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Synthesis of novel thiazolylpyrazoline derivatives and evaluation of their antimicrobial activities and cytotoxicities

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Abstract: Several novel thiazolylpyrazoline derivatives were synthesized by reacting substituted 3,5-diaryl-1-thiocarbamoyl-2-pyrazolines with phenacylbromides. The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and MS spectral data. Their antimicrobial activities against *Staphylococcus aureus* (ATCC-25923), *Enterococcus faecalis* (ATCC-29212), *Enterococcus faecalis* (ATCC-51922), *Listeria monocytogenes* (ATCC-1911), *Klebsiella pneumoniae* (ATCC-700603), *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-35218), *Escherichia coli* (ATCC-25922), *Candida albicans* (ATCC-90028), *Candida glabrata* (ATCC-90030), *Candida krusei* (ATCC-6258), and *Candida parapsilosis* (ATCC-22019) were investigated. The compounds were also studied for their cytotoxic effects using a MTT assay. Compound **7c** showed the highest antimicrobial activity, possessing the same potential as chloramphenicol against *K. pneumonia*, *P. aeruginosa*, and *E. coli* (ATCC-25923).

Key words: 2-Pyrazoline, thiazole, thiazolylpyrazoline, antimicrobial activity, cytotoxicity

1. Introduction

It has been reported that the morbidity, mortality, and costs related to the treatment of infectious diseases have been increased by antimicrobial resistance. The threat from resistance (particularly multiple resistance in bacterial strains that have disseminated widely) has never been so great. The main dynamics driving this threat are increased antibiotic use, bigger movement of people, and increased industrial and economic development.[1] The need for novel antibacterial and antifungal agents is greater than ever because of the emergence of multidrug resistance in common pathogens, the quick emergence of new infections, and the potential for use of multidrug-resistant agents in bioweapons.[2]

Every antimicrobial development candidate is considered "novel" by those who make it, but the multiple approaches to developing new compounds neither carry equal potential to overcome preexisting resistance mechanisms nor are they associated with equal development risk. There is a spectrum of innovation that ranges from developments within established classes, to completely new microbial pharmacophores and molecular targets.[3]

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General sources for novel antimicrobial agents include biological sources as well as large collections of different compounds collected from various laboratories. The recent advance of synthetic libraries represents a major advancement in the discovery of new lead compounds for antimicrobial drug development.[4]

Compounds having heterocyclic ring systems continue to attract considerable interest due to their wide range of biological activities. Amongst them, five-membered heterocyclic compounds, particularly azoles, occupy a unique place in the realm of natural and synthetic organic chemistry.[5]

Pyrazolines constitute a remarkable class of heterocycles due to their actual biological activities such as anticancer, antioxidant, antibacterial, antifungal, antidepressant, anti-inflammatory, anticonvulsant, antitumor, and analgesic properties.[6,7]

In addition, a thiazole ring is found in many potent biologically active compounds, such as sulfathiazole (antimicrobial drug), ritonavir (antiretroviral drug), abafungin (antifungal drug), bleomycine, and tiazofurin (antineoplastic drug). It has been observed over the years that thiazole derivatives have several biological activities such as antihypertensive, anti-inflammatory, antischizophrenia, antibacterial, anti-HIV, hypnotics, antiallergic, analgesic, antithrombotic, fibrinogen receptor antagonist, bacterial DNA gyrase B inhibitor, antitumor, and cytotoxic activities.[8]

Thus, the synthesis and biological activities of novel thiazolylpyrazoline derivatives activated a great deal of research. Remarkably, thiazolylpyrazoline derivatives were reported to display a variety of significant biological importance such as antimicrobial, antiviral, anti-inflammatory, antiamebic, and anticancer activities and β-ketoacyl-acyl carrier protein synthase III (FabH), epidermal growth factor receptor tyrosine kinase (EGFR TK) inhibitors, superoxidase inhibitors, and free radical scavengers.[9−16]

