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Synthesis of Some 1-[(*N,N*-Disubstituted thiocarbamoylthio)acetyl]-3-(2-thienyl)-5-aryl-2-pyrazoline Derivatives and Investigation of Their Antibacterial and Antifungal Activities

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Fourteen new 1-[(*N,N*-disubstituted thiocarbamoylthio)acetyl]-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives (**7a**−**n**) were synthesised by reacting 1-(chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines (**5a**−**g**) and appropriate sodium salts of *N,N*-disubstituted dithiocarbamoic acids (**6a**, **b**). The structures of the synthesised compounds were confirmed by elemental analyses, UV, IR, ^{1}H-NMR and FAB$^+$-MS spectral data. Their antibacterial activities against *Proteus vulgaris* (NRRL B-123), *Escherichia coli* (NRRL B-3704), *Aeromonas hydrophila* (Ankara University, Faculty of Veterinary Sciences), *Salmonella typhimurium* (NRRL B-4420), *Streptococcus feacalis* (NRRL B-14617), *Micrococcus luteus* (NRLL B-4375) were investigated and in this investigation, a significant level of activity was illustrated. Antifungal activities of the compounds against *Candida albicans and Candida globrata* (isolates obtained from Osmangazi Uni. Fac. of Medicine) were found to be inactive. Compounds **7c**−**n** were also evaluated for antituberculosis activity against *Mycobacterium tuberculosis* H_{37}Rv using the BACTEC 460 radiometric system and BACTEC 12B medium. The preliminary results indicated that all of the tested compounds were inactive against the test organism.

Keywords: 2-Pyrazoline; Sodium salts of *N,N*-disubstituted dithiocarbamoic acids; Antibacterial activity; Antifungal activity; Antituberculosis activity

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Introduction

Antibiotics are among the most prescribed drugs in the world today, and since their development and commercialisation, have saved countless millions of lives. The ideal antimicrobial agents are selective in only targeting the microorganism but not host cells. However, resistance to antimicrobial agents is now recognised as a major global public health problem. With the emergence of new bacterial strains resistant to many currently available antibiotic treatments, there is increasing interest in the discovery of novel antibacterial agents [1, 2].

Apart from this, because of the increased number of immunocompromised patients (AIDS, cancer and transplants), primary and opportunistic fungal infections continue to increase rapidly, and as a consequence, invasive fungal infections constitute a major cause of mortality for these patients. *Candida albicans* is one of the most common opportunistic fungi responsible for these kinds of infections. Although there are new classes of compounds that are now frequently used to treat fungal infections the frequency of deeply invasive candidiasis has increased 10-fold during the past decade. Moreover, many infections caused by the *Candida* spp are actually refractory to antifungal therapy [3, 4].

In order to overcome this rapid development of drug resistance, new agents should preferably consist of chemical characteristics that clearly differ from those of existing agents. In drug designing programs an essential component of the search for new leads is the synthesis of molecules, which is novel yet resembles known biologically active molecules by virtue of the presence of critical structural features. Certain small heterocyclic molecules act as highly functionalised scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules [5, 6].

Electron-rich nitrogen heterocyclies play an important role in diverse biological activities. Introducing a pyrazolidinone [7, 8] ring in place of the β-lactam ring (in penicillins and cephalosporins [9]) results in enhanced activity. A second

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nitrogen in the five-membered ring also influences the antibacterial or pharmacokinetic properties [10−14].

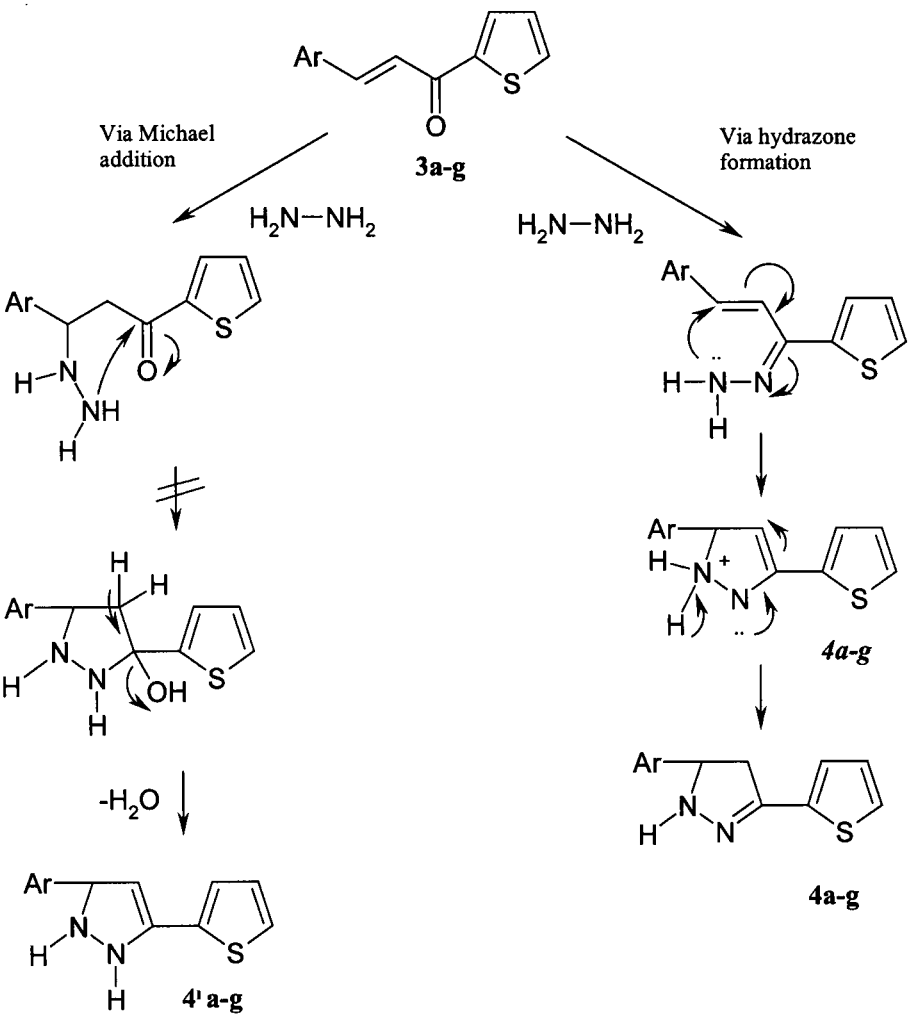
On the other hand, the structure-activity relationship study revealed that the antibacterial activity on thiocarbamoyl aromatic compounds was significantly affected by the lipophilicity, that is obtained by thiocarbamoyl moiety, especially the calculated log P value and the balance between hydrophilic substituent and hydrophobic substituent on the aromatic compounds [15]. Some thiocarbomoyl aromatics that were synthesised inspired by the above mentioned rationale were found to possess good *in vitro* antibacterial activity against gram-positive bacteria [15].

