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Original article

A novel series of thiazolyl–pyrazoline derivatives: Synthesis and evaluation of antifungal activity, cytotoxicity and genotoxicity

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ABSTRACT

In the current work, new thiazolyl–pyrazoline derivatives (**1**–**22**) were synthesized and evaluated for their antifungal effects against pathogenic yeasts and molds using a broth microdilution assay. Ames assay was carried out to determine the genotoxicity of the most effective antifungal derivatives. The cytotoxicity of the compounds (**1**–**22**) was also investigated against A549 human lung adenocarcinoma and NIH/3T3 mouse embryonic fibroblast cells. Among these derivatives, 2-[5-(4-fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]-4-(4-methylsulfonylphenyl)thiazole (**18**) can be identified as the most promising anticandidal derivative due to its notable inhibitory effect on *Candida zeylanoides* with a MIC value of 250 μg/mL when compared with ketoconazole (MIC = 250 μg/mL), low cytotoxicity against NIH/3T3 cells and non-mutagenic effect. On the other hand, 2-[5-(4-fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]-4-(4-bromophenyl)thiazole (**4**) can be considered as the most promising anticancer agent against A549 cancer cells owing to its notable inhibitory effect on A549 cells with an IC_{50} value of 62.5 μg/mL when compared with cisplatin (IC_{50} = 45.88 μg/mL) and low cytotoxicity against NIH/3T3 cells.

1. Introduction

Eukaryotic pathogens such as fungi pose a continuous and serious threat to public health since they share a close evolutionary relationship with their human hosts, limiting the number of drug targets that can be exploited to selectively kill fungal pathogens [1]. In recent years, the acquisition of multiple-drug resistance has resulted in a corresponding increase in demand for new effective antifungal agents with enhanced activity and limited toxicity [2–4]. On the other hand, in the last few decades, cancer has emerged as the second leading cause of death after cardiovascular disorders. The treatment of cancer is often complicated by high toxicity, low tolerability, and development of resistance [5–8].

Medicinal chemists have carried out considerable research on pyrazoline derivatives due to their diverse therapeutic applications extending from central nervous system applications to antimicrobials. The most predominant biological activity is observed for the class of 'antimicrobial agents' [9–11]. Furthermore, a considerable amount of research has reported that pyrazole-based heterocycles show promising activity against cancer cell lines including A549 human lung adenocarcinoma cell lines [12–16].

In terms of medicinal chemistry, thiazoles have also attracted a great deal of interest due to their presence in a large number of biologically active compounds, including natural products and pharmaceutical agents. The clinical efficacy of tiazofurin and its analogs, and bleomycins (BLMs) pointed out the importance of thiazole ring in the field of cancer treatment. Sulfathiazole (antimicrobial drug), abafungin (antifungal drug), and ritonavir (antiviral drug) are other examples of thiazole-based agents. Considerable research on thiazole and thiazolyl–pyrazoline derivatives in relation to their biological activity has been accomplished [17–32].

Prompted by the afore-mentioned findings and in the continuation of our ongoing research in the field of design, synthesis and biological evaluation of thiazolyl–pyrazoline derivatives [33,34], herein we described the synthesis and evaluation of a new series of thiophene substituted thiazolyl–pyrazolines as potential

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antifungal and anticancer agents.

2. Results and discussion

The synthesis of thiazolyl–pyrazoline derivatives (**1**–**22**) followed the general pathway outlined in Scheme 1. Initially, 1-(5-chloro/methylthiophen-2-yl)-3-(4-fluorophenyl)-2-propen-1-ones (**A**/**B**) were synthesized *via* the base-catalyzed Claisen–Schmidt condensation of 2-acetyl-5-chloro/methylthiophene with 4-fluorobenzaldehyde. Secondly, 3-(5-chloro/methylthiophen-2-yl)-5-(4-fluorophenyl)-1-thiocarbamoyl-2-pyrazolines (**C**/**D**) were obtained by the cyclization of chalcones (**A**/**B**) with thiosemicarbazide in the presence of sodium hydroxide. Finally, the ring closure reaction of 3-(5-chloro/methylthiophen-2-yl)-5-(4-fluorophenyl)-1-thiocarbamoyl-2-pyrazolines (**C**/**D**) with phenacyl bromides afforded thiazolyl–pyrazoline derivatives (**1**–**22**).

The structures of the newly synthesized compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analyses.

In the IR spectra of compounds **C** and **D**, the stretching bands for N–H group were observed in the region 3473–3350 cm^{-1}. C=N, C=C stretching and N–H bending vibrations were observed in the region 1575–1454 cm^{-1}. In the IR spectra of compounds **1**–**22**, C=N, C=C stretching and N–H bending vibrations were observed in the region 1633–1450 cm^{-1}. The aromatic and aliphatic C–H stretching vibrations gave rise to bands at 3140–3016 cm^{-1} and 2987–2839 cm^{-1}, respectively. In the IR spectra of the cyano-substituted compounds, the stretching bands for C≡N group occurred at 2223–2218 cm^{-1}.

In the ^{1}H NMR spectra of the compounds, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at δ 3.14–3.44 ppm (H_A), 3.98–4.04 ppm (H_M). The CH proton appeared as doublet of doublets at δ 5.64–5.72 (H_X) ppm due to vicinal coupling with two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring (J_{AM} = 17.50–18.00 Hz, J_{AX} = 6.50–7.50 Hz, J_{MX} = 11.50–12.00 Hz)

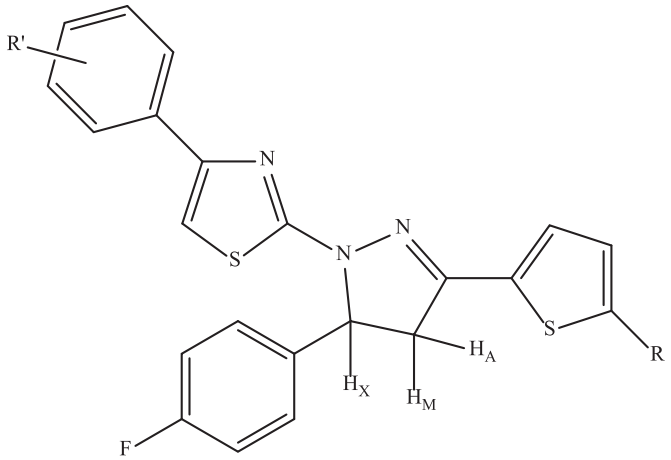


Fig. 1. AMX system of the pyrazoline ring.

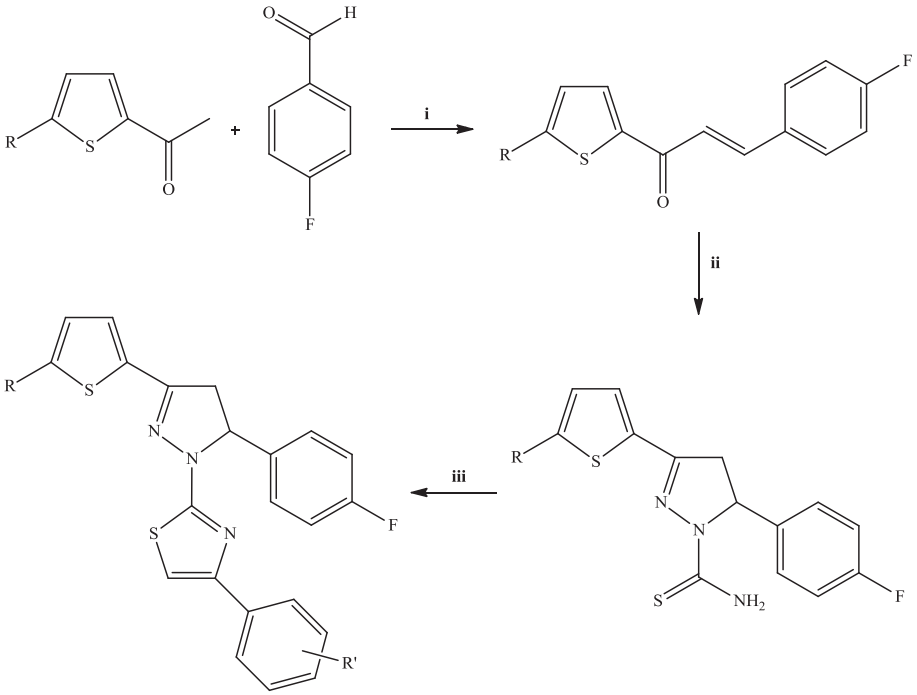
(Fig. 1). All the other aromatic and aliphatic protons were observed at expected regions.

In the ^{13}C NMR spectra of the compounds, the C_3, C_4 and C_5 carbons of the pyrazoline ring were observed at 148–151 ppm, 42–44 ppm and 62–65 ppm, respectively. In the ^{13}C NMR spectra of compounds **C** and **D**, C=S carbon appeared in the region 175–176 ppm. All the other aromatic and aliphatic carbons were observed at expected regions.

All of the synthetic compounds gave satisfactory mass spectroscopic data and elemental analysis, which were in full accordance with their depicted structures.

The synthesized compounds (**1**–**22**) were tested *in vitro* against pathogenic yeasts and molds. As shown in Table 1, the compounds exhibited more significant antifungal activity against yeasts than molds.

