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ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 403–409
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and antimicrobial activity of 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives

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Received 7 March 2006; received in revised form 3 August 2006; accepted 5 October 2006
Available online 27 November 2006

Abstract

Several 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives were synthesized by reacting substituted 3-(2-thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines with phenacyl bromides in ethanol. The structures of the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and EIMS spectral data. Their antimicrobial activities against *Escherichia coli* (NRRL B-3704), *Staphylococcus aureus* (NRLL B-767), *Salmonella typhimurium* (NRRL B-4420), *Bacillus cereus* (NRRL B-3711), *Streptococcus faecalis* (NRRL B-14617), *Aeromonas hydrophila* (Ankara Uni. Fac. of Veterinary), *Candida albicans* and *Candida glabrata* (isolates obtained from Osmangazi Uni. Fac. of Medicine) were investigated. A significant level of activity was observed.

Keywords: 2-Pyrazoline; Thiazole; Antimicrobial activity

1. Introduction

Combat against bacterial infections has resulted in the development of a wide variety of antibiotics. After years of misuse and overuse of antibiotics, bacteria are becoming antibiotic resistant, resulting in a potential global health crisis. There is already evidence that antibacterial resistance is associated with an increase in mortality. Frequently, it is recommended to use new antibacterial agents with enhanced broad-spectrum potency. Therefore, recent efforts have been directed toward exploring novel antibacterial agents [1].

Apart from this, during the past 20 years an increase of invasive fungal infections has been observed, particularly in immunosuppressed patients, which are now causes of morbidity and mortality. Autopsy data in fact indicate that more than half of the patients who die with malignancies are infected with *Candida* spp. and increasing numbers with other fungi. Since the discovery of amphotericin B a number of different classes of antifungal agents have been discovered. However, there is still a critical need for new antifungal agents to treat life threatening invasive mycoses [2].

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 are novel yet resemble known biologically active molecules by virtue of the presence of critical structural features. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules [3,4].

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doi:10.1016/j.ejmech.2006.10.001

Electron-rich nitrogen heterocyclics play an important role in diverse biological activities. Introducing a pyrazolidinone ring [5,6] in place of the β-lactam ring (in penicillins and cephalosporins [7]) results in enhanced activity. A second nitrogen in the five-membered ring also influences the antibacterial or pharmacokinetic properties [8–10]. 2-Pyrazoline derivatives have also been reported in the literature to exhibit various pharmacological activities such as antimicrobial [11–16], anti-inflammatory [17] and antihypertensive [18].

On the other hand, sulfur and/or nitrogen heterocycles that possess pharmaceutical activities widely occur in nature in the form of alkaloids, vitamins, pigments and as constituents of plant and animal cells. Penicillins containing a thiazole ring system (thiazolidine) [19] are also important naturally occurring products. Thiazoles and their derivatives are found to be associated with various biological activities such as antimicrobial [20–26], antituberculosis [27], and anti-HIV [28] activities.

In the interest of the above suggestion, we planned to synthesize a system that combines together two biolabile components which are 2-pyrazolines and thiazoles, to give a compact structure like the title compounds.

2. Chemistry

The synthetic route of compounds is outlined in Scheme 1. In the present work 1-(2-thienyl)-3-aryl-2-propen-1-ones (**3a–c**) were prepared by reacting 2-acetylthiophene (**1**) with aromatic aldehyde (**2a–c**) in accordance with the method described in the literature [29].