In a recent study, in silico molecular docking simulation was performed to position thiazolylpyrazoline derivatives in the DNA topoisomerase IV receptor structure active site to determine the probable binding model.[17] This study revealed that all the molecules showed good binding energy toward the target receptor DNA topoisomerase IV. Thiazolylpyrazoline derivatives have been tested for antimicrobial activity and some compounds showed good activity profiles against tested microbes. In another study, some thiazolylpyrazoline derivatives were synthesized and evaluated for their antifungal activity. According to the in silico molecular docking study, the compounds possessed the required binding energy to dock themselves with the binding pocket of Cytochrome P_{450} 14α-sterol demethylase (CYP51). The synthesized compounds showed significant antifungal activity, which has been fully supported by an in silico molecular docking study.[18]

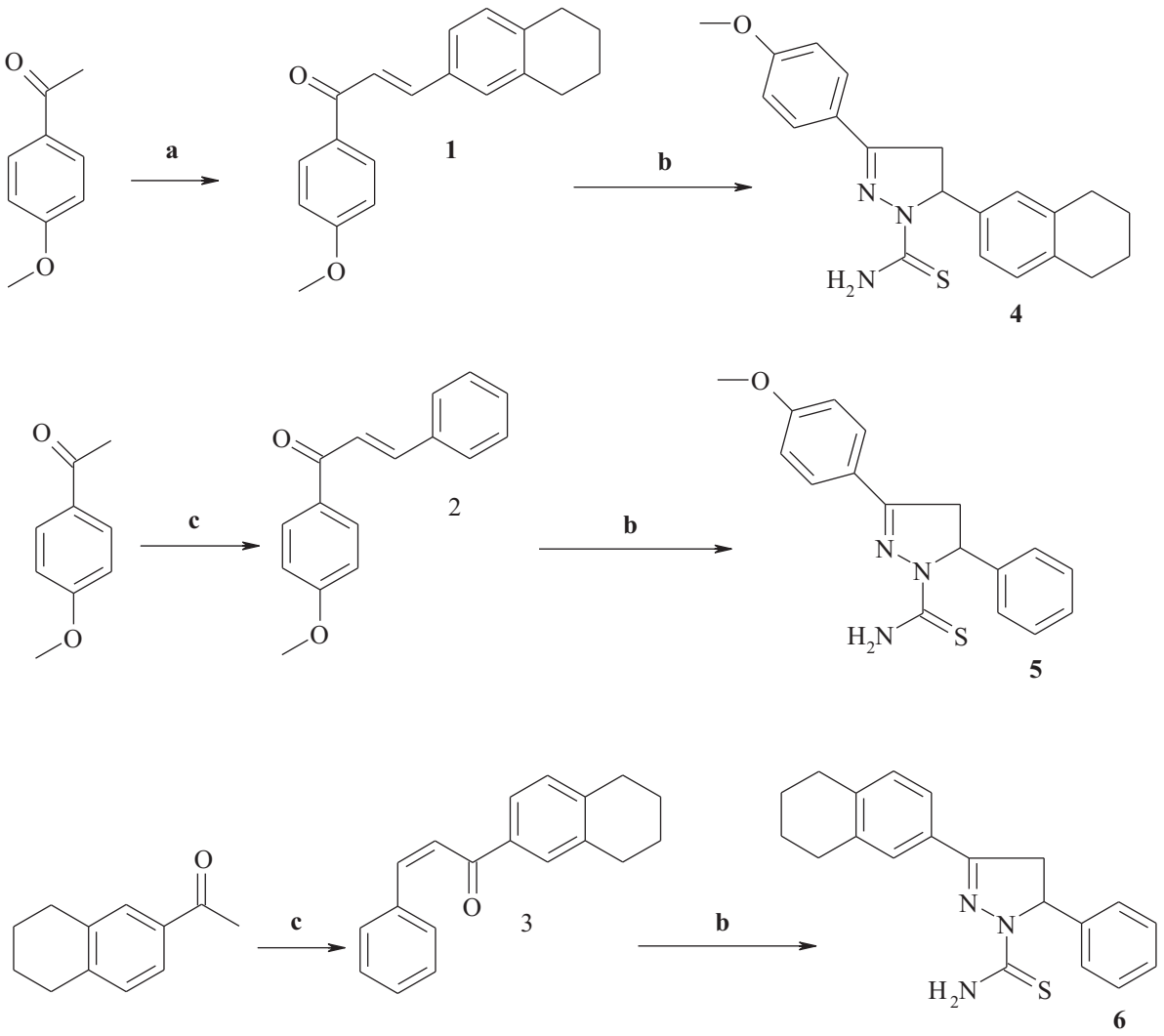
Keeping in view the therapeutic importance of thiazolylpyrazoline derivatives and in continuation of our work on the synthesis of biologically active thiazolylpyrazoline compounds, herein we describe the synthesis and evaluation of the antimicrobial and cytotoxic activities of novel molecules.[19−24]