Among the pathogenic fungi species, *Candida zeylanoides* was the most susceptible yeast to the tested compounds. Compounds



Scheme 1. The synthetic route for the preparation of thiazolyl–pyrazoline derivatives (**1**–**22**). Reagents and conditions: (i) 10% aqueous sodium hydroxide solution, ethanol, rt, 10 h; (ii) thiosemicarbazide, NaOH, ethanol, reflux, 8 h; (iii) substituted 2-bromoacetophenone, ethanol, reflux, 6 h.

Table 1
Antifungal effects of the compounds (**1**–**22**) as MIC values (μg/mL).

Compound	A	B	C	D	E	F	G	H	I	J
1	125	250	250	250	250	250	125	250	250	250
2	250	250	250	250	250	250	125	250	250	250
3	125	250	250	250	250	250	125	250	250	250
4	125	250	250	250	250	250	125	250	250	250
5	125	250	250	250	250	250	250	250	250	250
6	125	250	250	250	250	250	250	250	250	250
7	250	250	250	250	250	250	250	250	250	250
8	125	250	250	250	250	250	250	250	250	250
9	125	250	250	250	250	250	250	250	250	250
10	125	250	250	250	250	250	250	250	250	250
11	125	500	500	1000	500	500	125	250	250	250
12	125	500	500	500	500	500	250	250	250	250
13	125	250	250	250	250	250	250	250	250	250
14	125	250	250	250	250	250	250	250	250	250
15	125	250	250	250	250	250	250	250	250	250
16	125	250	250	250	250	250	250	250	250	250
17	125	250	250	250	250	250	125	250	250	250
18	125	250	250	250	250	250	125	250	250	250
19	250	500	500	500	500	500	250	250	250	250
20	250	500	500	500	500	500	250	250	250	250
21	250	250	250	250	250	250	250	250	250	250
22	250	250	250	250	250	250	250	250	250	250
Ketoconazole	62.5	62.5	125	125	250	15.62	15.62	31.25	7.81	62.5

A: *C. glabrata* (Clinical Isolate, Osmangazi University, Faculty of Medicine, Eskişehir, Turkey), **B**: *C. albicans* (ATCC-90028), **C**: *C. tropicalis* (NRLL Y-12968), **D**: *C. krusei* (NRLL Y-7179), **E**: *C. zeylanoides* (NRLL Y-1774), **F**: *C. parapsilosis* (NRLL Y-12696), **G:** *A. flavus* (NRRL-980), **H:** *A. niger* (ATCC-1094), **I:** *A. parasiticus* (NRRL-465), **J**: *F. solani* (NRRL-13414).

Table 2
AMES^{MPF} results of the compounds (**1**–**22**).

Compound	Concentrations (mg/mL)	Fold increase over the baseline			
		TA 98		TA 100	
		S9+	S9−	S9+	S9−
1	0.156	1.63*	0.00	1.85*	0.00
	0.3125	0.68	0.00	**3.23****	0.32
	0.625	1.08	0.67	**2.77****	0.96
	1.25	0.68	0.00	**4.62****	1.28
	2.5	0.27	0.00	**3.46****	**2.88****
	5	0.41	0.67	**4.38****	0.96
3	0.156	0.68	0.00	**3.23****	1.67*
	0.3125	0.81	0.00	**3.92****	0.67
	0.625	0.95	0.00	**3.46****	0.33
	1.25	0.81	0.67	**3.69****	0.33
	2.5	0.54	0.67	**3.00****	1.00*
	5	0.81	1.00	**3.92****	0.33
4	0.156	0.68	0.00	**2.77****	1.00
	0.3125	0.81	0.32	**3.92****	1.00
	0.625	0.95	0.00	**3.92****	0.33
	1.25	0.81	0.00	**5.08****	0.00
	2.5	0.68	0.00	**3.92****	0.33
	5	0.81	0.00	**2.54****	**2.00****
11	0.156	0.87	0.00	**2.08****	0.67
	0.3125	0.55	0.33	**2.31****	1.00
	0.625	1.09	0.67	**2.54****	1.33
	1.25	0.22	0.67	**2.77****	**3.00****
	2.5	0.33	0.00	**2.31****	1.33
	5	0.98	0.67	**2.31****	**2.67***
17	0.156	0.27	1.78*	1.01	0.80
	0.3125	0.18*	1.56*	0.94	0.74
	0.625	1.07*	1.11*	0.86	0.74
	1.25	0.44	1.11	0.70	0.31
	2.5	0.89	1.56*	0.55	0.31
	5	1.16	1.33*	1.01	0.80
18	0.156	0.41	0.67	0.73	0.78
	0.3125	0.27	0.22	0.73	1.04*
	0.625	1.63*	0.44	0.93	0.99
	1.25	0.95	0.89	0.80	0.52
	2.5	1.36*	0.67	1.26*	0.52
	5	0.81	0.89	0.93	1.09*

* t test p value (unpaired 1-sided) < 0.05; ** **t test p value (unpaired 1-sided) < 0.05 with a fold-induction over baseline > 2**.

11, **12**, **19** and **20** exhibited their inhibitory effects on *C. zeylanoides* with a MIC value of 500 μg/mL, whereas other derivatives and ketoconazole showed their anticandidal activity with a MIC value of 250 μg/mL. Compounds **1**, **3**, **4**, **5**, **6**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17** and **18** showed their antifungal activity against *Candida glabrata* with a MIC value of 125 μg/mL, whilst ketoconazole displayed its anticandidal activity with a MIC value of 62.5 μg/mL. Compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **13**, **14**, **15**, **16**, **17**, **18**, **21** and **22** showed their inhibitory effects on *Candida tropicalis* and *Candida krusei* with a MIC value of 250 μg/mL when compared with ketoconazole (MIC = 125 μg/mL). On the other hand, all derivatives showed their antifungal activity against *Aspergillus niger* and *Fusarium solani* with a MIC value of 250 μg/mL, whilst ketoconazole exhibited its inhibitory effects on *A. niger* and *F. solani* with MIC values of 31.25 μg/mL and 62.5 μg/mL, respectively. The microbiological results demonstrated that the antifungal effects of these compounds on *A. niger* and *F. solani* did not depend on the substituents. Compounds **1**, **2**, **3**, **4**, **11**, **17**, and **18** exhibited their antifungal activity against *Aspergillus flavus* with a MIC value of 125 μg/mL, whereas ketoconazole showed its antifungal activity with a MIC value of 15.62 μg/mL. Considering the antifungal effects of the compounds against pathogenic yeasts and molds, compounds **1**, **3**, **4**, **11**, **17** and **18** were selected to undergo further studies.

Ames assay was carried out to evaluate the genotoxicity of the most effective antifungal derivatives. In Ames MPF assay, more than 25 positive wells were observed with positive controls. This complied with the requirements in the Ames MPF assay manual. Negative controls showed less than 8 positive wells in the presence and absence of S9 with TA98 and TA100. These complied with the requirements in the Ames MPF assay manual and previous studies [35]. Our results were presented in Table 2.

Compounds **1**, **3** and **4** had a baseline of 2.46 with TA 98 in the presence of S9 and 1.0 in the absence of S9 with related negative control values. Furthermore, fold induction over baseline was less than 2 in each concentration of the compounds and the significant different results obtained did not show a dose-response tendency. So, the results of the Ames test indicated the nonmutagenic potentials of compounds **1**, **3** and **4** against TA 98. Compounds **1**, **3** and **4** showed a baseline of 1.44 and 1.04 against TA100 with/without

S9, respectively. Fold inductions over baseline were also more than 3 and statistically different results were obtained in the presence of S9 against TA 100. Partially, compound **4** showed 2-fold increase over baseline, which was also statistically significant, in its highest concentration without S9 against TA 100. According to these results, compounds **1**, **3** and **4** showed mutagenic properties against TA 100 in the presence of S9. Furthermore, compound **4** can be considered as a weak mutagen against TA 100 without S9.

Compound **11** had a baseline of 3.06 against TA 98 in the presence of S9 and 1.0 in the absence of S9. Fold inductions over baseline were also less than 2 in each concentration of the compounds and the significant different results obtained did not show a dose-response tendency. So, the Ames test indicated the non-mutagenic potential of compound **11** against TA 98. The baseline values obtained was 1.44 and 1.04 against TA100 in the presence and absence of S9, respectively. This compound also showed 2–3 fold increases over the baseline which were statistically significant and showed a dose-responce tendency. According to the criteria obtained from previous studies, compound **11** can be classified as weak positive against TA 100 with/without S9 (Fig. 2).

Mentioned-fold increases over the baseline according to the criteria were not determined with compounds **17** and **18**. Therefore, compounds **17** and **18** were classified as non-mutagenic compounds against TA 98 and 100 with/without S9. Although compounds **17** and **18** are 5-methylthiophene substituted thiazolyl–pyrazoline derivatives, methylsulfonyl-substituted compound **18** is less cytotoxic than cyano-substituted compound **17** against NIH/3T3 mouse embryonic fibroblast cells. It can be concluded that cyano substituent increased cytotoxicity.

According to the genotoxicity and cytotoxicity assays, compound **18** seems to be the most promising anticandidal derivative due to its notable inhibitory effect on *C. zeylanoides* with a MIC value of 250 μg/mL, low cytotoxicity against NIH/3T3 cells and non-mutagenic effect.

The compounds were investigated for their antiproliferative effects on A549 human lung adenocarcinoma cell line. Additionally, these compounds were tested for their cytotoxicity against NIH/3T3 mouse embryonic fibroblast cell line (Table 3). Compounds **3**, **4**, **13**, **16**, **17**, **20** and **21** showed higher antiproliferative activity against A549 cell line than the other derivatives. On the other hand, compounds **9**, **10**, **16**, **17**, **20** and **21** exhibited more cytotoxicity against NIH/3T3 cell line than the other compounds.