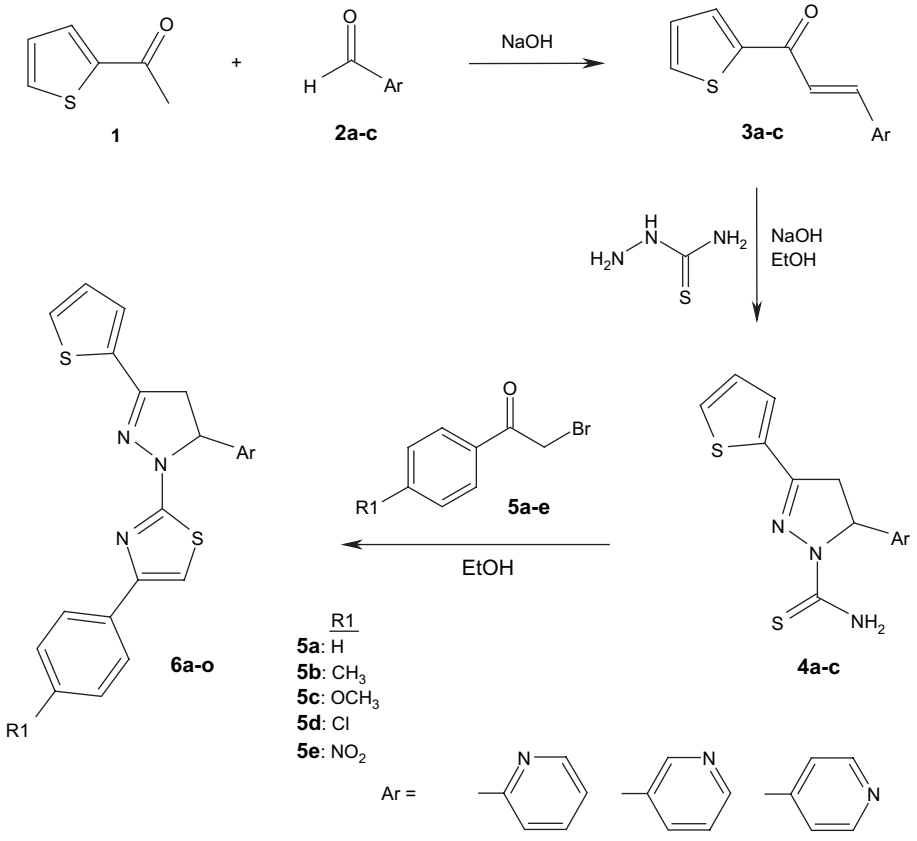
The 3-(2-thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines (**4a–c**) were prepared by reacting 1-(2-thienyl)-3-aryl-2-propen-1-ones (**3a–c**) with thiosemicarbazide. The condensation of 3-(2-thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines (**4a–c**) with appropriate phenacyl bromide resulted in the formation of 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives (**6a–o**) as shown in Scheme 1 [18].

Some characteristics of the synthesized compounds are shown in Table 1. Analytical and spectral data (^{1}H NMR, ^{13}C NMR, and EIMS) confirmed the structures of the new compounds.

3. Biology

3.1. Antimicrobial activity

Antimicrobial activities of compounds were tested using microbroth dilution method [30,31]. Tested microorganism strains were: *Escherichia coli* (NRRL B-3704), *Staphylococcus aureus* (NRLL B-767), *Salmonella typhimurium* (NRRL B-4420), *Bacillus cereus* (NRRL B-3711), *Streptococcus faecalis* (NRRL B-14617), *Aeromonas hydrophila* (Ankara Uni. Fac. of Veterinary), *Candida albicans* and *Candida glabrata* (isolates obtained from Osmangazi Uni. Fac. of Medicine).



Scheme 1. Synthetic route to the title compounds.

Table 1
Experimental data for compounds **6a–o**

Compound	Ar	R_1	Mol. for.	M. wt.	M.p. (°C)	Yield (%)
6a		–H	$C_{21}H_{16}N_4S_2$	388.52	280–282	26
6b		$-CH_3$	$C_{22}H_{18}N_4S_2$	402.54	227 dec.	34
6c		$-OCH_3$	$C_{22}H_{18}N_4OS_2$	418.54	220 dec.	36
6d		–Cl	$C_{21}H_{15}ClN_4S_2$	422.96	129–130	33
6e		$-NO_2$	$C_{21}H_{15}N_5O_2S_2$	433.51	198–200	52
6f		–H	$C_{21}H_{16}N_4S_2$	388.52	262	24
6g		$-CH_3$	$C_{22}H_{18}N_4S_2$	402.54	255	32
6h		$-OCH_3$	$C_{22}H_{18}N_4OS_2$	418.54	217–219	37
6i		–Cl	$C_{21}H_{15}ClN_4S_2$	422.96	258 dec.	38
6j		$-NO_2$	$C_{21}H_{15}N_5O_2S_2$	433.51	205	55
6k		–H	$C_{21}H_{16}N_4S_2$	388.52	159–160	27
6l		$-CH_3$	$C_{22}H_{18}N_4S_2$	402.54	126	38
6m		$-OCH_3$	$C_{22}H_{18}N_4OS_2$	418.54	122–124	41
6n		–Cl	$C_{21}H_{15}ClN_4S_2$	422.96	231–233	39
6o		$-NO_2$	$C_{21}H_{15}N_5O_2S_2$	433.51	235	53

Chloramphenicol and flucanazole were used as control drugs. The observed data on the antimicrobial activity of the compounds and control drugs are given in Table 2.

4. Results, discussion and conclusion

In this present work, a series of 15 new compounds were synthesized. Thus, starting from the 1-(2-thienyl)-3-aryl-2-propen-1-ones (**3a–c**), synthesized according to the literature [29], we have obtained 3-(2-thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines (**4a–c**) through treatment with thiosemicarbazide.