2. Results and discussion

2.1. Chemistry

The synthesis of thiazolylpyrazoline derivatives (**7a**–**7j**, **8a**, **8b**, **9a**, **9b**) was carried out according to the steps outlined in Schemes 1 and 2. The intermediate products, 1-(4'-methoxyphenyl)-3-(5,6,7,8,-tetrahydronaphthalen-2-yl)-2-propen-1-one (**1**) and 1-(4'-methoxyphenyl)-3-phenylprop-2-en-1-one (**2**), were synthesized via the base-catalyzed Claisen–Schmidt condensation of 4-methoxyacetophenone with 5,6,7,8-tetrahydronaphthalene-2-carbaldehyde and benzaldehyde, respectively. Likewise 3-phenyl-1-(5,6,7,8-tetrahydronaphthalene-2-yl)prop-2-en-1-one (**3**) was obtained by the condensation of 1-(5,6,7,8-tetrahydronaphthalen-2-yl)ethanone with benzalde-

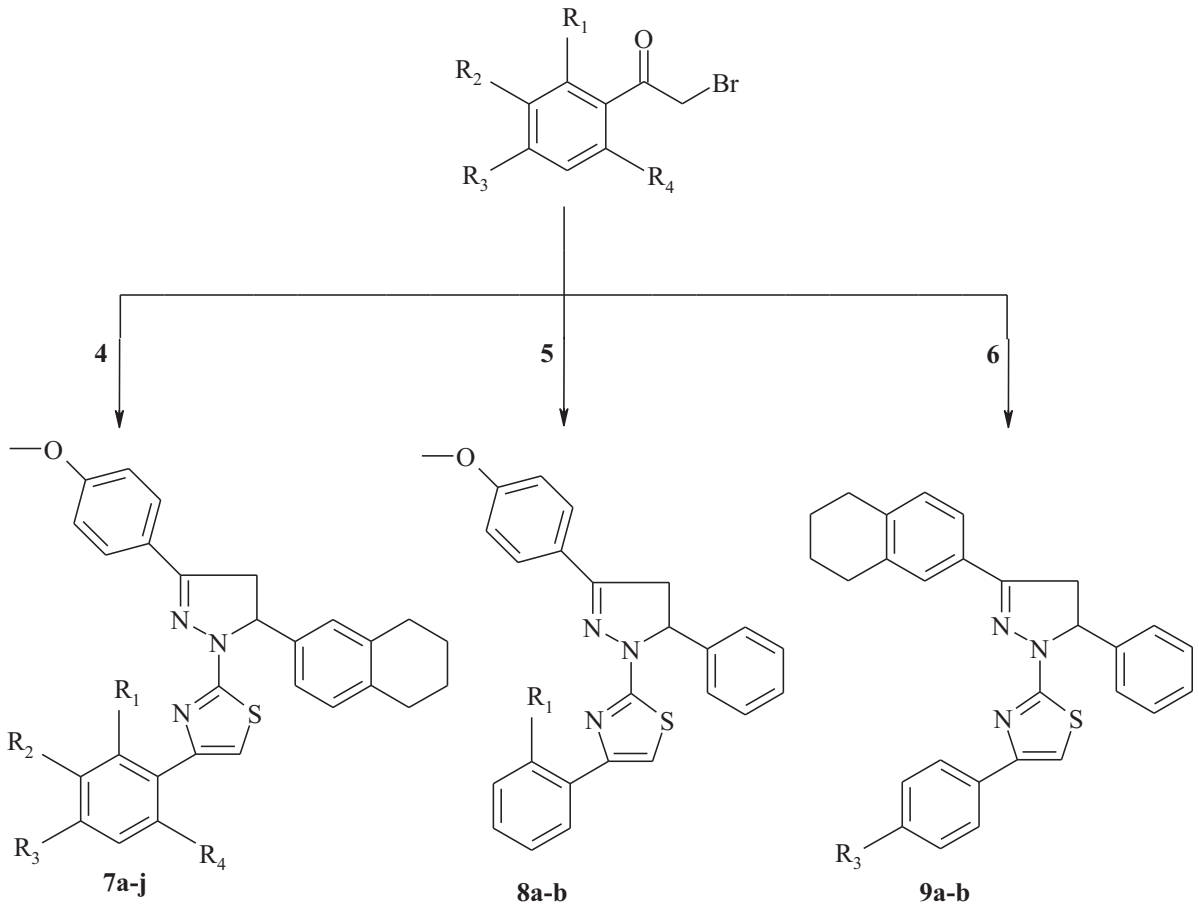
hyde. Secondly, the cyclization of chalcones (**1**–**3**) with thiosemicarbazide in the presence of sodium hydroxide led to 3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-1-thiocarbamoyl-2-pyrazoline (**4**), 3-(4'-methoxyphenyl)-5-phenyl-1-thiocarbamoyl-2-pyrazoline (**5**), and 5-phenyl-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-1-thiocarbamoyl-2-pyrazoline (**6**), respectively. Finally, reactions of **4**, **5**, and **6** with phenacylbromide derivatives gave compounds **7a**–**7j**, **8a**, **8b**, **9a**, and **9b** (Table 1).