Table 3
IC_{50} values of the compounds (**1**–**22**) against A549 and NIH/3T3 cell lines (μg/mL).

Compound	IC_{50} (μg/mL)	
	A549 cell line	NIH/3T3 cell line
1	>500	>500
2	>500	>500
3	125	>500
4	62.5	>500
5	>500	>500
6	>500	>500
7	>500	>500
8	>500	>500
9	>500	102.96
10	>500	73.77
11	>500	>500
12	>500	>500
13	125	>500
14	>500	>500
15	>500	>500
16	16.67	15.6
17	321.8	250
18	>500	373.83
19	>500	>500
20	183.65	146.5
21	173.44	125
22	>500	>500
Cisplatin	45.88	ND

ND: Not Determined.

In terms of their anticancer potential, compounds **3**, **4** and **13** can be considered to be selective cytotoxic agents against A549 cell line due to their low cytotoxicity against NIH/3T3 cell line.

Among these compounds, compound **4** bearing 5-chlorothiophene and *p*-bromophenyl moieties showed the highest cytotoxicity against A549 cell line with an IC_{50} value of 62.5 μg/mL, which was very similar to the positive control cisplatin (IC_{50} = 45.88 μg/mL). Although compound **15** also carries *p*-bromo substituent on phenyl ring, compound **15** showed low cytotoxicity against A549 cell line (IC_{50} > 500 μg/mL). This result pointed out

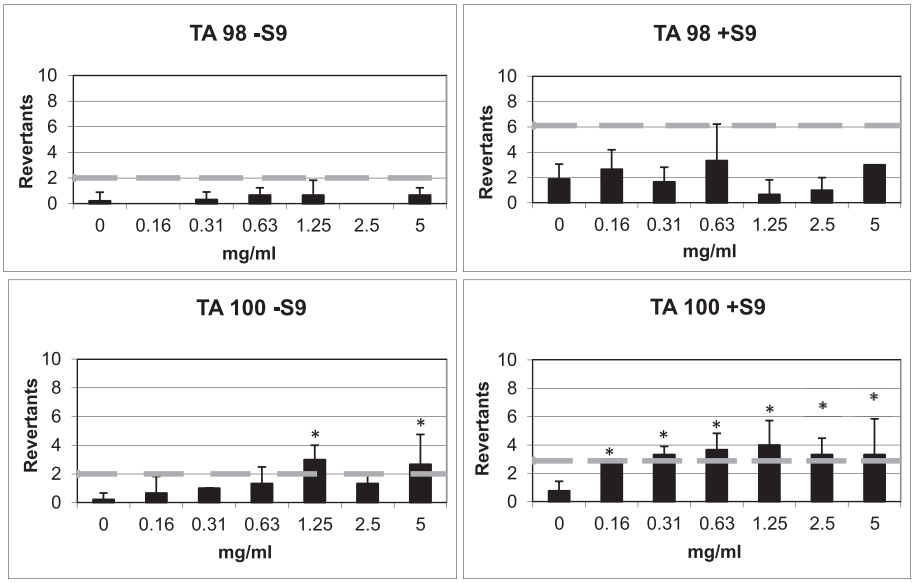


Fig. 2. Dose-response curve of compound **11** against TA98 and TA100 in the presence and absence of S9 according to AMES ^{MPF} test. * :t test p value (unpaired 1-sided) < 0.05 with a fold-induction over baseline > 2; ·····: 2-fold induction over baseline.

the importance of 5-chlorothiophene moiety for anticancer activity against A549 cell line.

According to our studies performed, compound **4** showed promising cytotoxic activity against A549 cell line with a similar dose of cisplatin without causing any toxicity on NIH/3T3 mouse embryonic fibroblast cell line.

3. Conclusion

In this study, new thiazolyl–pyrazoline derivatives were synthesized and investigated for their antifungal activity and cytotoxicity against A549 cell line.

An ideal drug is expected to exhibit high therapeutic effect and minimum toxicity. During early drug testing, Ames assay is widely used by the pharmaceutical industry to assess the mutagenic potential of the drug candidates for their reliability. For this purpose, Ames test was performed to determine the genotoxicity of the most effective antifungal compounds in the present study. Furthermore, a potential agent should have minimum or no side-effects on normal cells. So, in the current study the cytotoxic effects of all compounds were also investigated on NIH/3T3 cell lines.

In particular, compound **18** was the most promising antifungal derivative against *C. zeylanoides* with a MIC value of 250 μg/mL when compared with ketoconazole. In addition, this agent did not show any genotoxic potential and the cytotoxic dose of the compound was higher than its effective dose. Furthermore, compound **4** can be identified as the most promising anticancer agent owing to its antiproliferative effect on A549 cancer cell lines and non-toxic potential against NIH/3T3 cells. Further studies are required to evaluate the mechanism of action for the anticancer activity of compound **4** and anticandidal activity of compound **18**.

4. Experimental

4.1. Chemistry

All reagents were purchased from commercial suppliers and used without further purification. Melting points (Mp) were determined on an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected. IR spectra were recorded on an IRPrestige-21 Fourier Transform Infrared spectrophotometer (Shimadzu, Tokyo, Japan). ^{1}H NMR and ^{13}C NMR spectra of the synthesized compounds were recorded on a Bruker spectrometer (Bruker, Billerica, USA). Mass spectra were recorded on an Agilent LC-MSD-Trap-SL Mass spectrometer (Agilent, Minnesota, USA). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyzer (Perkin–Elmer, Norwalk, USA). The TLC was performed on Kieselgel 60 F_{254} (Merck) layer using petroleum ether:ethyl acetate (3:1 v/v) as eluents.

4.1.1. General procedure for the synthesis of the compounds

*4.1.1.1. 1-(5-Chloro/methylthiophen-2-yl)-3-(4-fluorophenyl)prop-2-en-1-ones (**A/B**).* A mixture of 2-acetyl-5-chloro/methylthiophene (0.05 mol), 4-fluorobenzaldehyde (0.05 mol) and 10% aqueous sodium hydroxide (10 mL) in ethanol (30 mL) was stirred at room temperature for 10 h. The progress of the reaction was checked by TLC. Upon completion, the reaction mixture was poured into crushed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol.

*4.1.1.2. 3-(5-Chloro/methylthiophen-2-yl)-5-(4-fluorophenyl)-1-thiocarbamoyl-2-pyrazolines (**C/D**).* A mixture of chalcone (**A/B**) (0.01 mol), thiosemicarbazide (0.012 mol) and sodium hydroxide (0.01 mol) was refluxed in ethanol (25 mL) for 8 h. The solution was poured into crushed ice. The precipitate was filtered and crystallized from ethanol.

*4.1.1.2.1. 3-(5-Chlorothiophen-2-yl)-5-(4-fluorophenyl)-1-thiocarbamoyl-2-pyrazoline (**C**).* Yield: 85%. Mp 230–231 °C.

IR ν_{max} (cm^{-1}): 3473.80, 3350.35 (N–H stretching), 1575.84, 1508.33, 1475.54 (C=N and C=C stretching), 1438.90, 1415.75, 1365.60, 1338.60 (C–H bending), 1215.15, 1163.08, 1091.71, 1012.63 (C–N stretching and aromatic C–H in plane bending), 835.18, 794.67 (aromatic C–H out of plane bending).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.14 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 3.6 Hz, C_4-H_A pyrazoline), 3.88 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 11.6 Hz, C_4-H_M pyrazoline), 5.95 (1H, dd, J_{MX} = 11.2 Hz, J_{AX} = 3.6 Hz, C_5-H_X pyrazoline), 7.15–7.37 (6H, m, aromatic protons), 7.68 (1H, brs, N–H), 8.11 (1H, brs, N–H).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 42.34 (CH_2), 62.41 (CH), 115.07 and 115.29 (2CH), 127.31 (CH), 127.39 (CH), 127.94 (C), 130.83 (C), 132.10 (CH), 132.81 (CH), 138.68 and 138.71 (C), 150.11 (C), 159.90 and 162.32 (C), 175.89 (C).

For $C_{14}H_{11}ClFN_3S_2$ Calculated: C, 49.48; H, 3.26; N, 12.36. Found: C, 49.49; H, 3.24; N, 12.35.

MS (*ESI*) (*m/z*): (M^++1) 340.8.

*4.1.1.2.2. 3-(5-Methylthiophen-2-yl)-5-(4-fluorophenyl)-1-thiocarbamoyl-2-pyrazoline (**D**).* Yield: 78%. Mp 225–226 °C.

IR ν_{max} (cm^{-1}): 3473.80, 3352.28 (N–H stretching), 3051.39 (aromatic C–H), 2916.37 (aliphatic C–H), 1575.84, 1506.41, 1489.05, 1454.33 (C=N and C=C), 1361.74, 1342.46 (C–H bending), 1213.23, 1163.08, 1091.71 (C–N stretching and aromatic C–H in plane bending), 835.18, 798.53 (aromatic C–H out of plane bending).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.48 (3H, s, CH_3), 3.10 (1H, dd, J_{AM} = 17.6 Hz, J_{AX} = 3.2 Hz, C_4-H_A pyrazoline), 3.87 (1H, dd, J_{MA} = 17.6 Hz, J_{MX} = 11.6 Hz, C_4-H_M pyrazoline), 5.93 (1H, dd, J_{MX} = 11.6 Hz, J_{AX} = 3.2 Hz, C_5-H_X pyrazoline), 6.85 (1H, dd, J = 3.6, 1.2 Hz, thiophene C_4–H), 7.14–7.29 (5H, m, aromatic protons), 7.52 (1H, brs, N–H), 8.04 (1H, brs, N–H).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.21 (CH_3), 42.77 (CH_2), 62.13 (CH), 115.05 and 115.26 (2CH), 126.56 (C), 127.27 (CH), 127.36 (CH), 131.30 (CH), 131.43 (CH), 138.80 and 138.83 (C), 144.41 (C), 151.06 (C), 159.88 and 162.29 (C), 175.57 (C).