Compounds (**6a–o**) were obtained by reacting compounds (**4a–c**) with phenacyl bromide or its derivatives in ethanol [18]. The substitution of the *para* position of phenacyl bromide played an important role in the thiazole formation step. Namely, the phenacyl bromide derivatives with a strong electron-withdrawing group like NO_2 in *para* position of the benzene ring, gave higher yield compared to the other groups.

The structure of compounds (**6a–o**) was confirmed by ^1H NMR, ^{13}C NMR and EIMS spectral data. In the 400 MHz ^1H NMR spectrum of the compounds, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at δ 3.23–3.58 ppm (Ha), 3.80–3.96 ppm (Hb). The CH (Hx) proton appeared as a doublet of doublets at δ 5.57–5.74 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring ($J_{AB} = 17.1–17.6$ Hz, $J_{AX} = 7.0–7.5$ Hz, $J_{BX} = 12.0–12.1$ Hz). The H_5-proton of thiazole was observed as a singlet between 6.70 and 6.85 ppm. All the other aromatic and aliphatic protons were observed at expected regions. ^{13}C NMR chemical shift values of the carbon atoms at 42.21–43.90 ppm (C-4), 62.66–65.72 ppm (C-5) and about 150.37–151.61 ppm (C-3) corroborate the 2-pyrazoline character deduced from the ^1H NMR data.

The mass spectra (EIMS) of compounds (**6a–o**) are also in agreement with their molecular formula. One can distinguish two groups with significant differences. In the first

Table 2
MIC values (μg/mL) of compounds **6a–o**

Compound	A	B	C	D	E	F	G	H
6a	250	15.6	62.5	31.25	31.25	31.25	250	250
6b	250	125	125	62.5	250	125	125	125
6c	125	125	125	62.5	250	125	62.5	62.5
6d	125	62.5	62.5	62.5	62.5	62.5	125	62.5
6e	125	125	125	62.5	250	125	125	125
6f	250	125	250	250	250	250	250	250
6g	250	125	125	125	250	250	125	125
6h	250	250	125	250	250	125	125	125
6i	250	250	250	3.9	125	250	250	250
6j	250	31.25	125	62.5	125	31.25	62.5	62.5
6k	125	250	125	125	250	62.5	62.5	62.5
6l	125	250	125	125	250	62.5	62.5	62.5
6m	125	125	125	62.5	250	62.5	62.5	62.5
6n	125	250	250	250	250	125	125	125
6o	125	250	250	250	500	125	125	125
Reference-1	15.60	31.25	31.25	31.25	250	125	–	–
Reference-2	–	–	–	–	–	–	250	250

Reference-1: Chloramphenicol, **Reference-2**: flucanazole; A: *Escherichia coli* (NRRL B-3704); B: *Staphylococcus aureus* (NRRL B-767); C: *Salmonella typhimurium* (NRRL B-4420); D: *Bacillus cereus* (NRRL B-3711); E: *Streptococcus faecalis* (NRRL B-14617); F: *Aeromonas hydrophila* (Ankara Uni. Fac. of Veterinary); G: *Candida albicans* (isolates obtained from Osmangazi Uni. Fac. of Medicine); H: *Candida glabrata* (isolates obtained from Osmangazi Uni. Fac. of Medicine).

group of compounds (**6a–j**), the mass spectra exhibit a very strong molecular ion (*m/z* 388, 402, 418,…) without notable fragmentations. On the other hand, in the second group (**6k–o**), the mass spectra are rather different. Besides the molecular ion ([M^+] 20–50%) one observes two strong fragments, the first one corresponding to the loss of the pyridine nucleus [$M^+ - 78$] and especially the second ion which is the result of a complex fragmentation of the pyrazoline nucleus ([*m/z* 199] 100%).

MICs were recorded as the minimum concentration of a compound that inhibits the growth of tested microorganisms. All of the compounds tested illustrated significant antibacterial and antifungal activity when compared with reference drugs. The antibacterial assessment revealed that the compounds possess weak activities. The MIC values are generally within the range of 3.9–250 μg/ml against all evaluated strains.

In comparing their MIC values with chloramphenicol, all compounds were effective against *S. faecalis*. Compounds **6a** and **6d** especially showed very high activity. Compounds **6i** and **6j** showed strong activity. Compounds **6b**, **6c**, **6e**, **6f**, **6g**, **6h**, **6k**, **6l**, **6m** and **6n** showed a similar level of activity with chloramphenicol and **6o** showed moderate activity when compared with the reference agent. All compounds were effective against *A. hydrophila*. Compounds **6a** and **6j** showed very high activity. Compounds **6d**, **6k**, **6l** and **6m** especially showed strong activity. Compounds **6b**, **6c**, **6e**, **6h**, **6n** and **6o** showed a similar level of activity with chloramphenicol and **6f**, **6g** and **6i** showed moderate activity.