a: 5,6,7,8-Tetrahydronaphthalene-2-carbaldehyde / NaOH, **b**: Thiosemicarbazide / NaOH,
c: Benzaldehyde / NaOH

Scheme 1. Synthesis of intermediate compounds.

The structures of the compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, and MS spectral data. The spectral analysis for intermediate original compound **4** was given, but the spectral analysis for compounds **5** and **6**, which were examined in previous studies, was not given.[19,25] In the IR spectra of the final compounds **7a**–**7j**, **8a**, **8b**, **9a**, and **9b**, C=N and C=C stretching vibrations were observed in the region 1630–1450 cm^{-1}. The aromatic C–H stretching vibrations gave rise to bands at 3117–3015 cm^{-1}.



Scheme 2. Synthesis of title compounds.

Table 1. Some properties of the compounds.

Compounds	R_1	R_2	R_3	R_4	Molecular formula	Yield (%)	Mp (°C)
7a	H	H	F	H	$C_{29}H_{26}FN_3OS$	89	192
7b	H	H	Cl	H	$C_{29}H_{26}ClN_3OS$	91	193
7c	H	H	Br	H	$C_{29}H_{26}BrN_3OS$	87	187
7d	H	H	CH_3	H	$C_{30}H_{29}N_3OS$	82	178
7e	H	H	OCH_3	H	$C_{30}H_{29}N_3O_2S$	80	172
7f	H	H	NO_2	H	$C_{29}H_{26}N_4O_3S$	91	221
7g	H	Cl	Cl	H	$C_{29}H_{25}Cl_2N_3OS$	80	171
7h	H	O–CH_2–O		H	$C_{30}H_{27}N_3O_3S$	85	194
7i	H	NO_2	H	H	$C_{29}H_{26}N_4O_3S$	84	170
7j	OCH_3	H	H	OCH_3	$C_{31}H_{31}N_3O_3S$	88	164
8a	H	H	H	H	$C_{25}H_{21}N_3OS$	82	213
8b	OH	H	H	H	$C_{25}H_{21}N_3O_2S$	80	212
9a	H	H	Cl	H	$C_{28}H_{26}ClN_3S$	80	176
9b	H	H	CH_3	H	$C_{29}H_{27}N_3S$	78	185

In the ^{1}H NMR spectra, C_6 and C_7 protons resonated as multiplets at δ 1.77–1.83 ppm and C_5 and C_8 protons at 2.70–2.80 ppm, corresponding to tetrahydronaphthalenes; the methylene of the pyrazoline ring

resonated as a pair of doublets of doublets at δ 3.30–3.40 ppm (H_A) and 3.85–3.92 ppm (H_B). The CH proton (H_X) at position 5 of the pyrazoline ring appeared as a doublet of doublets or a broad signal at δ 5.60–5.68 ppm due to vicinal coupling with the two magnetically nonequivalent protons of the methylene group at position 4 of pyrazoline (Figure). All the other aromatic and aliphatic protons were observed at expected regions.