2-Pyrazoline derivatives [16-26] and *N*-substituted/*N,N*-disubstituted dithiocarbamoic acid esters [27-33] have been reported in the literature to exhibit various pharmacological activities such as antibacterial, antifungal, herbicidal and anticholinergic. In the interest of the above suggestion, we planned to synthesise a system that combines these two biolabile components which are 2-pyrazolines and *N*-substituted/*N,N*-disubstituted dithiocarbamoic acid esters, together to give a compact structure like title compounds.

Results and discussion

In this study, the chalcones (1-(2-Thienyl)-3-aryl-2-propen-1-ones) (**3a**−**g**) were synthesised by literature methods as described [34] and treated with hydrazine hydrate (80%) to obtain 5-Aryl-3-(2-thienyl)-2-pyrazolines (**4a**−**g**) (Figure 1).

This reaction 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 two different pyrazolines (**4a**−**g or 4′a**−**g**), as shown in Figure 1. According to the currently accepted mechanism [35] the formation



Scheme 1. Proposed mechanisms of pyrazoline formation.

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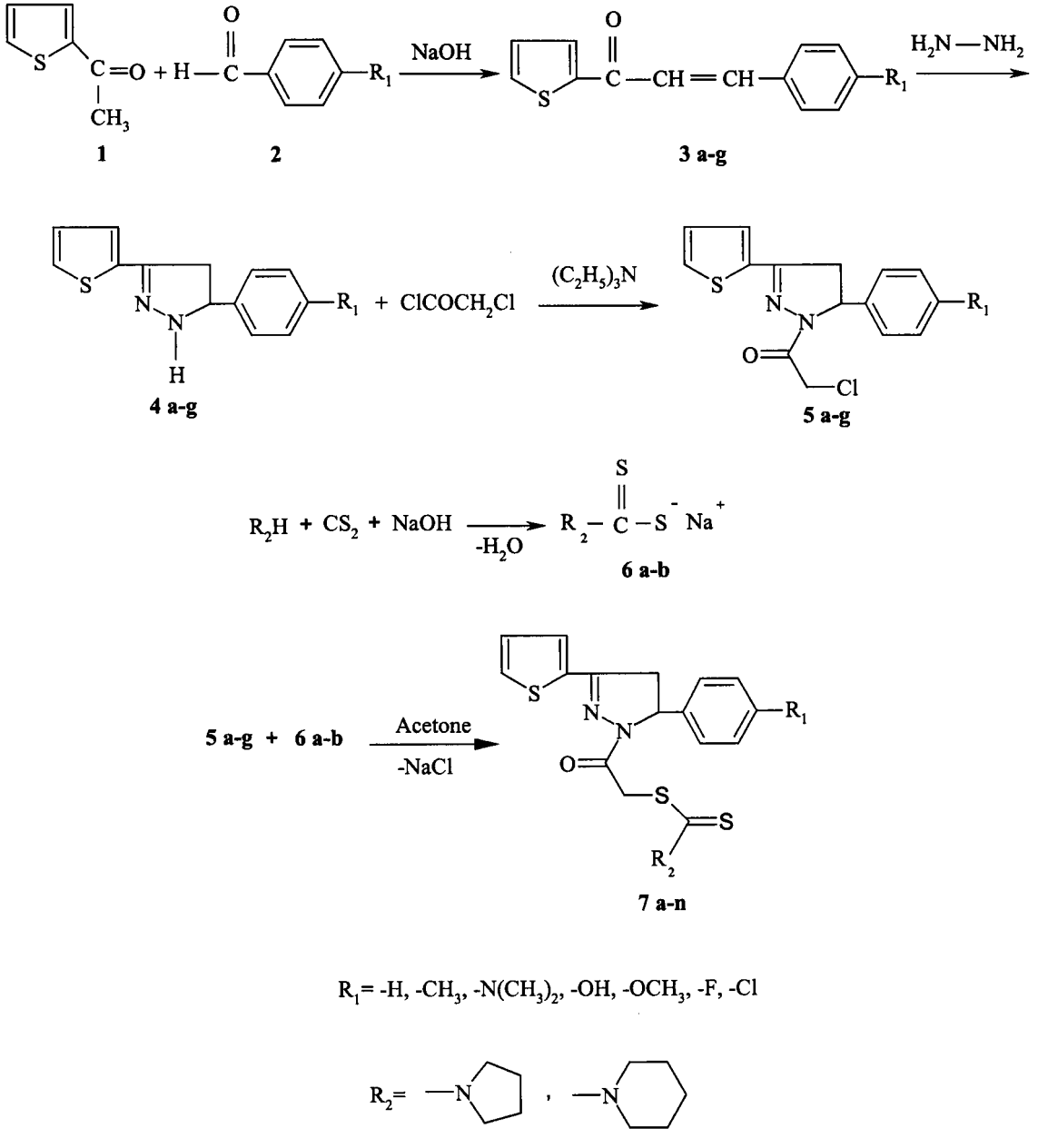
of (**4a**−**g**), instead of the regioisomer (**4′a**−**g**), is favoured via hydrazone formation. Under these reaction conditions, the product stereochemistry is determined by the stereochemistry of the step **4a**−**g** → **4a**−**g** where a stereoselective enamine-imine tautomerism may take place [36] 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. But the lower J values compared to what is described in the literature [37] may be attributed to electronic environment effects.