In comparing their MIC values with chloramphenicol, all compounds were effective against *B. cereus*. Compound **6i** especially showed high activity. Compound **6a** showed a same level of activity with chloramphenicol and **6b**, **6c**, **6d**, **6e**, **6j** and **6m** showed moderate activity. On the other hand the compounds exhibited comparable activities against *S. aureus*. Compound **6a** showed high activity and compounds **6j** showed equal activity. Compound **6d** showed moderate activity and the other compounds were found less active than the reference agent.

From the similar results obtained with *S. typhimurium*, compounds **6a** and **6d** showed moderate activity, whereas all other compounds showed less activity when compared with chloramphenicol. The compounds were less active against *E. coli*.

The antifungal activity of the compounds was studied with two pathogenic fungi. The results are summarized in Table 2. Flucanazole has been used as reference for inhibitory activity against fungi. All compounds showed good antifungal activity. When compared to flucanazole, twelve compounds are more active (MIC < 250 μg/mL) and three compounds are equipotent (250 μg/mL) against *C. albicans* and *C. glabrata*.

5. Experimental

5.1. 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. Thin-layer chromatography (TLC) was performed with glass plates (0.25 mm) precoated with Merck silica gel 60 F_{254}, and flash chromatography separations (FC) were carried out with Merck silica gel 60 (200–450 mesh), using 50:50 EtOAc/hexanes as eluents. ^{1}H NMR and ^{13}C NMR spectra of $CDCl_3$ solutions (TMS as internal standard) were respectively recorded at 400 and 100 MHz with a Bruker apparatus. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift (δ) values are given in parts per million and coupling constants (J) in Hertz. GC–MS was performed with an Agilent Technologie 6890N GC apparatus (equipped with a 12 m × 0.20 mm dimethylpolysiloxane capillary column) linked to an Agilent 5973 EIMS mass spectrometer.

5.1.1. General procedure for the synthesis of the compounds

*5.1.1.1. 1-(2-Thienyl)-3-aryl-2-propen-1-ones (**3a–c**).* A mixture of 2-acetylthiophene (0.04 mol) (**1**), aromatic aldehyde (0.04 mol) (**2a–c**) 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 [29].

*5.1.1.2. 3-(2-Thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines (**4a–c**).* To a suspension of 1-(2-thienyl)-3-aryl-2-propen-1-one derivatives (**3a–c**) (0.01 mol) and sodium hydroxide (1 g, 0.025 mol) in ethanol (50 mL), thiosemicarbazide (0.012 mol) was added. The mixture was refluxed for 8 h. The products were poured into crushed ice and the solid mass which separated out was filtered, dried and crystallized from an appropriate solvents [18].

*5.1.1.3. 1-(4-Aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline (**6a–o**).* To a suspension of compounds (**4a–c**) (0.01 mol) in ethanol (15 mL) phenacyl bromide derivatives (0.01 mol) were added and heated to reflux for 1 h. After cooling, the precipitate was collected by suction filtration and purified by flash chromatography on silica gel with EtOAc/hexanes (50:50, v/v). The product was crystallized from appropriate solvent.

*5.1.1.3.1. 1-(4-Phenyl-2-thiazolyl)-3-(2-thienyl)-5-(4-pyridyl)-2-pyrazoline (**6a**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.25 (1H, dd, $J = 17.2$, 7.5 Hz), 3.93 (1H, dd, $J = 17.2$, 12.0 Hz), 5.62 (1H, dd, $J = 12.0$, 7.5 Hz), 6.85 (1H, s), 7.06 (1H, dd, $J = 5.0$, 3.5 Hz), 7.19 (1H, dd, $J = 3.5$, 1.0 Hz), 7.21–7.38 (5H, m), 7.42 (1H, dd, $J = 5.0$, 1.5 Hz), 7.58–7.62 (2H, m), 8.59–8.62 (2H, m).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 43.76 (CH_2), 63.92 (CH), 104.05 (CH), 121.60 (2CH), 125.80 (2CH), 127.63 (CH), 127.65 (CH), 128.06 (CH), 128.48 (2CH), 128.55 (CH), 134.51 (C), 134.69 (C), 147.35 (C), 150.02 (2CH), 150.57 (C), 151.54 (C), 164.74 (C).

EIMS (*m/z*): 388 (M^+, 100%), 355 (2), 310 (8), 278 (12), 264 (5), 237 (4), 213 (4), 194 (4), 175 (33), 174 (12).