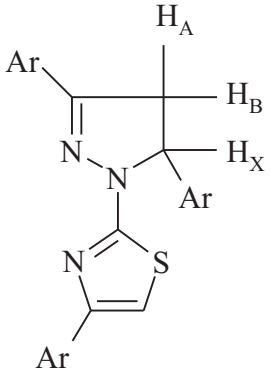


Figure. ABX system of pyrazoline ring.

^{13}C NMR chemical shift values of the carbon atoms at 43.61–44.62 ppm (pyrazoline C_4), 64.42–64.88 ppm (pyrazoline C_5), and about 148.20–161.10 ppm (pyrazoline C_3) corroborate the 2-pyrazoline character deduced from the ^{1}H NMR data. All the other aromatic and aliphatic carbon atoms were observed at expected regions.

The mass spectra (EIMS) of the compounds (**7a**–**7j**, **8a**, **8b**, **9a**, and **9b**) are also in agreement with their molecular formula.

2.2. Biology

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. When compared with chloramphenicol (MIC = 200 μg/mL), all of the compounds and chloramphenicol showed the same level of activity against *K. pneumoniae* (ATCC-700603) and *P. aeruginosa* (ATCC-27853) (Table 2). A similar result was obtained from *E. coli* (ATCC-25923): compound **7c** showed the same level of activity when compared with chloramphenicol. When compared with ketoconazole (MIC = 2 and 3.125 μg/mL), all of the compounds showed low activity against the tested fungi.

The necessity when generating a chemotherapeutic agent is to show minimal or no side-effects on healthy cells in patients receiving chemotherapy. The cytotoxic activities of these compounds were evaluated against a normal mouse embryonic fibroblast cell line, NIH/3T3, for determining the selectivity of potential antimicrobial agents.

When we evaluated the effects of the synthesized compounds against the NIH/3T3 cell line (healthy), most of the compounds were found to have higher IC_{50} values (Table 2) than their effective doses (MIC = 200 μg/mL), which were also the same as the positive control, chloramphenicol, against *K. pneumoniae* and *P. aeruginosa*. Thus, they may be regarded as nontoxic at their effective antibacterial doses. Only compounds **8a** and **8b** exhibited antimicrobial activity with MIC values lower than their IC_{50} values against *K. pneumonia* and *P. aeruginosa* as a result of cytotoxicity.

Table 2. Antimicrobial activity and cytotoxicity of the compounds (μg/mL).

	A	B	C	D	E	F	G	H	I	J	K	L	Cyt.
7a	400	400	400	400	200	200	400	400	200	200	100	100	> 500
7b	400	400	400	400	200	200	400	400	200	200	100	100	> 500
7c	400	400	400	400	200	200	400	200	200	200	100	100	> 500
7d	400	400	400	400	200	200	400	400	200	200	100	100	> 500
7e	400	400	400	400	200	200	400	400	200	200	100	100	> 500
7f	400	400	400	400	200	200	400	400	200	200	100	100	> 500
7g	400	400	400	400	200	200	400	400	200	200	100	100	> 500
7h	400	400	400	400	200	200	400	400	200	200	100	100	> 500
7i	400	400	400	400	200	200	400	400	200	200	100	100	> 500
7j	400	400	400	400	200	200	400	400	200	200	100	100	> 500
8a	400	400	400	400	200	200	400	400	200	200	100	100	301.63
8b	400	400	400	400	200	200	400	400	200	200	100	100	371.60
9a	400	400	400	400	200	200	400	400	200	200	100	100	> 500
9b	400	400	400	400	200	200	400	400	200	200	100	100	> 500
Ref.1	6.25	25	200	25	200	200	200	200	-	-	-	-	-
Ref.2	-	-	-	-	-	-	-	-	2	2	3.125	3.125	-