For $C_{15}H_{14}FN_3S_2$ Calculated: C, 56.40; H, 4.42; N, 13.16. Found: C, 56.41; H, 4.40; N, 13.17.

MS (*ESI*) (*m/z*): (M^++1) 320.4.

*4.1.1.3. 2-[5-(4-Fluorophenyl)-3-(5-chloro/methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-phenylthiazole derivatives (**1–22**).* A mixture of 3-(5-chloro/methylthiophen-2-yl)-5-(4-fluorophenyl)-1-thiocarbamoyl-2-pyrazoline (**C**/**D**) (0.001 mol) and 2-bromoacetophenone/4′-substituted-2-bromoacetophenone (0.001 mol) in ethanol (20 mL) was refluxed for 6 h. The reaction mixture was cooled and filtered.

*4.1.1.3.1. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-phenylthiazole (**1**).* Yield: 93%. Mp 149 °C.

IR ν_{max} (cm^{-1}): 3113.11, 3039.81 (aromatic C–H), 1600.92, 1535.34, 1508.33 (aromatic C=N and C=C stretching), 1444.68 (C–H bending), 1230.58, 1138.00, 1053.13, 1008.77 (C–N stretching and aromatic C–H out of plane bending), 827.46, 792.74, 704.02 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.38 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 4.04 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.71 (1H, dd, J_{MX} = 12 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 7.19–7.48 (10H, m, aromatic protons), 7.70 (2H, dd, J = 7.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 43.52 (CH_2), 64.38 (CH), 105.09 (CH), 115.67 and 115.89 (2CH), 125.93 (2CH), 128.01 (C), 128.40 (C), 128.94 (2CH), 129.22 (CH), 129.30 (CH), 129.82 (CH), 131.43 (CH), 133.50 (CH), 134.81 (C), 138.02 and 138.05 (C), 148.54

(C), 150.93 (C), 160.76 and 163.18 (C), 164.38 (C).

For $C_{22}H_{15}ClFN_3S_2$ Calculated: C, 60.06; H, 3.44; N, 9.55. Found: C, 60.04; H, 3.43; N, 9.56.

MS (*ESI*) (*m/z*): (M^++1) 440.9.

*4.1.1.3.2. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-fluorophenyl)thiazole (**2**).* Yield: 94%. Mp 159 °C.

IR ν_{max} (cm^{-1}): 3113.11 (aromatic C–H), 2972.31 (aliphatic C–H), 1604.77, 1539.20, 1521.84, 1508.33, 1485.19 (C=N and C=C stretching), 1444.68 (C–H bending), 1313.52, 1220.94, 1155.36, 1047.35, 1010.70 (C–N stretching and aromatic C–H out of plane bending), 827.46, 800.46, 732.95 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.38 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 6.5 Hz, C_4-H_A pyrazoline), 4.02 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.70 (1H, dd, J_{MX} = 11.5 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 7.19 (2H, dd, J = 8.5 Hz), 7.23–7.75 (9H, m, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 43.05 (CH_2), 63.86 (CH), 104.34 (CH), 115.22 and 115.43 (4CH), 127.37 and 127.45 (2CH), 127.93 (C), 128.74 and 128.82 (2CH), 129.42 (CH), 130.92 and 130.95 (C), 131.00 (2C), 132.97 (CH), 137.51 and 137.54 (C), 148.15 (C), 149.39 (C), 160.27–160.37 and 162.70–162.80 (C), 163.97 (C).

For $C_{22}H_{14}ClF_2N_3S_2$ Calculated: C, 57.70; H, 3.08; N, 9.18. Found: C, 57.72; H, 3.07; N, 9.18.

MS (*ESI*) (*m/z*): (M^++1) 458.9.

*4.1.1.3.3. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-chlorophenyl)thiazole (**3**).* Yield: 88%. Mp 171 °C.

IR ν_{max} (cm^{-1}): 3138.18 (aromatic C–H), 2891.30 (aliphatic C–H), 1575.84, 1537.27, 1475.54 (C=N and C=C stretching), 1446.61, 1369.46 (C–H bending), 1294.24, 1269.16, 1220.94, 1085.92, 1053.13 (C–N stretching and aromatic C–H out of plane bending), 817.82, 788.89, 734.88 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.38 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 6.5 Hz, C_4-H_A pyrazoline), 4.00 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 11.5 Hz, C_4-H_M pyrazoline), 5.70 (1H, dd, J_{MX} = 12 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 7.14–7.48 (9H, m, aromatic protons), 7.71 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 43.05 (CH_2), 63.81 (CH), 105.41 (CH), 115.23 and 115.45 (2CH), 127.12 (2CH), 127.98 (C), 128.51 (2CH), 128.77 and 128.84 (2CH), 129.53 (CH), 131.00 (C), 131.97 (C), 132.93 (CH), 133.15 (C), 137.44 and 137.47 (C), 148.29 (C), 149.16 (C), 160.27 and 162.69 (C), 163.97 (C).

For $C_{22}H_{14}Cl_2FN_3S_2$ Calculated: C, 55.70; H, 2.97; N, 8.86. Found: C, 55.69; H, 2.95; N, 8.86.

MS (*ESI*) (*m/z*): (M^++1) 475.4.

*4.1.1.3.4. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-bromophenyl)thiazole (**4**).* Yield: 90%. Mp 191 °C.

IR ν_{max} (cm^{-1}): 3132.40, 3055.24 (aromatic C–H), 1604.77, 1535.34, 1508.33 (C=N and C=C stretching), 1448.54, 1398.39 (C–H bending), 1294.24, 1213.23, 1155.36, 1099.43, 1051.20, 1006.84 (C–N stretching and aromatic C–H out of plane bending), 827.46, 788.89, 732.95 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.38 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 4.01 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.70 (1H, dd, J_{MX} = 12 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 7.17–7.35 (4H, m, aromatic protons), 7.41 (1H, s, thiazole), 7.44–7.47 (2H, m, aromatic protons), 7.55 (2H, d, J = 8.5 Hz, aromatic protons), 7.65 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 42.93 (CH_2), 63.80 (CH), 105.37 (CH), 115.10 and 115.31 (2CH), 120.48 (C), 127.34 (2CH), 127.79 (C), 128.67 and 128.75 (2CH), 129.24 (C), 130.97 (C), 131.29 (2CH), 132.88 (CH), 133.46 (CH), 137.31 (C), 148.09 (C), 149.18 (C), 160.21 and 162.63 (C), 163.94 (C).

For $C_{22}H_{14}BrClFN_3S_2$ Calculated: C, 50.93; H, 2.72; N, 8.10. Found: C, 50.91; H, 2.74; N, 8.10.

MS (*ESI*) (*m/z*): (M^++1) 519.8.

*4.1.1.3.5. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-nitrophenyl)thiazole (**5**).* Yield: 95%. Mp 212 °C.

IR ν_{max} (cm^{-1}): 3103.46, 3045.60 (aromatic C–H), 1595.13, 1546.91, 1506.41 (C=N and C=C stretching), 1446.61, 1328.95 (C–H bending), 1224.80, 1132.21, 1053.13, 1008.77 (C–N stretching and aromatic C–H out of plane bending), 852.54, 829.39, 794.67, 707.88 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.40 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 6.5 Hz, C_4-H_A pyrazoline), 4.03 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.72 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 7.0 Hz, C_5-H_X pyrazoline), 7.20–7.24 (3H, m, aromatic protons), 7.35 (1H, d, J = 3.5 Hz, aromatic proton), 7.47–7.50 (2H, m, aromatic protons), 7.72 (1H, s, thiazole), 7.95 (2H, d, J = 8.5 Hz, aromatic protons), 8.22 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 43.00 (CH_2), 63.81 (CH), 109.36 (CH), 115.21 and 115.43 (2CH), 123.92 (2CH), 126.19 (2CH), 127.93 (C), 128.85 and 128.92 (2CH), 129.59 (CH), 131.11 (CH), 132.79 (C), 137.17 and 137.21 (C), 140.23 (C), 146.18 (C), 148.32 (C), 148.59 (C), 160.28 and 162.71 (C), 164.14 (C).

For $C_{22}H_{14}ClFN_4O_2S_2$ Calculated: C, 54.49; H, 2.91; N, 11.55. Found: C, 54.50; H, 2.90; N, 11.54.

MS (*ESI*) (*m/z*): (M^++1) 485.9.

*4.1.1.3.6. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-cyanophenyl)thiazole (**6**).* Yield: 72%. Mp 204 °C.