*5.1.1.3.2. 1-(4-(p-Methylphenyl)-2-thiazolyl)-3-(2-thienyl)-5-(4-pyridyl)-2-pyrazoline (**6b**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.31 (3H, s), 3.23 (1H, dd, $J = 17.1$, 7.5 Hz), 3.89 (1H, dd, $J = 17.1$, 12.1 Hz), 5.58 (1H, dd, $J = 12.1$, 7.5 Hz), 6.78 (1H, s), 7.05 (1H, dd, $J = 5.0$, 3.5 Hz), 7.09 (2H, d, $J = 8.0$ Hz), 7.14–7.20 (1H, m), 7.30–7.34 (2H, m), 7.40 (1H, d, $J = 5.0$), 7.49 (2H, d, $J = 8.0$ Hz), 8.57–8.62 (2H, m).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 21.24 (CH_3), 43.72 (CH_2), 63.94 (CH), 103.21 (CH), 121.54 (2CH), 125.73 (2CH), 127.64 (CH), 128.00 (CH), 128.48 (CH), 129.17 (2CH), 132.04 (C), 134.58 (C), 137.39 (C), 147.26 (C), 150.26 (2CH), 150.31 (C), 151.61 (C), 164.66 (C).

EIMS (*m/z*): 402 (M^+, 100%), 369 (3), 324 (5), 292 (14), 278 (5), 252 (3), 189 (33), 143 (15).

*5.1.1.3.3. 1-(4-(p-Methoxyphenyl)-2-thiazolyl)-3-(2-thienyl)-5-(4-pyridyl)-2-pyrazoline (**6c**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.25 (1H, dd, $J = 17.1$, 7.5 Hz), 3.79 (3H, s), 3.93 (1H, dd, $J = 17.1$, 12.1 Hz), 5.62 (1H, dd, $J = 12.1$, 7.5 Hz), 6.70 (2H, d, $J = 7.0$ Hz), 6.83 (2H, d, $J = 9.0$ Hz), 7.07 (1H, dd, $J = 5.0$, 3.5 Hz), 7.20 (1H, dd, $J = 3.5$, 1.5 Hz), 7.37 (1H, d, $J = 6.5$ Hz), 7.42 (1H, dd, $J = 5.0$, 1.0 Hz), 7.52 (2H, d, $J = 9.0$ Hz), 8.61 (2H, d, $J = 6.0$ Hz).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 43.75 (CH_2), 55.29 (CH_3), 63.92 (CH), 102.23 (CH), 113.87 (2CH), 116.22 (2CH), 121.70 (CH), 127.07 (2CH), 127.64 (CH), 128.02 (CH), 128.53 (CH), 134.52 (C), 147.25 (C), 149.76 (C), 149.87 (CH), 150.80 (C), 151.30 (C), 159.22 (C), 164.73 (C).

EIMS (*m/z*): 418 (M^+, 100%), 385 (2), 340 (5), 308 (10), 294 (3), 276 (1), 268 (1), 213 (3), 205 (25), 189 (13), 159 (21).

*5.1.1.3.4. 1-(4-(p-Chlorophenyl)-2-thiazolyl)-3-(2-thienyl)-5-(4-pyridyl)-2-pyrazoline (**6d**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.26 (1H, dd, $J = 17.1$, 7.5 Hz), 3.94 (1H, dd, $J = 17.1$, 12.0 Hz), 5.60 (1H, dd, $J = 12.0$, 7.5 Hz), 6.83 (1H, s), 7.07 (1H, dd, $J = 5.3$, 3.5 Hz), 7.20 (1H, dd, $J = 3.5$, 1.0 Hz), 7.23–7.28 (2H, m), 7.32–7.36 (2H, m), 7.43 (1H, dd, $J = 5.5$, 1.5 Hz), 7.49–7.54 (2H, m), 8.58–8.61 (2H, m).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 43.81 (CH_2), 63.87 (CH), 104.39 (CH), 121.55 (2CH), 127.05 (2CH), 127.68 (CH), 128.18 (CH), 128.62 (2CH), 128.66 (CH), 133.18 (C), 133.26 (C), 134.39 (C), 147.52 (C), 150.00 (2CH), 150.39 (C), 150.52 (C), 164.85 (C).

EIMS (*m/z*): 422 + 424 (M^+, 100 + 42%), 389 (5), 344 (7), 312 (11), 298 (4), 271 (3), 221 (17), 173 (17).

*5.1.1.3.5. 1-(4-(p-Nitrophenyl)-2-thiazolyl)-3-(2-thienyl)-5-(4-pyridyl)-2-pyrazoline (**6e**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.35 (1H, dd, $J = 17.1$, 7.5 Hz), 4.12 (1H, dd, $J = 17.1$, 12.0 Hz), 5.71 (1H, dd, $J = 12.0$, 7.5 Hz), 7.10–7.45 (4H, m), 7.65–7.72 (2H, m), 7.90 (2H, d, $J = 9.0$ Hz), 8.18 (2H, d, $J = 9.0$ Hz), 8.54–8.60 (2H, m).