In the present work, 14 new compounds were synthesised. 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**) (Scheme 2, Table 1). The formulas of compounds (**5a**−**g**, **7a**−**n**) were by elemental analyses and their structures were determined by UV, IR, ^{1}H-NMR and FAB$^+$-MS spectral data. The IR data were very informative and provided evidence for the formation of the expected



Scheme 2. Synthetic route of the title compounds.

Table 1. Physicochemical data of compounds **5a**–**g** and **7a**–**n**.

Compd.	R_1	R_2	Molecular Formula	MW	Mp [°C]	Yield [%]	λ_{max}^{EtOH} [nm]
5a	H	–	$C_{15}H_{13}ClN_2OS$	304.8	126	76	208.8, 318.4
5b	CH_3	–	$C_{16}H_{15}ClN_2OS$	318.8	130	62	208.4, 313.4
5c	$N(CH_3)_2$	–	$C_{17}H_{18}ClN_3OS$	347.8	145	78	207.6, 317.4
5d	OH	–	$C_{15}H_{13}ClN_2O_2S$	320.8	250	69	207.8, 313.6
5e	OCH_3	–	$C_{16}H_{15}ClN_2O_2S$	334.8	120†	68	207.4, 315.4
5f	F	–	$C_{15}H_{12}ClFN_2OS$	322.7	143–144	64	208.4, 312.4
5g	Cl	–	$C_{15}H_{12}Cl_2N_2OS$	339.2	118†	73	208.4, 319.4
7a	H	Pyrrolidine	$C_{20}H_{21}N_3OS_3$	415.6	149	45	208.4, 318.2
7b	H	Piperidine	$C_{21}H_{23}N_3OS_3$	429.6	105–106	52	207.6, 315.3
7c	CH_3	Pyrrolidine	$C_{21}H_{23}N_3OS_3$	429.6	98	41	210.4, 320.4
7d	CH_3	Piperidine	$C_{22}H_{25}N_3OS_3$	443.6	110–113	47	213.6, 318.7
7e	$N(CH_3)_2$	Pyrrolidine	$C_{22}H_{26}N_4OS_3$	458.6	83–84	65	208.4, 315.4
7f	$N(CH_3)_2$	Piperidine	$C_{23}H_{28}N_4OS_3$	472.7	89	68	207.4, 317.9
7g	OH	Pyrrolidine	$C_{20}H_{21}N_3O_2S_3$	431.6	121–123	49	208.2, 319.6
7h	OH	Piperidine	$C_{21}H_{23}N_3O_2S_3$	445.6	180–182	50	220.6, 315.2
7i	OCH_3	Pyrrolidine	$C_{21}H_{23}N_3O_2S_3$	445.6	73	56	207.8, 319.8
7j	OCH_3	Piperidine	$C_{22}H_{25}N_3O_2S_3$	459.6	79	53	207.6, 318.9
7k	F	Pyrrolidine	$C_{20}H_{20}FN_3OS_3$	433.5	139	49	207.6, 318.2
7l	F	Piperidine	$C_{21}H_{22}FN_3OS_3$	447.6	77–78	51	208.6, 319.7
7m	Cl	Pyrrolidine	$C_{20}H_{20}ClN_3OS_3$	450.0	108	64	208.4, 320.4
7n	Cl	Piperidine	$C_{21}H_{22}ClN_3OS_3$	464.0	109	63	208.6, 320.2

† Literature Mp from [38].

structures. C=O, C=N, C=C and C=S functions absorbed strongly in the expected regions: C=O at 1677–1643 cm^{-1}, C=N and C=C at 1614–1402 cm^{-1} and C=S at 1245–1221 cm^{-1}, respectively. The ^{1}H-NMR spectral data were also consistent with the assigned structures. In the 250 MHz ^{1}H-NMR spectrum of compounds, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at δ 3.10–3.40 ppm, 3.80–4.00 ppm. The CH proton appeared as doublet of doublets at δ 5.40-5.65 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring (J_{AM} = 17.82–18.08 Hz, J_{AX} = 4.22–4.78 Hz, J_{MX} = 11.44–11.75 Hz). The CH_2 protons of acetyl which are on 1 position of pyrazoline (**5a**–**g**) and (**7a**–**n**) are observed at 4.60–4.70 ppm as double doublets (J = 13.71–16.18 Hz, J = 13.77–16.21) this geminal coupling is result from steric structure of compound. These geminal protons are observed as double doublet because of possible two different conformation since rigid protons were occurred. All the other aromatic and aliphatic protons were observed at expected regions. All compounds gave satisfactory elemental analysis. The mass spectra (MS (FAB)) of compounds showed [M+1] peaks, in agreement with their molecular formula.

MICs were recorded as the minimum concentration of a compound that inhibits the growth of tested microorganisms. All of the compounds tested were illustrated significant antibacterial activity when compared with chloramphenicole.

The antibacterial assessment revealed that the compounds possesses significant activity. The MIC values are generally within the range of 62.5-500 μg/mL against all evaluated strains.