IR ν_{max} (cm^{-1}): 3118.90 (aromatic C–H), 2987.74, 2900.94 (aliphatic C–H), 2223.92 (C≡N stretching), 1604.77, 1544.98, 1508.33, 1452.40 (C=N and C=C stretching), 1408.04 (C–H bending), 1311.59, 1215.15, 1047.35, 1014.56 (C–N stretching and aromatic C–H out of plane bending), 833.25, 785.03, 740.67, 702.09 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.39 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 4.01 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.71 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 7.0 Hz, C_5-H_X pyrazoline), 7.18–7.22 (3H, m, aromatic protons), 7.33 (1H, d, J = 3.5 Hz, aromatic proton), 7.44–7.48 (2H, m, aromatic protons), 7.63 (1H, s, thiazole), 7.81 (2H, d, J = 8.5 Hz, aromatic protons), 7.87 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 43.11 (CH_2), 63.80 (CH), 108.44 (CH), 109.62 (C), 115.26 and 115.48 (2CH), 118.90 (C), 126.00 (2CH), 128.00 (CH), 128.76 and 128.84 (2CH), 129.66 (CH), 131.10 (C), 132.59 (2CH), 132.84 (C), 137.35 and 137.38 (C), 138.37 (C), 148.56 (C), 148.68 (C), 160.28 and 162.71 (C), 164.09 (C).

For $C_{23}H_{14}ClFN_4S_2$ Calculated: C, 59.41; H, 3.03; N, 12.05. Found: C, 59.40; H, 3.03; N, 12.06.

MS (*ESI*) (*m/z*): (M^++1) 465.9.

*4.1.1.3.7. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-methylsulfonylphenyl)thiazole (**7**).* Yield: 67%. Mp 254 °C.

IR ν_{max} (cm^{-1}): 3113.11, 3039.81 (aromatic C–H), 2931.80 (aliphatic C–H), 1593.20, 1531.48, 1508.33 (C=N and C=C stretching), 1404.18 (C–H bending), 1300.02, 1278.81, 1213.23, 1147.65, 1051.20, 1008.77 (C–N stretching and aromatic C–H out of plane bending), 964.41, 833.25, 773.46, 732.95 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.21 (3H, s, SO_2CH_3), 3.38 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 4.03 (1H, dd,

J_{MA} = 17.5 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.72 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 7.0 Hz, C_5-H_X pyrazoline), 7.20–7.23 (3H, m, aromatic protons), 7.34 (1H, d, J = 4 Hz, aromatic proton), 7.47–7.50 (2H, m, aromatic protons), 7.63 (1H, s, thiazole), 7.90 (2H, d, J = 8.5 Hz, aromatic protons), 7.94 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 42.95 (CH_2), 43.49 (CH_3), 63.87 (CH), 108.03 (CH), 115.14 and 115.35 (2CH), 125.93 (2CH), 127.27 (2CH), 127.88 (CH), 128.81 and 128.90 (2CH), 129.45 (CH), 131.03 (C), 132.83 (C), 137.21 and 137.24 (C), 138.75 (C), 139.23 (C), 148.43 (C), 148.68 (C), 160.24 and 162.67 (C), 164.08 (C).

For $C_{23}H_{17}ClFN_3O_2S_3$ Calculated: C, 53.32; H, 3.31; N, 8.11. Found: C, 53.30; H, 3.30; N, 8.14.

MS (*ESI*) (*m/z*): (M^++1) 519.0.

*4.1.1.3.8. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-methylphenyl)thiazole (**8**).* Yield: 68%. Mp 166 °C.

IR ν_{max} (cm^{-1}): 3140.11, 3043.67 (aromatic C–H), 2910.58 (aliphatic C–H), 1633.71, 1577.77, 1523.76, 1508.33, 1487.12 (C=N and C=C stretching), 1446.61, 1369.46 (C–H bending), 1294.24, 1219.01, 1211.30, 1051.20 (C–N stretching and aromatic C–H out of plane bending), 829.39, 817.82, 786.96, 731.02 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.28 (3H, s, CH_3), 3.32 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 3.98 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.68 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 7.0 Hz, C_5-H_X pyrazoline), 7.14–7.47 (9H, m, aromatic protons), 7.59 (2H, d, J = 8.0 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 20.64 (CH_3), 42.91 (CH_2), 63.85 (CH), 103.58 (CH), 115.07 and 115.28 (2CH), 125.32 (2CH), 127.81 (CH), 128.67 and 128.75 (2CH), 128.93 (2CH), 129.16 (CH), 130.83 (C), 131.64 (C), 132.99 (C), 136.73 (C), 137.46 and 137.49 (C), 147.87 (C), 150.47 (C), 160.20 and 162.62 (C), 163.74 (C).

For $C_{23}H_{17}ClFN_3S_2$ Calculated: C, 60.85; H, 3.77; N, 9.26. Found: C, 60.85; H, 3.76; N, 9.27.

MS (*ESI*) (*m/z*): (M^++1) 454.9.

*4.1.1.3.9. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-methoxyphenyl)thiazole (**9**).* Yield: 69%. Mp 209 °C.

IR ν_{max} (cm^{-1}): 3109.25 (aromatic C–H), 2937.59 (aliphatic C–H), 1608.63, 1539.20, 1519.91, 1506.41, 1485.19 (C=N and C=C stretching), 1442.75 (C–H bending), 1286.52, 1249.87, 1219.01, 1170.79, 1051.20, 1029.99, 1008.77 (C–N stretching and aromatic C–H out of plane bending), 829.39, 798.53, 736.81 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.32 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 3.76 (3H, s, OCH_3), 3.99 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.68 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 7.0 Hz, C_5-H_X pyrazoline), 6.90 (2H, d, J = 8.5 Hz, aromatic protons), 7.15 (1H, s, thiazole), 7.18–7.21 (3H, m, aromatic protons), 7.30 (1H, d, J = 4.0 Hz, aromatic proton), 7.44–7.47 (2H, m, aromatic protons), 7.63 (2H, d, J = 9.0 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 42.93 (CH_2), 55.00 (CH_3), 63.87 (CH), 102.36 (CH), 113.79 (2CH), 115.09 and 115.30 (2CH), 126.72 (2CH), 127.18 (CH), 127.83 (CH), 128.66 and 128.74 (2CH), 129.17 (C), 130.81 (C), 133.00 (C), 137.51 and 137.54 (C), 147.84 (C), 150.26 (C), 158.74 (C), 160.19 and 162.62 (C), 163.75 (C).

For $C_{23}H_{17}ClFN_3OS_2$ Calculated: C, 58.78; H, 3.65; N, 8.94. Found: C, 58.76; H, 3.66; N, 8.95.

MS (*ESI*) (*m/z*): (M^++1) 470.9.

*4.1.1.3.10. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(1,3-benzodioxol-5-yl)thiazole (**10**).* Yield: 73%. Mp 185 °C.

IR ν_{max} (cm^{-1}): 3111.18 (aromatic C–H), 2895.15 (aliphatic C–H), 1604.77, 1543.05, 1508.33, 1477.47 (C=N and C=C stretching), 1446.61, 1354.03 (C–H bending), 1307.74, 1222.87, 1107.14, 1035.77 (C–N stretching and aromatic C–H out of plane bending), 939.33, 835.18, 790.81, 732.95 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.14 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 4.00 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.68 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 7.0 Hz, C_5-H_X pyrazoline), 6.02 (2H, s, O–CH_2–O), 6.88 (1H, m, aromatic proton), 7.15–7.23 (6H, m, aromatic protons), 7.31 (1H, d, J = 4.0 Hz, aromatic proton), 7.44–7.47 (2H, m, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 43.02 (CH_2), 63.91 (CH), 100.97 (CH_2), 103.06 (CH), 105.79 (CH), 108.21 (CH), 115.16 and 115.37 (2CH), 119.30 (CH), 127.88 (C), 128.68 (CH), 128.76 and 128.81 (2CH), 129.27 (CH), 130.92 (C), 133.00 (C), 137.57 and 137.60 (C), 146.68 (C), 147.41 (C), 147.97 (C), 150.09 (C), 160.24 and 162.66 (C), 163.63 (C).

For $C_{23}H_{15}ClFN_3O_2S_2$ Calculated: C, 57.08; H, 3.12; N, 8.68. Found: C, 57.07; H, 3.11; N, 8.68.

MS (*ESI*) (*m/z*): (M^++1) 484.9.

*4.1.1.3.11. 2-[5-(4-Fluorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-trifluoromethylphenyl)thiazole (**11**).* Yield: 77%. Mp 176 °C.

IR ν_{max} (cm^{-1}): 3115.04 (aromatic C–H), 1606.70, 1546.91, 1525.69, 1508.33, 1450.47 (C=N and C=C stretching), 1411.89 (C–H bending), 1323.17, 1309.67, 1224.80, 1153.43, 1103.28, 1066.64, 1008.77 (C–N stretching and aromatic C–H out of plane bending), 827.46 (aromatic C–H out of plane bending), 698.23 (C–S stretching).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.38 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 4.01 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.71 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 7.0 Hz, C_5-H_X pyrazoline), 7.18–7.24 (3H, m, aromatic protons), 7.32 (1H, d, J = 4.0 Hz, aromatic proton), 7.46–7.49 (2H, m, aromatic protons), 7.56 (1H, s, thiazole), 7.70 (2H, d, J = 8.0 Hz, aromatic protons), 7.90 (2H, d, J = 8.0 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 42.93 (CH_2), 63.86 (CH), 107.21 (CH), 115.10 and 115.31 (2CH), 122.82 (C), 125.28 and 125.32 (CH), 125.52 (C), 125.86 (2CH), 127.73 (CH), 128.72 and 128.80 (2CH), 129.26 (CH), 131.07 (C), 132.87 (CH), 137.21 and 137.23 (C), 137.92 (C), 148.20 and 148.23 (C), 148.86 (2C), 160.26 and 162.68 (C), 164.08 (C).