EIMS (*m/z*): 433 (M^+, 100%), 417 (1), 403 (14), 410 (10), 355 (7), 323 (9), 309 (8), 282 (4), 220 (25).

*5.1.1.3.6. 1-(4-Phenyl-2-thiazolyl)-3-(2-thienyl)-5-(3-pyridyl)-2-pyrazoline (**6f**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.32 (1H, dd, $J = 17.1$, 7.5 Hz), 3.96 (1H, dd, $J = 17.1$, 12.0 Hz), 5.68 (1H, dd, $J = 12.0$, 7.5 Hz), 6.84 (1H, s), 7.18 (1H, dd, $J = 5.0$, 3.5 Hz), 7.20–7.33 (5H, m), 7.43 (1H, dd, $J = 5.0$, 1.5 Hz), 7.60–7.66 (2H, m), 7.73 (1H, m), 8.55 (1H, dd, $J = 5.0$, 1.5 Hz), 8.78 (1H, d, $J = 2.0$ Hz).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 30.93 (CH), 43.86 (CH_2), 62.68 (CH), 103.86 (CH), 123.79 (CH), 125.79 (2CH), 127.57 (CH), 127.63 (CH), 128.46 (CH), 128.49 (2CH), 134.24 (CH), 134.68 (C), 134.71 (C), 137.09 (C), 147.30 (C), 148.74 (CH), 149.09 (CH), 151.51 (C), 164.69 (C).

EIMS (*m/z*): 388 (M^+, 100%), 353 (3), 310 (7), 278 (17), 264 (8), 246 (10), 175 (20), 129 (17).

*5.1.1.3.7. 1-(4-(p-Methylphenyl)-2-thiazolyl)-3-(2-thienyl)-5-(3-pyridyl)-2-pyrazoline (**6g**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.24 (3H, s), 3.25 (1H, dd, $J = 17.6$, 7.0 Hz), 3.86 (1H, dd, $J = 17.6$, 12.0 Hz), 5.60 (1H, dd, $J = 12.0$, 7.0 Hz), 6.70 (1H, s), 6.98–7.05 (3H, m), 7.14 (1H, dd, $J = 3.5$, 1.0 Hz), 7.23 (1H, dd, $J = 8.0$, 5.0 Hz), 7.35 (1H, dd, $J = 5.0$, 1.0 Hz), 7.42–7.47 (2H, m), 7.67 (1H, m), 8.47 (1H, dd, $J = 4.5$, 1.5 Hz), 8.69 (1H, d, $J = 2.0$ Hz).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 21.23 (CH_3), 43.82 (CH_2), 62.66 (CH), 103.13 (CH), 116.24 (CH), 123.92 (CH), 125.70

(2CH), 127.63 (CH), 127.96 (CH), 128.45 (CH), 129.19 (2CH), 132.00 (C), 134.54 (CH), 134.67 (C), 137.36 (C), 147.23 (C), 148.51 (CH), 149.78 (C), 151.58 (C), 164.65 (C).

EIMS (*m/z*): 402 (M^+, 100%), 369 (5), 324 (6), 292 (18), 278 (8), 260 (11), 189 (23), 143 (18).

*5.1.1.3.8. 1-(4-(p-Methoxyphenyl)-2-thiazolyl)-3-(2-thienyl)-5-(3-pyridyl)-2-pyrazoline (**6h**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.35 (1H, dd, $J = 17.1$, 7.5 Hz), 3.80 (3H, s), 3.95 (1H, dd, $J = 17.1$, 12.0 Hz), 5.67 (1H, dd, $J = 12.0$, 7.5 Hz), 6.70 (1H, s), 6.82–6.87 (2H, m), 7.08 (1H, dd, $J = 5.0$, 3.5 Hz), 7.22 (1H, dd, $J = 3.5$, 1.0 Hz), 7.29 (1H, dd, $J = 7.5$, 4.5 Hz), 7.43 (1H, dd, $J = 5.0$, 1.5 Hz), 7.54–7.59 (2H, m), 7.72 (1H, m), 8.55 (1H, dd, $J = 5.0$, 1.5 Hz), 8.78 (1H, d, $J = 2.0$ Hz).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 43.85 (CH_2), 55.28 (CH_3), 62.71 (CH), 102.02 (CH), 113.86 (2CH), 123.76 (CH), 127.06 (2CH), 127.63 (CH), 127.90 (CH), 128.41 (CH), 134.05 (C), 134.15 (CH), 134.76 (C), 137.10 (C), 147.19 (C), 148.88 (CH), 149.16 (CH), 151.27 (C), 159.17 (C), 164.65 (C).