In comparing their MIC values with chloramphenicole, all of the compounds were effective against *Proteus vulgaris;* **7a**, **7e**, **7f**, **7g** and **7m** especially showed strong activity. **7b**, **7c**, **7d**, **7h**, **7i**, **7k** and **7n** showed a similar level of activity with chloramphenicole and **7l** showed moderate activity. All of the compounds were effective against *Aeromonas hydrophil.* Compounds **7d**, **7g**, **7h** and **7m** especially showed strong activity. **7a**, **7c**, **7e**, **7f**, **7i**, **7j**, **7k** and **7n** showed a similar level of activity and **7b**, **7l** showed moderate activity when compared with the reference agent.

In comparing their MIC values with chloramphenicole, all of the compounds were effective against *Escherichia coli*; The compound **7g** especially showed strong activity. **7a**, **7c**, **7d**, **7e**, **7f**, **7h**, **7i**, **7j**, **7k**, **7l**, **7m** and **7n** showed a same level of activity with chloramphenicole and **7b** showed moderate activity.

On the other hand the compounds exhibited comparable activities against *Salmonella typhimurium;* Compounds **7g**, **7h** and **7m** showed equal activity and the other compounds

were found less active than the reference agent. When compared with chloramphenicole, the compounds **7a**, **7d**, **7e**, **7f**, **7g** and **7n** showed similar activity against *Micrococcus luteus,* whereas all other compounds showed less activity. From the similar results obtained from *Streptococcus feacalis*, compounds **7b**, **7l** and **7n** showed moderate activity, whereas remained compounds have same activity values when compared with chloramphenicole. The compounds were found inactive against *Candida albicans and Candida globrata* when compared with ketoconazole.

In conclusion, 1-[(*N,N*-disubstitutedthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives showed significant activities against gram-positive and gram negative bacteria. However, all of the tested compounds were inactive against *Candida* species and *Mycobacterium tuberculosis* $H_{37}Rv$.

Acknowledgments

We thanks D. Joseph A. Maddry from the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), National Institute of Allergy and Infectious Diseases Southern Research Institute, GWL Hansen's Disease Center, Colorado State University, Birmingham, Alabama, USA for the in vitro evaluation of antituberculosis activity.

Experimental

Chemistry

All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected. The compounds were checked for purity by TLC on silica gel 60 F_{254}. Spectroscopic data were recorded on the following instruments: UV, Shimadzu Model 160A UV spectrophotometer; IR, Shimadzu 435 IR spectrophotometer; ^{1}H-NMR, Bruker 250 MHz NMR spectrometer in DMSO-d_6 using TMS as internal standard; Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser; MS-FAB, VG Quattro mass spectrometer.

*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 crystallised from ethanol [34].

*5-Aryl-3-(2-thienyl)-2-pyrazolines (**4a**–**g**)*

A solution of the appropriate thienyl chalcone (0.01 mol) (**3a**–**g**) 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 crystallised from ethanol [34].

*1-(Chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines (**5a**–**g**)*

The 5-Aryl-3-(2-thienyl)-2-pyrazolines (**4a**–**g**) (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 recrystallised from ethanol [38].

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

Sodium hydroxide (0.01mol) was dissolved in ethanol (80 mL) with constant stirring. After addition of the secondary amine (0.01 mol) the mixture was cooled in an ice bath and carbon disulphide (0.10 mol) was added dropwise with stirring. Further agitation of the reaction mixture thus obtained for 1 h at room temperature, evaporation of the solvent under reduced pressure, and subsequent addition of dry ether until precipitation reached completion, filtration afforded products which were recrystallised from ethanol [39].

*1-[(N,N-Disubstituted thiocarbamoylthio)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 1 h. The solvent was evaporated, washed with water and recrystallised from ethanol.

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

*1-(Chloroacetyl)-3-(2-thienyl)-5-phenyl-2-pyrazoline (**5a**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.8 (4.80), 318.4 (4.65). IR [ν, cm^{-1}, KBr]: 1658 (C=O), 1591-1417 (C=N, C=C).

*1-(Chloroacetyl)-3-(2-thienyl)-5-(4-methylphenyl)-2-pyrazoline (**5b**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.4 (5.06), 313.4 (4.92). IR [ν, cm^{-1}, KBr]: 1672 (C=O), 1514-1403 (C=N, C=C). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 2.30 (3H, s, Ar-CH_3), 3.40 (1H, dd J_{AM} = 17.98 Hz, J_{AX} = 4.47 Hz, C_4-H_A of pyrazoline ring), 4.00 (1H, dd J_{MA} = 17.98 Hz, J_{MX} = 11.62 Hz, C_4-H_M of pyrazoline ring), 4.65 (1H, d J = 13.78 Hz, CO*CH* geminal proton), 4.70 (1H, d J = 13.77 Hz, CO*CH* geminal proton), 5.60 (1H, dd J_{MX} = 11.60 Hz, J_{AX} = 4.46 Hz, C_5-H_X of pyrazoline ring), 7.15–7.25 (5H, m, phenyl protons and thiophene C_4-H), 7.55 (1H, dd J = 2.64 Hz, J = 1.01 Hz, thiophene C_3-H), 7.80 (1H, dd J= 5.04 Hz, J = 1.03 Hz thiophene C_5-H). MS-FAB$^+$: m/z: 318 [M$^+$], 319 [M+1], 320 [M+2]

*1-(Chloroacetyl)-3-(2-thienyl)-5-(4-dimethylaminophenyl)-2-pyrazoline (**5c**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 207.6 (5.20), 317.4 (5.15). IR [ν, cm^{-1}, KBr]: 1672 (C=O), 1610-1415 (C=N, C=C).