For $C_{23}H_{14}ClF_4N_3S_2$ Calculated: C, 54.38; H, 2.78; N, 8.27. Found: C, 54.36; H, 2.80; N, 8.25.

MS (*ESI*) (*m/z*): (M^++1) 508.9.

*4.1.1.3.12. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-phenylthiazole (**12**).* Yield: 87%. Mp 146 °C.

IR ν_{max} (cm^{-1}): 3113.11, 3064.89 (aromatic C–H), 2914.44 (aliphatic C–H), 1600.92, 1537.27, 1508.33, 1475.54 (C=N and C=C stretching), 1442.75, 1381.03 (C–H bending), 1232.51, 1138.00, 1051.20, 1026.13 (C–N stretching and aromatic C–H out of plane bending), 842.89, 825.53, 796.60, 771.53, 705.95 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.51 (3H, s, CH_3), 3.32 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 6.5 Hz, C_4-H_A pyrazoline), 4.01 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 11.5 Hz, C_4-H_M pyrazoline), 5.67 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.87 (1H, m, aromatic proton), 7.18–7.36 (7H, m, aromatic protons), 7.44–7.47 (2H, m, aromatic protons), 7.70 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.10 (CH_3), 43.43 (CH_2), 63.56 (CH), 104.18 (CH), 115.07 and 115.29 (2CH), 125.37 (2CH), 126.33 (C), 127.38 (CH), 128.35 (2CH), 128.57 (CH), 128.65 (CH), 129.80 (CH), 131.57 (CH), 134.37 (C), 137.68 and 137.71 (C), 143.08

(C), 148.77 (C), 150.37 (C), 160.16 and 162.58 (C), 164.12 (C).

For $C_{23}H_{18}FN_3S_2$ Calculated: C, 65.85; H, 4.32; N, 10.02. Found: C, 65.85; H, 4.30; N, 10.03.

MS (*ESI*) (*m/z*): (M^++1) 420.5.

*4.1.1.3.13. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-fluorophenyl)thiazole (**13**).* Yield: 88%. Mp 168 °C.

IR ν_{max} (cm^{-1}): 3111.18, 3055.24 (aromatic C–H), 2927.24 (aliphatic C–H), 1591.27, 1535.34, 1508.33, 1481.33 (C=N and C=C stretching), 1313.52, 1276.88, 1217.08, 1153.43, 1112.93, 1043.49 (C–N stretching and aromatic C–H out of plane bending), 827.46, 802.39, 732.95 (aromatic C–H out of plane bending), 696.30 (C–S stretching).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.52 (3H, s, CH_3), 3.32 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 4.01 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.67 (1H, dd, J_{MX} = 11.5 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.87 (1H, m, aromatic proton), 7.17 (2H, d, J = 9.0 Hz, aromatic protons), 7.21 (2H, d, J = 9.0 Hz, aromatic protons), 7.24 (1H, d, J = 3.5 Hz, aromatic proton), 7.29 (1H, s, thiazole), 7.43–7.46 (2H, m, aromatic protons), 7.72–7.75 (2H, m, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.09 (CH_3), 43.44 (CH_2), 63.55 (CH), 103.90 (CH), 115.09 and 115.30 (4CH), 126.33 (C), 127.29 and 127.37 (2CH), 128.55 and 128.63 (2CH), 129.83 (CH), 130.97 and 131.00 (2C), 131.54 (CH), 137.63 and 137.66 (C), 143.12 (C), 148.84 (C), 149.32 (C), 160.16–160.28 and 162.58–162.71 (C), 164.22 (C).

For $C_{23}H_{17}F_2N_3S_2$ Calculated: C, 63.14; H, 3.92; N, 9.60. Found: C, 63.13; H, 3.92; N, 9.61.

MS (*ESI*) (*m/z*): (M^++1) 438.5.

*4.1.1.3.14. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-chlorophenyl)thiazole (**14**).* Yield: 75%. Mp 172 °C.

IR ν_{max} (cm^{-1}): 3095.75 (aromatic C–H), 2922.16 (aliphatic C–H), 1604.77, 1546.91, 1508.33, 1471.69 (C=N and C=C stretching) 1311.59, 1224.80, 1089.78, 1049.28, 1012.63 (C–N stretching and aromatic C–H out of plane bending), 825.53, 802.39, 729.09 (aromatic C–H out of plane bending), 692.44 (C–S stretching).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.51 (3H, s, CH_3), 3.32 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 6.5 Hz, C_4-H_A pyrazoline), 4.01 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.67 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.86 (1H, m, aromatic proton), 7.20 (2H, m, aromatic protons), 7.25 (1H, d, J = 3.5 Hz, aromatic proton), 7.38 (1H, s, thiazole), 7.41 (2H, d, J = 8.5 Hz, aromatic protons), 7.44–7.46 (2H, m, aromatic protons), 7.72 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.09 (CH_3), 43.43 (CH_2), 63.51 (CH), 104.96 (CH), 115.09 and 115.30 (2CH), 126.33 (C), 127.04 (2CH), 128.38 (2CH), 128.57 and 128.65 (2CH), 129.88 (C), 131.50 (CH), 131.84 (CH), 133.21 (C), 137.55 and 137.58 (C), 143.16 (C), 148.94 (C), 149.11 (C), 160.16 and 162.58 (C), 164.22 (C).

For $C_{23}H_{17}ClFN_3S_2$ Calculated: C, 60.85; H, 3.77; N, 9.26. Found: C, 60.86; H, 3.75; N, 9.25.

MS (*ESI*) (*m/z*): (M^++1) 454.9.

*4.1.1.3.15. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-bromophenyl)thiazole (**15**).* Yield: 85%. Mp 194 °C.

IR ν_{max} (cm^{-1}): 3132.40, 3043.67 (aromatic C–H), 2887.44 (aliphatic C–H), 1604.77, 1531.48, 1508.33, 1471.69 (C=N and C=C stretching), 1398.39 (C–H bending), 1219.01, 1155.36, 1097.50, 1045.42, 1006.84 (C–N stretching and aromatic C–H out of plane bending), 819.75, 804.32, 790.81, 732.95 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.51 (3H, s, CH_3), 3.32 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 6.5 Hz, C_4-H_A pyrazoline), 4.00 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.66 (1H, dd, J_{MX} = 11.5 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.86 (1H, m, aromatic proton), 7.19 (2H, m, aromatic protons), 7.24 (1H, d, J = 3.0 Hz, aromatic proton), 7.37 (1H, s, thiazole), 7.43–7.45 (2H, m, aromatic protons), 7.54 (2H, d, J = 8.5 Hz, aromatic protons), 7.65 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.09 (CH_3), 43.41 (CH_2), 63.51 (CH), 105.04 (CH), 115.08 and 115.29 (2CH), 120.41 (C), 126.31 (C), 127.34 (2CH), 128.58 and 128.66 (2CH), 129.85 (C), 131.29 (2CH), 131.50 (CH), 133.55 (CH), 137.52 and 137.55 (C), 143.14 (C), 148.91 (C), 149.16 (C), 160.16 and 162.58 (C), 164.21 (C).

For $C_{23}H_{17}BrFN_3S_2$ Calculated: C, 55.42; H, 3.44; N, 8.43. Found: C, 55.41; H, 3.45; N, 8.43.

MS (*ESI*) (*m/z*): (M^++1) 499.4.

*4.1.1.3.16. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-nitrophenyl)thiazole (**16**).* Yield: 83%. Mp 210 °C.

IR ν_{max} (cm^{-1}): 3115.04 (aromatic C–H), 2918.30 (aliphatic C–H), 1595.13, 1533.41, 1504.48 (C=N and C=C stretching), 1336.67, 1315.45, 1222.87, 1155.36, 1105.21, 1047.35 (C–N stretching and aromatic C–H out of plane bending), 835.18, 790.81, 731.02 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.51 (3H, s, CH_3), 3.44 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 4.01 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.67 (1H, dd, J_{MX} = 11.5 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.86 (1H, m, aromatic proton), 7.21 (2H, m, aromatic protons), 7.25 (1H, d, J = 3.5 Hz, aromatic proton), 7.46 (2H, dd, J = 8.5, 5.5 Hz, aromatic protons), 7.67 (1H, s, thiazole), 7.94 (2H, d, J = 9.0 Hz, aromatic protons), 8.21 (2H, d, J = 9.0 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.09 (CH_3), 43.43 (CH_2), 63.52 (CH), 108.95 (CH), 115.13 and 115.34 (2CH), 123.82 (2CH), 126.12 (2CH), 126.34 (C), 128.69 and 128.77 (2CH), 130.02 (CH), 131.40 (CH), 137.35 and 137.38 (C), 140.29 (C), 143.31 (C), 146.11 (C), 148.30 (C), 149.26 (C), 160.22 and 162.64 (C), 164.37 (C).

For $C_{23}H_{17}FN_4O_2S_2$ Calculated: C, 59.47; H, 3.69; N, 12.06. Found: C, 59.46; H, 3.70; N, 12.05.

MS (*ESI*) (*m/z*): (M^++1) 465.5.

*4.1.1.3.17. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-cyanophenyl)thiazole (**17**).* Yield: 84%. Mp 203 °C.