EIMS (*m/z*): 418 (M^+, 100%), 383 (6), 340 (6), 308 (14), 294 (7), 276 (9), 268 (3), 213 (9), 2055 (18), 189 (17), 159 (26).

*5.1.1.3.9. 1-(4-(p-Chlorophenyl)-2-thiazolyl)-3-(2-thienyl)-5-(3-pyridyl)-2-pyrazoline (**6i**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.25 (1H, dd, $J = 17.6$, 7.5 Hz), 3.87 (1H, dd, $J = 17.6$, 12.0 Hz), 5.57 (1H, dd, $J = 12.0$, 7.5 Hz), 6.73 (1H, s), 7.00 (1H, dd, $J = 5.0$, 4.0 Hz), 7.14 (1H, dd, $J = 4.0$, 1.0 Hz), 7.17–7.23 (3H, m), 7.36 (1H, dd, $J = 5.0$, 1.0 Hz), 7.45–7.49 (2H, m), 7.63 (1H, m), 8.47 (1H, dd, $J = 4.5$, 1.5 Hz), 8.68 (1H, d, $J = 1.5$ Hz).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 43.90 (CH_2), 62.67 (CH), 104.24 (CH), 123.79 (CH), 127.04 (2CH), 127.65 (CH), 128.07 (CH), 128.55 (CH), 128.63 (2CH), 133.20 (C), 133.21 (C), 134.13 (CH), 134.57 (C), 136.95 (C), 147.46 (C), 148.76 (CH), 149.19 (CH), 150.37 (C), 164.79 (C).

EIMS (*m/z*): 422 + 424 (M^+, 100 + 40%), 389 (8), 387 (8), 344 (7), 312 (17), 298 (8), 272 (7), 227 (9), 211 (11), 209 (14), 173 (17), 163 (17).

*5.1.1.3.10. 1-(4-(p-Nitrophenyl)-2-thiazolyl)-3-(2-thienyl)-5-(3-pyridyl)-2-pyrazoline (**6j**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.26 (1H, dd, $J = 17.5$, 7.5 Hz), 3.90 (1H, dd, $J = 17.5$, 12.0 Hz), 5.60 (1H, dd, $J = 12.0$, 7.5 Hz), 6.77 (1H, s), 7.02 (1H, dd, $J = 5.3$, 4.0 Hz), 7.16 (1H, dd, $J = 4.0$, 1.0 Hz), 7.42 (1H, dd, $J = 5.3$, 1.0 Hz), 7.48–7.50 (2H, m), 7.86 (2H, d, $J = 9.0$ Hz), 8.12 (2H, d, $J = 9.0$ Hz), 8.47 (1H, dd, $J = 4.5$, 2.0 Hz), 8.67 (1H, d, $J = 2.0$ Hz).

EIMS (*m/z*): 433 (M^+, 100%), 403 (10), 387 (2), 368 (4), 323 (12), 309 (10), 291 (6), 277 (3), 227 (12), 220 (13), 174 (12).

*5.1.1.3.11. 1-(4-Phenyl-2-thiazolyl)-3-(2-thienyl)-5-(2-pyridyl)-2-pyrazoline (**6k**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.53 (1H, dd, $J = 17.1$, 7.0 Hz), 3.80 (1H, dd, $J = 17.1$, 12.0 Hz), 5.68 (1H, dd, $J = 12.0$, 7.0 Hz), 6.70 (1H, s), 6.99 (1H, dd, $J = 5.0$, 4.0 Hz), 7.17 (1H, dd, $J = 4.0$, 1.0 Hz), 7.30–7.42 (5H, m), 7.45 (1H, dd, $J = 5.0$, 1.0 Hz), 7.52–7.55 (2H, m), 7.60–7.70 (1H, m), 8.55–8.57 (1H, m).

EIMS (*m/z*): 388 (M^+, 46%), 310 (57), 246 (5), 231 (3), 199 (100), 102 (12).