*1-(Chloroacetyl)-3-(2-thienyl)-5-(4-hydroxyphenyl)-2-pyrazoline (**5d**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 207.8 (4.91), 313.6 (4.94). IR [ν, cm^{-1}, KBr]: 3245 (O-H), 1654 (C=O), 1614-1423 (C=N, C=C). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 3.15 (1H, dd J_{AM} = 17.96 Hz, J_{AX} = 4.33 Hz, C_4-H_A of pyrazoline ring), 3.90 (1H, dd J_{MA} = 17.94 Hz, J_{MX} = 11.56 Hz, C_4-H_M of pyrazoline ring), 4.55 (1H, d J = 13.71 Hz, CO*CH* geminal proton), 4.60 (1H, d J = 13.78 Hz, CO*CH* geminal proton), 5.45 (1H, dd J_{MX} = 11.46 Hz, J_{AX} = 4.22 Hz, C_5-H_X of pyrazoline ring), 6.65 (2H, d J = 8.50 Hz, phenyl

$C_{2,6}$-H), 7.10 (2H, d J = 8.52 Hz, phenyl $C_{3,5}$-H), 7.15 (1H, t J = 3.69 Hz, thiophene C_4-H), 7.45 (1H, d J = 2.62 Hz, thiophene C_3-H), 7.75 (1H, d J = 5.07 Hz, thiophene C_5-H), 9.40 (1H, s, Ar-OH). MS-FAB$^+$: m/z: 320 [M$^+$], 321 [M+1], 322 [M+2]

*1-(Chloroacetyl)-3-(2-thienyl)-5-(4-methoxyphenyl)-2-pyrazoline (**5e**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 207.4 (4.71), 315.4 (4.61). IR [ν, cm^{-1}, KBr]: 1658 (C=O), 1610-1406 (C=N, C=C).

*1-(Chloroacetyl)-3-(2-thienyl)-5-(4-fluorophenyl)-2-pyrazoline (**5f**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.4 (4.87), 312.4 (4.83). IR [ν, cm^{-1}, KBr]: 1674 (C=O), 1602−1402 (C=N, C=C). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6): 3.30 (1H, dd J_{AM} = 18.03 Hz, J_{AX} = 4.62 Hz, C_4-H_A of pyrazoline ring), 3.95 (1H, dd J_{MA} = 18.06 Hz, J_{MX} = 11.68 Hz, C_4-H_M of pyrazoline ring), 4.70 (1H, d J= 13.85 Hz, CO*CH* geminal proton), 4.75 (1H, d J = 13.84 Hz, CO*CH* geminal proton), 5.65 (1H, dd J_{MX} = 11.64 Hz, J_{AX} = 4.60 Hz, C_5-H_X of pyrazoline ring), 7.20−7.35 (5H, m, phenyl protons and thiophene C_4-H), 7.55 (1H, d J = 3.63 Hz, thiophene C_3-H), 7.85 (1H, dd J = 5.04 Hz, J = 0.96 Hz thiophene C_5-H). MS-FAB$^+$: m/z: 322 [M], 323 [M+1], 324 [M+2]

*1-(Chloroacetyl)-3-(2-thienyl)-5-(4-chlorophenyl)-2-pyrazoline (**5g**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.4 (4.80), 319.4 (4.65). IR [ν, cm^{-1}, KBr]: 1664 (C=O), 1595−1413 (C=N, C=C). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 3.25 (1H, dd J_{AM} = 18.08 Hz, J_{AX} = 4.71 Hz, C_4-H_A of pyrazoline ring), 3.95 (1H, dd J_{MA} = 18.07 Hz, J_{MX} = 11.74 Hz, C_4-H_M of pyrazoline ring), 4.65 (1H, d J= 13.88 Hz, CO*CH* geminal proton), 4.70 (1H, d J = 13.85 Hz, CO*CH* geminal proton), 5.60 (1H, dd J_{MX} = 11.75 Hz, J_{AX} = 4.68 Hz, C_5-H_X of pyrazoline ring), 7.20 (1H, t J = 3.70 Hz, thiophene C_4-H), 7.30 (2H, d J = 8.48 Hz, phenyl $C_{2,6}$-H), 7.45 (2H, d J= 8.47 Hz, phenyl $C_{3,5}$-H), 7.50 (1H, d J = 2.68 Hz, thiophene C_3-H), 7.80 (1H, d J= 5.08 Hz, thiophene C_5-H). MS-FAB$^+$: m/z: 339 [M], 340 [M+1], 341 [M+2]

*1-[(1-Pyrrolidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-phenyl-2-pyrazoline (**7a**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.4 (4.94), 318.2 (4.74). IR [ν, cm^{-1}, KBr]: 1654 (C=O), 1519−1406 (C=N, C=C), 1222 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.80−2.10 (4H, two m, pyrrolidine $C_{3,4}$-H), 3.17 (1H, dd J_{AM} = 18.00 Hz, J_{AX} = 4.60 Hz, C_4-H_A of pyrazoline ring), 3.55−3.75 (4H, two d, pyrrolidine $C_{2,5}$-H), 3.91 (1H, dd J_{MA} = 18.00 Hz, J_{MX} = 11.70 Hz, C_4-H_M of pyrazoline ring), 4.60 (1H, d J = 16.10 Hz, CO*CH* geminal proton), 4.65 (1H, d J = 16.08 Hz, CO*CH* geminal proton), 5.57 (1H, dd J_{MX} = 11.70 Hz, J_{AX} = 4.60 Hz, C_5-H_X of pyrazoline ring), 6.60 (2H, d J = 8.49 Hz, phenyl $C_{2,6}$-H), 7.20−7.40 (4H, m, phenyl protons and thiophene C_4-H), 7.55 (1H, s, thiophene C_3-H), 7.75 (1H, s, thiophene C_5-H). MS-FAB$^+$: m/z: 415 [M$^+$], 416 [M+1]