IR ν_{max} (cm^{-1}): 3115.04 (aromatic C–H), 2970.38, 2918.30 (aliphatic C–H), 2218.14 (C≡N stretching), 1606.70, 1539.20, 1508.33, 1479.40 (C=N and C=C stretching), 1301.95, 1222.87, 1155.36, 1047.35 (C–N stretching and aromatic C–H out of plane bending), 835.18, 792.74,740.67 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.54 (3H, s, CH_3), 3.34 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 6.5 Hz, C_4-H_A pyrazoline), 4.01 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.67 (1H, dd, J_{MX} = 11.5 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.86 (1H, m, aromatic proton), 7.19 (2H, m, aromatic protons), 7.24 (1H, d, J = 3.5 Hz, aromatic proton), 7.45 (2H, dd, J = 8.5, 5.5 Hz, aromatic protons), 7.60 (1H, s, thiazole), 7.80 (2H, d, J = 8.5 Hz, aromatic protons), 7.87 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.09 (CH_3), 43.47 (CH_2), 63.49 (CH), 107.97 (CH), 109.50 (C), 115.12 and 115.33 (2CH), 118.80 (C), 125.92 (2CH), 126.33 (C), 128.56 and 128.64 (2CH), 129.97 (CH), 131.42 (CH), 132.42 (2CH), 137.44 and 137.48 (C), 138.40 (C), 143.27 (C), 148.62 (C), 149.16 (C), 160.18 and 162.60 (C), 164.32 (C).

For $C_{24}H_{17}FN_4S_2$ Calculated: C, 64.84; H, 3.85; N, 12.60. Found: C, 64.85; H, 3.83; N, 12.60.

MS (*ESI*) (*m/z*): (M^++1) 445.5.

*4.1.1.3.18. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-methylsulfonylphenyl)thiazole (**18**).* Yield: 70%. Mp 241 °C.

IR ν_{max} (cm^{-1}): 3113.11, 3016.67 (aromatic C–H), 2953.02 (aliphatic C–H), 1593.20, 1529.55, 1508.33 (C=N and C=C stretching), 1404.18 (C–H bending), 1298.09, 1278.81, 1213.23, 1147.65, 1087.85, 1049.28 (C–N stretching and aromatic C–H out of plane bending), 958.62, 835.18, 798.53, 773.46, 731.02 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.51 (3H, s, CH_3), 3.21 (3H, s, SO_2CH_3), 3.37 (1H, dd, J_{AM} = 18.0 Hz, J_{AX} = 7.0 Hz, C_4-H_A pyrazoline), 4.02 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.68 (1H, dd, J_{MX} = 11.5 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.87 (1H, m, aromatic proton), 7.21 (2H, m, aromatic protons), 7.26 (1H, d, J = 3.5 Hz, aromatic proton), 7.47 (2H, dd, J = 8.5, 5.5 Hz, aromatic protons), 7.60 (1H, s, thiazole), 7.90 (2H, d, J = 8.5 Hz, aromatic protons), 7.95 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.25 (CH_3), 43.50 (CH_2), 43.54 (CH_3), 63.60 (CH), 107.83 (CH), 115.24 and 115.45 (2CH), 126.00 (2CH), 126.49 (C), 127.39 (2CH), 128.83 and 128.92 (2CH), 130.23 (CH), 131.49 (CH), 137.53 and 137.57 (C), 138.90 (C), 139.19 (C), 143.39 (C), 148.72 (C), 149.34 (C), 160.27 and 162.69 (C), 164.35 (C).

For $C_{24}H_{20}FN_3O_2S_3$ Calculated: C, 57.93; H, 4.05; N, 8.44. Found: C, 57.92; H, 4.04; N, 8.46.

MS (*ESI*) (*m/z*): (M^++1) 498.6.

4.1.1.3.19. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-methylphenyl)thiazole (***19***). Yield: 73%. Mp 178 °C.

IR ν_{max} (cm^{-1}): 3140.11, 3057.17 (aromatic C–H), 2912.51 (aliphatic C–H), 1604.77, 1523.76, 1508.33, 1481.33 (C=N and C=C stretching), 1294.24, 1219.01, 1155.36, 1097.50, 1045.42 (C–N stretching and aromatic C–H out of plane bending), 831.32, 817.82, 790.81, 732.95 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.29 (3H, s, CH_3), 2.51 (3H, s, CH_3), 3.31 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 6.5 Hz, C_4-H_A pyrazoline), 3.98 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.65 (1H, dd, J_{MX} = 11.5 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.85 (1H, m, aromatic proton), 7.14–7.23 (6H, m, aromatic protons), 7.44 (2H, dd, J = 8.5, 5.5 Hz, aromatic protons), 7.60 (2H, d, J = 8.0 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.09 (CH_3), 20.64 (CH_3), 43.39 (CH_2), 63.57 (CH), 103.24 (CH), 115.04 and 115.25 (2CH), 125.32 (2CH), 126.31 (C), 128.58 and 128.66 (2CH), 128.92 (2CH), 129.31 (CH), 131.60 (CH), 131.74 (C), 136.66 (C), 137.69 and 137.71 (C), 143.03 (C), 148.66 (C), 150.45 (C), 160.15 and 162.57 (C), 164.05 (C).

For $C_{24}H_{20}FN_3S_2$ Calculated: C, 66.49; H, 4.65; N, 9.69. Found: C, 66.50; H, 4.64; N, 9.68.

MS (*ESI*) (*m/z*): (M^++1) 434.5.

4.1.1.3.20. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-methoxyphenyl)thiazole (***20***). Yield: 78%. Mp 186 °C.

IR ν_{max} (cm^{-1}): 3109.25 (aromatic C–H), 2929.87, 2839.22 (aliphatic C–H), 1606.70, 1521.84, 1508.33, 1485.19 (C=N and C=C stretching), 1317.38, 1286.52, 1246.02, 1215.15, 1170.79, 1047.35, 1028.06 (C–N stretching and aromatic C–H out of plane bending), 829.39, 804.32, 736.81, 698.23 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.51 (3H, s, CH_3), 3.28 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 7.5 Hz, C_4-H_A pyrazoline), 3.76 (3H, s, OCH_3), 3.99 (1H, dd, J_{MA} = 18.0 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.65 (1H, dd, J_{MX} = 12.0 Hz, J_{AX} = 7.0 Hz, C_5-H_X pyrazoline), 6.85 (1H, m, aromatic protons), 6.91 (2H, d, J = 9.0 Hz, aromatic proton), 7.13 (1H, s, thiazole), 7.15–7.24 (3H, m, aromatic protons), 7.44 (2H, dd, J = 8.5, 6.0 Hz, aromatic protons), 7.63 (2H, d, J = 9.0 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.12 (CH_3), 43.42 (CH_2), 55.01 (CH_3), 63.61 (CH), 102.05 (CH), 113.79 (2CH), 115.09 and 115.30 (2CH), 126.33 (CH), 126.74 (2CH), 127.31 (CH), 128.60 and 128.68 (2CH), 129.75 (C), 131.64 (C), 137.75 and 137.78 (C), 143.05 (C), 148.64 (C), 150.26 (C), 158.72 (C), 160.17 and 162.59 (C), 164.07 (C).

For $C_{24}H_{20}FN_3OS_2$ Calculated: C, 64.12; H, 4.48; N, 9.35. Found: C, 64.14; H, 4.45; N, 9.34.

MS (*ESI*) (*m/z*): (M^++1) 450.5.

4.1.1.3.21. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(1,3-benzodioxol-5-yl)thiazole (***21***). Yield: 82%. Mp 205 °C.

IR ν_{max} (cm^{-1}): 3116.97 (aromatic C–H), 2972.31, 2900.94 (aliphatic C–H), 1602.85, 1544.98, 1508.33, 1479.40 (C=N and C=C stretching), 1357.89 (C–H bending), 1303.88, 1251.80, 1222.87, 1103.28, 1039.63 (C–N stretching and aromatic C–H out of plane bending), 835.18, 792.74, 738.74 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.54 (3H, s, CH_3), 3.29 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 8.0 Hz, C_4-H_A pyrazoline), 3.98 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 12.0 Hz, C_4-H_M pyrazoline), 5.64 (1H, dd, J_{MX} = 11.5 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.02 (2H, s, O–CH_2–O), 6.82–6.89 (2H, m, aromatic protons), 7.08–7.27 (6H, m, aromatic protons), 7.44 (2H, dd, J = 8.0, 5.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.12 (CH_3), 43.46 (CH_2), 63.62 (CH), 100.91 (CH_2), 102.71 (CH), 105.79 (CH), 108.16 (CH), 115.09 and 115.31 (2CH), 119.26 (CH), 126.34 (C), 128.56 and 128.64 (2CH), 128.91 (C), 129.79 (CH), 131.60 (CH), 137.76 and 137.79 (C), 143.09 (C), 146.63 (C), 147.37 (C), 148.73 (C), 150.06 (C), 160.17 and 162.60 (C), 163.92 (C).

For $C_{24}H_{18}FN_3O_2S_2$ Calculated: C, 62.19; H, 3.91; N, 9.06. Found: C, 62.20; H, 3.90; N, 9.05.

MS (*ESI*) (*m/z*): (M^++1) 464.5.

4.1.1.3.22. 2-[5-(4-Fluorophenyl)-3-(5-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-trifluoromethylphenyl)thiazole (***22***). Yield: 68%. Mp 158 °C.