*5.1.1.3.12. 1-(4-(p-Methylphenyl)-2-thiazolyl)-3-(2-thienyl)-5-(2-pyridyl)-2-pyrazoline (**6l**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.25 (3H, s), 3.58 (1H, dd, $J = 17.1$, 7.0 Hz), 3.84 (1H, dd, $J = 17.1$, 12.0 Hz), 5.74 (1H, dd, $J = 12.0$, 7.0 Hz), 6.71 (1H, s), 6.98 (1H, dd, $J = 5.0$, 4.0 Hz), 7.04 (2H, d, $J = 8.0$ Hz), 7.10–7.16 (2H, m), 7.18 (1H, s), 7.30 (1H, dd, $J = 5.0$, 1.0 Hz), 7.45–7.51 (2H, m), 7.57–7.69 (1H, m), 8.53–8.56 (1H, m).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 21.23 (CH_3), 42.21 (CH_2), 65.72 (CH), 102.92 (CH), 122.32 (CH), 122.71 (CH), 125.76 (2CH), 127.49 (CH), 127.93 (CH), 128.14 (CH), 129.10 (2CH), 132.25 (C), 135.00 (C), 136.77 (CH), 137.20 (C), 148.11 (C), 149.46 (CH), 151.47 (C), 159.56 (C), 165.11 (C).

EIMS (*m/z*): 402 (M^+, 49%), 369 (2), 325 (13), 324 (63), 292 (2), 260 (4), 213 (3), 199 (100), 175 (4), 147 (7), 116 (13).

*5.1.1.3.13. 1-(4-(p-Methoxyphenyl)-2-thiazolyl)-3-(2-thienyl)-5-(2-pyridyl)-2-pyrazoline (**6m**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.52 (1H, dd, $J = 17.1$, 7.5 Hz), 3.80 (3H, s), 3.95 (1H, dd, $J = 17.1$, 12.1 Hz), 5.67 (1H, dd, $J = 12.1$, 7.5 Hz), 6.72 (1H, s), 6.85 (2H, d, $J = 9.0$ Hz), 7.05 (1H, dd, $J = 5.0$, 4.0 Hz), 7.18 (1H, dd, $J = 4.0$, 1.5 Hz), 7.40 (1H, dd, $J = 5.0$, 1.5 Hz), 7.50–7.52 (2H, m), 7.58 (2H, d, $J = 9.0$ Hz), 7.68–7.72 (1H, m), 8.52–8.55 (1H, m).

EIMS (*m/z*): 418 (M^+, 49%), 399 (1), 340 (52), 324 (1), 308 (2), 276 (3), 264 (1), 213 (2), 199 (100), 159 (8), 132 (16).

*5.1.1.3.14. 1-(4-(p-Chlorophenyl)-2-thiazolyl)-3-(2-thienyl)-5-(2-pyridyl)-2-pyrazoline (**6n**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.55 (1H, dd, $J = 17.1$, 7.0 Hz), 3.86 (1H, dd, $J = 17.1$, 12.0 Hz), 5.73 (1H, dd, $J = 12.0$, 7.0 Hz), 6.63 (1H, s), 6.75 (1H, s), 6.98 (1H, dd, $J = 5.0$, 4.0 Hz), 7.08–7.21 (5H, m), 7.50–7.54 (2H, m), 7.59–7.65 (1H, m), 8.53–8.57 (1H, m).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 42.41 (CH_2), 65.68 (CH), 104.10 (CH), 116.24 (CH), 122.15 (CH), 122.84 (CH), 127.10 (2CH), 128.12 (CH), 128.33 (CH), 128.56 (2CH), 133.08 (C), 133.39 (C), 134.81 (C), 136.98 (CH), 148.36 (C), 149.40 (CH), 150.27 (C), 159.47 (C), 165.28 (C).

EIMS (*m/z*): 424 + 422 (M^+, 12 + 29%), 389 (2), 346 (15), 344 (37), 280 (3), 211 (4), 200 (16), 199 (100), 136 (8).

*5.1.1.3.15. 1-(4-(p-Nitrophenyl)-2-thiazolyl)-3-(2-thienyl)-5-(2-pyridyl)-2-pyrazoline (**6o**).* ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.56 (1H, dd, $J = 17.1$, 7.5 Hz), 3.88 (1H, dd, $J = 17.1$, 12.0 Hz), 5.70 (1H, dd, $J = 12.0$, 7.5 Hz), 6.88–6.92 (1H, m), 7.00–7.20 (5H, m), 8.00 (2H, d, $J = 9.0$ Hz), 8.20 (2H, d, $J = 9.0$ Hz), 8.62–8.65 (2H, m).

EIMS (*m/z*): 433 (M^+, 18%), 403 (9), 355 (17), 325 (7), 309 (6), 200 (17), 199 (100), 167 (5), 144 (4), 117 (6), 79 (14).

5.2. Biology

5.2.1. Antimicrobial activity

Microdilution broth susceptibility assay was used for the antibacterial evaluation of the compounds [30], whereas antifungal susceptibility of the fungus yeasts was examined according to NCCLS reference method for broth dilution antifungal susceptibility testing of yeasts [31]. Chloramphenicol was used as standard antibacterial agent whereas

flucanazol was used as antifungal agent and both are prepared as described in the related references.

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