*1-[(1-Piperidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-phenyl-2-pyrazoline (**7b**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 207.6 (4.96), 315.3 (4.85). IR [ν, cm^{-1}, KBr]: 1666 (C=O), 1471−1404 (C=N, C=C), 1228 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.50−1.70 (6H, br. s, piperidine $C_{3,4,5}$-H), 3.17 (1H, dd J_{AM} = 17.90 Hz, J_{AX} = 4.60 Hz, C_4-H_A of pyrazoline ring), 3.80 (1H, dd J_{MA}= 17.90 Hz, J_{MX} = 11.70 Hz, C_4-H_M of pyrazoline ring), 3.90−4.20 (4H, two br. s, piperidine $C_{2,6}$-H), 4.60 (1H, d J = 16.17 Hz, CO*CH* geminal proton), 4.65 (1H, d J = 16.20 Hz, CO*CH* geminal proton), 5.57 (1H, dd J_{MX} = 11.70 Hz, J_{AX} = 4.60 Hz, C_5-H_X of pyrazoline ring), 7.10−7.20 (6H, m, phenyl protons and thiophene C_4-H), 7.45 (1H, d J= 2.69 Hz, thiophene C_3-H), 7.75 (1H, d J = 4.14 Hz, thiophene C_5-H). MS-FAB$^+$: m/z: 430 [M+1]

*1-[(1-Pyrrolidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-methylphenyl)-2-pyrazoline (**7c**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 210.4 (5.12), 320.4 (4.96). IR [ν, cm^{-1}, KBr]: 1668 (C=O), 1587−1406 (C=N, C=C), 1226 (C=S).

*1-[(1-Piperidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-methylphenyl)-2-pyrazoline (**7d**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 213.6 (4.97), 318.7 (4.50). IR [ν, cm^{-1}, KBr]: 1674 (C=O), 1515−1411 (C=N, C=C), 1224 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.55 (6H, m, piperidine $C_{3,4,5}$-H), 2.20 (3H, s, Ar-CH_3), 3.15 (1H, dd J_{AM} = 17.96 Hz, J_{AX} = 4.51 Hz, C_4-H_A of pyrazoline ring), 3.90 (1H, dd J_{MA} = 17.87 Hz, J_{MX} = 11.64 Hz, C_4-H_M of pyrazoline ring), 3.95−4.15 (4H, two br. s, piperidine $C_{2,6}$-H), 4.70 (1H, d J = 16.05 Hz, CO*CH* geminal proton), 4.75 (1H, d J = 16.04 Hz, CO*CH* geminal proton), 5.55 (1H, dd J_{MX} = 11.61 Hz, J_{AX} = 4.50 Hz, C_5-H_X of pyrazoline ring), 7.00−7.20 (5H, m, phenyl protons and thiophene C_4-H), 7.45 (1H, d J = 2.69 Hz, thiophene C_3-H), 7.75 (1H, d J= 4.14 Hz, thiophene C_5-H). MS-FAB$^+$: m/z: 443 [M], 444 [M+1]

*1-[(1-Pyrrolidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-dimethylaminophenyl)-2-pyrazoline (**7e**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.4 (4.93), 315.4 (4.84). IR [ν, cm^{-1}, KBr]: 1658 (C=O), 1614−1413 (C=N, C=C), 1224 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.85−2.15 (4H, two m, pyrrolidine $C_{3,4}$-H), 2.90 (6H, s, $N(CH_3)_2$), 3.20 (1H, dd J_{AM} = 17.84 Hz, J_{AX} = 4.34 Hz, C_4-H_A of pyrazoline ring), 3.70−3.80 (4H, two t, pyrrolidine $C_{2,5}$-H), 3.90 (1H, dd J_{MA} = 17.83 Hz, J_{MX} = 11.63 Hz, C_4-H_M of pyrazoline ring), 4.65 (1H, d J = 15.97 Hz, CO*CH* geminal proton), 4.70 (1H, d J = 16.00 Hz, CO*CH* geminal proton), 5.50 (1H, dd J_{MX} = 11.66 Hz, J_{AX} = 4.37 Hz, C_5-H_X of pyrazoline ring), 6.70 (2H, d J = 8.44 Hz, phenyl $C_{2,6}$-H), 7.10 (2H, d J = 8.65 Hz, phenyl $C_{3,5}$-H), 7.20 (1H, t J = 3.73 Hz, thiophene C_4-H), 7.55 (1H, d J = 2.93 Hz, thiophene C_3-H), 7.80 (1H, d J= 4.46 Hz, thiophene C_5-H). MS-FAB$^+$: m/z: 458 [M], 459 [M+1]

*1-[(1-Piperidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-dimethylaminophenyl)-2-pyrazoline (**7f**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 207.4 (5.11), 317.9 (4.76). IR [ν, cm^{-1}, KBr]: 1656 (C=O), 1613−1413 (C=N, C=C), 1225 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.55 (6H, m, piperidine $C_{3,4,5}$-H), 2.85 (6H, s, $N(CH_3)_2$), 3.15 (1H, dd J_{AM} = 17.82 Hz, J_{AX} = 4.42 Hz, C_4-H_A of pyrazoline ring), 3.80 (1H, dd J_{MA} = 17.88 Hz, J_{MX} = 11.52 Hz, C_4-H_M of pyrazoline ring), 4.15 (4H, m, piperidine $C_{2,6}$-H), 4.60 (1H, d J = 15.92 Hz, CO*CH* geminal proton), 4.65 (1H, d J = 15.90 Hz, CO*CH* geminal proton), 5.45 (1H, dd J_{MX} = 11.44 Hz, J_{AX} = 4.30 Hz, C_5-H_X of pyrazoline ring), 6.60 (2H, d J = 8.70 Hz, phenyl $C_{2,6}$-H), 7.00 (3H, d J = 8.65 Hz, phenyl $C_{3,5}$-H and thiophene C_4-H), 7.45 (1H, d J = 2.86 Hz, thiophene C_3-H), 7.75 (1H, d J = 4.30 Hz, thiophene C_5-H). MS-FAB$^+$: m/z: 472 [M], 473 [M+1]

