3-pyrazoline isomerizes to the more stable 2-pyrazoline. However, the lower J values compared to what is described in the literature[10] may be attributed to electronic environment effects.

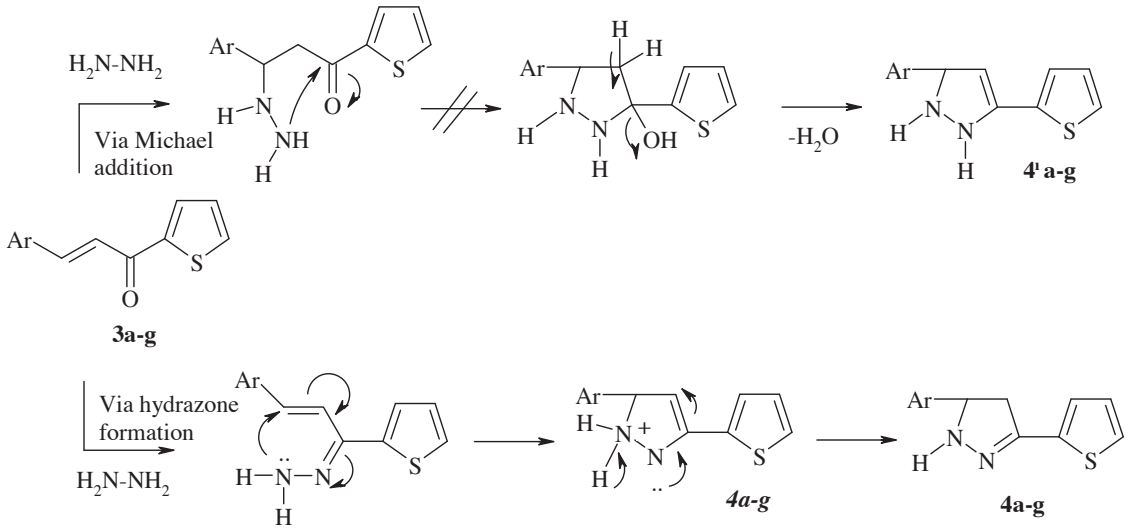

Figure 2.

In this study, 14 new compounds were synthesized. The reaction of 1-(chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines (**5a-g**) with appropriate sodium salts of N,N-disubstituted dithiocarbamoic acids (**6a,b**) resulted 1-[(N,N-disubstituted thiocarbamoylthio)acetyl]-3-(2-thienyl)-5-aryl-2-pyrazolines **(7a-n)** (Figure 1, Table 1). The chemical structures of the compounds **(5a-g, 7a-n)** were confirmed by UV, IR, ^{1}H-NMR, and FAB$^+$-MS spectral data. The electronic spectra showed characteristic maxima for $\pi - \pi^*$ corresponding

to the conjugated aromatic rings in the 2-pyrazoline derivatives in the range of 207-212 and 312-320 nm for **5a-g** and **7a-n,** respectively. The IR data were very informative and provided evidence for the formation of the expected structures. The ^{1}H-NMR spectral data were also consistent with the assigned structures. Mass spectra (MS(FAB)) of the compounds showed M+1 peaks, which were in agreement with their molecular formula.

The antimycobacterial activities of the synthesized compounds were screened in vitro using BACTEC 460 radiometric system against *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) at 6.25 μg/ml. Rifampicin was used as the standard in this test. The preliminary results showed that all of the tested compounds were inactive against the test organism (Table 2).

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