IR ν_{max} (cm^{-1}): 3111.18 (aromatic C–H), 2920.23 (aliphatic C–H), 1612.49, 1550.77, 1525.69, 1512.19 (C=N and C=C stretching), 1323.17, 1234.44, 1157.29, 1114.86, 1066.64, 1014.56 (C–N stretching and aromatic C–H out of plane bending), 829.39, 848.68, 804.32, 705.95 (aromatic C–H out of plane bending).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.51 (3H, s, CH_3), 3.37 (1H, dd, J_{AM} = 17.5 Hz, J_{AX} = 6.5 Hz, C_4-H_A pyrazoline), 4.02 (1H, dd, J_{MA} = 17.5 Hz, J_{MX} = 11.5 Hz, C_4-H_M pyrazoline), 5.69 (1H, dd, J_{MX} = 11.5 Hz, J_{AX} = 6.5 Hz, C_5-H_X pyrazoline), 6.87 (1H, m, aromatic proton), 7.21 (2H, m, aromatic protons), 7.26 (1H, d, J = 3.5 Hz, aromatic proton), 7.46 (2H, dd, J = 8.5, 5.5 Hz, aromatic protons), 7.56 (1H, s, thiazole), 7.72 (2H, d, J = 8.0 Hz, aromatic protons), 7.91 (2H, d, J = 8.5 Hz, aromatic protons).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.23 (CH_3), 43.52 (CH_2), 63.57 (CH), 107.08 (CH), 115.23 and 115.45 (2CH), 122.95 (C), 125.47 and 125.51 (CH), 125.65 (C), 125.96 (2CH), 126.48 (CH), 127.39 (C), 128.76 and 128.85 (2CH), 130.18 (CH), 131.52 (CH), 137.58 and 137.61 (C), 138.09 (C), 143.36 (C), 148.87 (C), 149.25 (C), 160.27 and 162.69 (C), 164.37 (C).

For $C_{24}H_{17}F_4N_3S_2$ Calculated: C, 59.13; H, 3.51; N, 8.62. Found: C, 59.11; H, 3.53; N, 8.62.

MS (*ESI*) (*m/z*): (M^++1) 488.5.

4.2. Microbiology

The antifungal assay was carried out according to the microbroth dilution method with some modifications [36,37]. The compounds (**1**–**22**) were tested for their *in vitro* antifungal activity against *C. glabrata* (Clinical Isolate, Osmangazi University, Faculty of

Medicine, Eskişehir, Turkey), *Candida albicans* (ATCC 90028), *C. tropicalis* (NRLL Y-12968), *C. krusei* (NRLL Y-7179), *C. zeylanoides* (NRLL Y-1774), *Candida parapsilosis* (NRLL Y-12696), *A. flavus* (NRRL-980), *A. niger* (ATCC-1094), *Aspergillus parasiticus* (NRRL 465), *F. solani* (NRRL-13414).

Microbroth dilution-susceptibility assay was used for antifungal evaluation of the compounds. The stock solutions of the samples were prepared in dimethyl sulfoxide (DMSO, Merck). Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.0039 mg/mL in micro-test tubes that were transferred to 96-well microtiter plates. Fungal strains grown on Potato Dextrose Agar (PDA) at 25 °C for 5 days for molds and Sabouraud Dextrose Agar (SDA) at 37 °C for 24 h for yeasts suspensions in double-strength Potato Dextrose Broth (PDB) were standardized to 10^5 spores/mL. 100 μL of each spore suspension was then added into the wells. The last well-chain without a fungus was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 25 °C (for molds) for 72 h and 37 °C (for yeasts) for 48 h, antifungal activity was detected by the investigation of mycelia growing under stereo microscope for molds. After incubation at 37 °C for 48 h, antifungal activities against to yeasts were detected by spraying of 0.5% triphenyl tetrazolium chloride (TTC, Merck) aqueous solution. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of compounds that inhibited visible growth, as indicated by the TTC staining. Ketoconazole was used as an antifungal agent.

4.3. Genotoxicity test

4.3.1. Ames MPF

Ames assay was carried out to determine the mutagenicity of the compounds using Ames MPF 98/100 mutagenicity assay sample kit (Xenometrix AG, Gewerbertrasse, Switzerland) [38]. *Salmonella typhimurium* strains, TA98 (frameshift mutations) and TA100 (base-pair substitutions), were thawed and growth medium (200 mL) was added to obtain homogenous suspensions of *Salmonella* strains (TA98 and TA100). The suspension (25 mL) was added to a mixture of 10 mL growth medium and 10 μL ampicillin (50 mg/mL). Negative control, which was devoid of *Salmonella* strains, was also prepared. The culture tubes were incubated in a shaker (SI-600, Jeio Tech, Korea) at 37 °C, 250 rpm for 14–16 h. The 'overnight grown' cultures were diluted 1:10 with growth medium and the absorbance was measured at 600 nm. The absorbance for the 'overnight grown' culture and negative control should be 0.25 and 0.005, respectively. Dose range for the chemicals with the tester strains according to the previous guidelines at a concentration range of 16–5000 μg/mL [39]. Compounds **1**, **3**, **4**, **11**, **17** and **18** were prepared in six different concentrations (5, 2.5, 1.25, 0.625, 0.3125, 0.156 mg/mL) in DMSO. Mutagenic potential of the compounds was determined in the absence and the presence of Aroclor™-1254 induced male Sprague–Dawley rat liver microsomal enzyme (S9) mix (Xenometrix AG, Gewerbertrasse, Switzerland) in sterile medium. The final concentration of S9 in the assay was 4.5% v/v. 2-nitrofluorene (2 μg/mL) and 4-nitroquinoline *N*-oxide (0.1 μg/mL) were used as positive controls in the absence of S9 mix. In the presence of S9 with TA 98 and TA100, 2-aminoanthracene was used as a positive control in the concentrations of 1 μg/mL and 2.5 μg/mL, respectively. 4% DMSO was used as a solvent control. Then, each well of a 24-well plate received 10 μL of each test chemical solution. Then, exposure medium (Xenometrix AG, Gewerbertrasse, Switzerland) was mixed with the bacterial culture at a ratio of 1:10 (TA98) and 1:20 (TA100). For the experiments with S9 mix, the volume of the exposure medium was reduced accordingly. 240 μL of this mixture was added to the wells of the 24-well-plate. Then, plates were incubated in the environmental shaker at 37 °C, 250 rpm for 90 min. Each sample was analyzed in triplicate. At the end of 90 min, 2.8 mL of indicator medium (Xenometrix AG, Gewerbertrasse, Switzerland) was added to each well of the 24-well plates. 50 μL aliquots were distributed into 48 wells of a 384-well-plate for each concentration and was incubated at 37 °C in a dry incubator for 48 h. The presence of the revertant bacteria dropped the pH of solution resulting in color change of the indicator from purple to yellow. The number of positive (yellow) wells out of 48 wells in triplicates were counted and compared with the negative control. Fold induction over the negative control and fold induction over the baseline were calculated. Fold induction over the negative control is the ratio of the mean number of positive wells for the dose concentration divided by the mean number of positive wells for the zero dose (negative) control. Fold induction over the baseline is the ratio of the mean number of positive wells for the dose concentration divided by zero dose baseline. The zero dose baseline is obtained by adding one standard deviation to the mean number of positive wells of the zero dose control.

Mutagenity was determined according to the criteria from previous studies [35] as follows:

If the baseline was ≤3, significant increases between 2 and 3-fold the baseline were classified as weak positive, and increases of greater than threefold the baseline were classified as positive. If the baseline was >3, significant increases between 1.5 and 2.5-fold the baseline were classified as weak positive, and increases of greater than 2.5-fold the baseline were classified as positive. To be classified as a mutagenic compound, there should be at least two adjacent doses with significant increases or a significant increase at the highest dose level. All doses were tested for according to Student's t-test at $p < 0.05$ for statistical significance. Compounds which did not have any of above properties were classified as negative.

4.4. Cytotoxicity

NIH/3T3 mouse embryonic fibroblast and A549 human lung adenocarcinoma cell lines were used for cytotoxicity assay. NIH/3T3 cells were incubated in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Thermo Scientific, USA) supplemented with fetal calf serum (Hyclone, Thermo Scientific, USA), 100 IU/mL penicillin and 100 mg/mL streptomycin (Hyclone, Thermo Scientific, USA) and 7.5% $NaHCO_3$ at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. A549 cells were incubated in RPMI medium (Hyclone, Thermo Scientific, USA) supplemented with fetal calf serum, 100 IU/mL penicillin and 100 mg/mL streptomycin and 7.5% $NaHCO_3$ at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. NIH/3T3 and A549 cells were seeded at 20,000 cells into each well of 96-well plates. After 24 h of incubating period, the culture mediums were removed and the compound was added to culture medium at 500–3.9 μg/mL doses. After 24 h of incubation, the cytotoxicity test was performed using the In Cytotox-XTT 1 Parameter Cytotoxicity Kit (Xenometrix AG, Gewerbertrasse 25, Switzerland), which measures mitochondrial activity (tetrazolium hydroxide (XTT)) in NIH/3T3 and A549 cells. Firstly, the cells were washed with phosphate buffered saline (PBS) and were added 200 μL/well of fresh culture medium. XTTI and XTTII solution were mixed at 1:100 ratio. Then, 50 μL of this mixture was added to all wells. The plate was incubated for 3 h at 37 °C, 5% CO_2. After 3 h, the content of the well was mixed by pipetting up and down. Then, OD of the plate was read at 480 nm with a reference wave length at 680 nm. Inhibition% was calculated at each concentration of the compounds. IC_{50} value was estimated by non-linear regression analysis. Cisplatin was used as a positive control. The stock solutions of the compounds were prepared in DMSO and further dilutions were made with fresh

culture medium. The final DMSO concentration was under 0.1%. All experiments were performed in triplicates [38].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.12.055.

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