*1-[(1-Pyrrolidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-hydroxyphenyl)-2-pyrazoline (**7g**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.2 (4.90), 319.6 (4.55). IR [ν, cm^{-1}, KBr]: 3247 (O-H), 1643 (C=O), 1614−1421 (C=N, C=C), 1228

(C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.85−2.00 (4H, two m, pyrrolidine $C_{3,4}$-H), 3.10 (1H, dd J_{AM} = 17.96 Hz, J_{AX} = 4.25 Hz, C_4-H_A of pyrazoline ring), 3.55−3.75 (4H, two t, pyrrolidine $C_{2,5}$-H), 3.85 (1H, dd J_{MA} = 17.91 Hz, J_{MX} = 11.58 Hz, C_4-H_M of pyrazoline ring), 4.60 (1H, d J = 16.03 Hz, CO*CH* geminal proton), 4.65 (1H, d J = 16.00 Hz, CO*CH* geminal proton), 5.40 (1H, dd J_{MX} = 11.61 Hz, J_{AX} = 4.32 Hz, C_5-H_X of pyrazoline ring), 6.60 (2H, d J = 8.49 Hz, phenyl $C_{2,6}$-H), 7.00 (2H, d J = 8.52 Hz, phenyl $C_{3,5}$-H), 7.10 (1H, t J = 3.71 Hz, thiophene C_4-H), 7.45 (1H, d J= 2.70 Hz, thiophene C_3-H), 7.75 (1H, d J = 4.96 Hz, thiophene C_5-H), 9.30 (1H, s, Ar-OH). MS-FAB$^+$: m/z: 431 [M], 432 [M+1]

*1-[(1-Piperidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-hydroxyphenyl)-2-pyrazoline (**7h**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 220.6 (5.14), 315.2 (5.08). IR [ν, cm^{-1}, KBr]: 3307 (O-H), 1647 (C=O), 1595−1417 (C=N, C=C), 1226 (C=S).

*1-[(1-Pyrrolidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-methoxyphenyl)-2-pyrazoline (**7i**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 207.8 (4.92), 319.8 (4.54). IR [ν, cm^{-1}, KBr]: 1662 (C=O), 1610−1411 (C=N, C=C), 1245 (C=S).

*1-[(1-Piperidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-methoxyphenyl)-2-pyrazoline (**7j**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 207.6 (4.89), 318.9 (4.54). IR [ν, cm^{-1}, KBr]: 1662 (C=O), 1610−1415 (C=N, C=C), 1228 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.55 (6H, m, piperidine $C_{3,4,5}$-H), 3.20 (1H, dd J_{AM} = 17.87 Hz, J_{AX} = 4.47 Hz, C_4-H_A of pyrazoline ring), 3.70 (3H, s, Ar-OCH_3), 3.90 (1H, dd J_{MA} = 17.88 Hz, J_{MX} = 11.52 Hz, C_4-H_M of pyrazoline ring), 4.15 (4H, m, piperidine $C_{2,6}$-H), 4.70 (1H, d J = 16.02 Hz, CO*CH* geminal proton), 4.75 (1H, d J = 16.00 Hz, CO*CH* geminal proton), 5.50 (1H, dd J_{MX} = 11.57 Hz, J_{AX} = 4.30 Hz, C_5-H_X of pyrazoline ring), 6.85 (2H, d J = 8.68 Hz, phenyl $C_{2,6}$-H), 7.15 (3H, d J = 8.59 Hz, phenyl $C_{3,5}$-H and thiophene C_4-H), 7.50 (1H, d J = 3.63 Hz, thiophene C_3-H), 7.75 (1H, d J = 4.16 Hz, thiophene C_5-H). MS-FAB$^+$: m/z: 459 [M], 460 [M+1]

*1-[(1-Pyrrolidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-fluorophenyl)-2-pyrazoline (**7k**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 207.6 (5.13), 318.2 (4.67). IR [ν, cm^{-1}, KBr]: 1657 (C=O), 1603−1409 (C=N, C=C), 1221 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.85−2.00 (4H, m, pyrrolidine $C_{3,4}$-H), 3.20 (1H, dd J_{AM} = 18.05 Hz, J_{AX} = 4.69 Hz, C_4-H_A of pyrazoline ring), 3.55−3.70 (4H, two t, pyrrolidine $C_{2,5}$-H), 3.85 (1H, dd J_{MA}= 17.94 Hz, J_{MX} = 11.66 Hz, C_4-H_M of pyrazoline ring), 4.65 (1H, d J = 16.18 Hz, CO*CH* geminal proton), 4.70 (1H, d J = 16.15 Hz, CO*CH* geminal proton), 5.55 (1H, dd J_{MX} = 11.75 Hz, J_{AX} = 4.67 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 = 2.72 Hz, thiophene C_3-H), 7.75 (1H, d J = 4.12 Hz, thiophene C_5-H). MS-FAB$^+$: m/z: 433 [M], 434 [M+1], 456 [M+Na]

*1-[(1-Piperidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-fluorophenyl)-2-pyrazoline (**7l**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.6 (5.43), 319.7 (5.26). IR [ν, cm^{-1}, KBr]: 1652 (C=O), 1508−1415 (C=N, C=C), 1224 (C=S).

*1-[(1-Pyrrolidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-chlorophenyl)-2-pyrazoline (**7m**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.4 (5.07), 320.4 (4.68). IR [ν, cm^{-1}, KBr]: 1677 (C=O), 1595−1406 (C=N, C=C), 1234 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.80−2.00 (4H, m, pyrrolidine $C_{3,4}$-H), 3.20 (1H, dd J_{AM} = 17.97 Hz, J_{AX} = 4.76 Hz, C_4-H_A of pyrazoline ring), 3.55−3.75 (4H, two t, pyrrolidine $C_{2,5}$-H), 3.90 (1H, dd J_{MA} = 18.01 Hz, J_{MX} = 11.79 Hz, C_4-H_M of pyrazoline ring), 4.60 (1H, d J = 16.18 Hz, CO*CH* geminal proton), 4.65 (1H, d J = 16.19 Hz, CO*CH* geminal proton), 5.55 (1H, dd J_{MX} = 11.66 Hz, J_{AX} = 4.67 Hz, C_5-H_X of pyrazoline ring), 7.10 (1H, t J = 2.51 Hz, thiophene C_4-H), 7.20 (2H, d J = 8.51 Hz, phenyl $C_{2,6}$-H), 7.40 (2H, d J = 8.48 Hz, phenyl $C_{3,5}$-H), 7.45 (1H, d J = 2.73 Hz, thiophene C_3-H), 7.75 (1H, d J = 5.04 Hz, thiophene C_5-H). MS-FAB$^+$: m/z: 450 [M], 451 [M+1], 452 [M+2]

*1-[(1-Piperidinylthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(4-chlorophenyl)-2-pyrazoline (**7n**)*

UV [λ_{max}^{EtOH} (nm), log ε]: 208.6 (5.11), 320.2 (4.77). IR [ν, cm^{-1}, KBr]: 1664 (C=O), 1593−1409 (C=N, C=C), 1224 (C=S). ^{1}H-NMR (250 MHz, δ ppm, DMSO-d_6,): 1.60 (6H, m, piperidine $C_{3,4,5}$-H), 3.25 (1H, dd J_{AM} = 17.96 Hz, J_{AX} = 4.78 Hz, C_4-H_A of pyrazoline ring), 3.95 (1H, dd J_{MA} = 17.90 Hz, J_{MX} = 11.80 Hz, C_4-H_M of pyrazoline ring), 4.20 (4H, m, piperidine $C_{2,6}$-H), 4.70 (1H, d J = 16.18 Hz, CO*CH* geminal proton), 4.75 (1H, d J = 16.21 Hz, CO*CH* geminal proton), 5.60 (1H, dd J_{MX} = 11.55 Hz, J_{AX} = 4.72 Hz, C_5-H_X of pyrazoline ring), 7.20 (1H, t J = 4.35 Hz, thiophene C_4-H), 7.30 (2H, d J = 8.52 Hz, phenyl $C_{2,6}$-H), 7.45 (2H, d J = 8.42 Hz, phenyl $C_{3,5}$-H), 7.50 (1H, d J = 3.54 Hz, thiophene C_3-H), 7.80 (1H, d J = 4.97 Hz, thiophene C_5-H). MS-FAB$^+$: m/z: 464 [M], 465 [M+1], 466 [M+2].

Microbiology

Antimicrobial activities of compounds were tested using microbroth dilution method [40]. Tested microorganism strains were; *Proteus vulgaris* (NRRL B-123), *Escherichia coli* (NRRL B-3704), *Aeromonas hydrophila* (Ankara University, Faculty of Veterinary Sciences), *Salmonella typhimurium* (NRRL B-4420), *Streptococcus feacalis* (NRRL B-14617), *Micrococcus luteus* (NRLL B-4375), *Candida albicans and Candida globrata* (isolates obtained from Osmangazi University, Faculty of Medicine). Microbroth dilution susceptibility assay was used for the antimicrobial evaluation of the compounds. Stock solutions of the samples were prepared in dimethylsulfoxide. Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.007 mg/mL in micro-test tubes that were transferred to 96-well microtitre plates. Overnight grown bacterial and *Candida* suspensions in double-strength Mueller-Hinton broth were standardised to 10^8 CFU/mL using McFarland No: 0.5 standard solution. 100 µL of each microorganism suspension was then added into the wells. The last well-chain without microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37°C for 18−24 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Chloramphenicol was used as standard antibacterial agent whereas ketoconazole was used as antifungal. The observed data on the antimicrobial activity of the compounds and control drugs are given in Table 2.

Compounds **7c**−**n** were also evaluated for antituberculosis activity against *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) using the BACTEC 460 radiometric system and BACTEC 12B medium. The preliminary results indicated that all of the tested compounds were inactive against the test organism.

Table 2. MIC values μg/mL of compounds **7a**−**n**[†].

Compounds	A	B	C	D	E	F	G	H
7a	125	250	250	250	250	62.5	250	250
7b	250	500	500	500	500	250	500	500
7c	250	250	250	250	250	250	250	250
7d	250	250	125	250	250	62.5	250	250
7e	125	250	250	250	250	62.5	125	125
7f	125	250	250	250	250	62.5	125	125
7g	125	125	125	125	250	62.5	125	62.5
7h	250	250	125	125	250	125	250	250
7i	250	250	250	250	250	125	250	250
7j	250	250	250	250	250	125	250	250
7k	250	250	250	250	250	125	250	250
7l	500	250	500	500	500	125	500	250
7m	125	250	125	125	250	125	250	250
7n	250	250	250	250	500	62.5	250	250
Reference-1	250	250	250	125	250	62.5	–	–
Reference-2	–	–	–	–	–	–	15.6	3.9

[†] **Reference-1:** Chloramphenicole, **Reference-2:** Ketoconazole; **A:** *Proteus vulgaris* (NRRL B-123), **B:** *Escherichia coli* (NRRL B-3704), **C:** *Aeromonas hydrophila* (Ankara Uni. Fac.of Veterinary), **D:** *Salmonella typhimurium* (NRRL B-4420), **E:** *Streptococcus feacalis* (NRRL B-14617), **F:** *Micrococcus luteus* (NRRL B-4375), **G:** *Candida albicans* (isolates obtained from Osmangazi Uni. Fac.of Medicine), **H:** *Candida globrata* (isolates obtained from Osmangazi University, Faculty of Medicine).

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