A: *S. aureus*, **B:** *E. faecalis* (ATCC-29212), **C:** *E. faecalis* (ATCC-51922), **D:** *L. monocytogenes,* **E:** *K. pneumonia,* **F:** *P. aeruginosa,* **G:** *E. coli* (ATCC-35218), **H:** *E. coli* (ATCC-25923), **I:** *C. albicans,* **J:** *C. glabrata*, **K:** *C. krusei*, **L:** *C. parapsilopsis*,

Ref.1: Chloramphenicol, **Ref.2:** Ketoconazole, **Cyt (**Cytotoxicity**):** IC_{50} values for cell lines (NIH3T3)

3. Conclusion

All the synthesized compounds showed antibacterial activity against *K. pneumoniae* and *P. aeruginosa*, with a MIC value of 200 μg/mL. They did not show any cytotoxicity against fibroblasts. The results mentioned above suggest that thiazolylpyrazolines have potential as antibacterial compounds that are worth being investigated further for the development of new drugs to treat infectious diseases.

4. Experimental

4.1. General remarks

All chemicals were purchased from commercial suppliers and used without purification. Melting point (mp) was determined on an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected. Spectroscopic data were recorded with the following instruments: IR, Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan); NMR, Bruker 500 MHz spectrometer in $CDCl_3$ using TMS internal standard; and MS, LC/MS/MS Mass Spectrometer (3200 Q TRAP, AB Sciex Instruments, USA).

4.2. Chemistry

Chalcones (**1**, **2**, **3**): All chalcone derivatives were synthesized according to the literature.[19,25]

4.2.1. General procedure for the synthesis of the intermediate compounds (4, 5, 6)

A mixture of chalcone (0.01 mol), thiosemicarbazide (0.012 mol), and sodium hydroxide (0.01 mol) was refluxed in ethanol (30 mL) for 8 h. The solution was poured into crushed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol.

4.2.1.1 3-(4'-Methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-1-thiocarbamoyl-2-pyrazoline (4)

Yield: 87%, mp 230 °C. IR ν_{max} (cm^{-1}): 3470.7, 3375.2 (N–H stretching), 1560.9, 1510.4, 1467.5 (C=N and C=C stretching), 1210.2, 1168.0, 1095.5, 1010.0 (C–N stretching and aromatic C–H bending). ^{1}H NMR (500 MHz, $CDCl_3$)δ (ppm): 1.72–1.83 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.70–2.78 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.20 (1H, dd, J = 17.5 Hz, J = 3.4 Hz, pyrazoline C_4–H), 3.74 (1H, dd, J = 17.41 Hz, J = 11.31 Hz, pyrazoline C_4–H), 3.88 (3H, s, OCH_3), 5.99 (1H, dd, J = 11.12 Hz, J = 2.68 Hz, pyrazoline C_5–H), 6.91 (1H, s, Ar–H), 6.95 (3H, d, J = 8.86 Hz, Ar–H), 7.03 (1H, d, J = 7.86 Hz, Ar–H), 7.70 (2H, d, J = 8.82 Hz, Ar–H).

4.2.2. General procedure for compounds 7a–7j

A mixture of 3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-1-thiocarbamoyl-2-pyrazoline (**4**) (0.001 mol) and appropriate 2-bromoacetophenone derivative (0.001 mol) was refluxed in ethanol (20 mL) for 4 h. The reaction mixture was cooled and filtered.

4.2.2.1. 4-(4'-Fluorophenyl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7a)

IR ν_{max} (cm^{-1}): 3125.7, 3045.3 (aromatic C–H), 1620.9, 1500.3 (C=N and C=C stretching), 1220.4, 1170.1, 1055.2 (C–N stretching and aromatic C–H bending). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.75–1.86 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.73–2.81 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.35 (1H , dd, J = 17.37 Hz, J = 6.42 Hz, pyrazoline C_4–H), 3.89 (1H, dd, J = 16.75 Hz, J = 12.90 Hz, pyrazoline C_4–H), 3.90 (3H, s, OCH_3), 5.62–5.73 (1H, br, pyrazoline C_5–H), 6.73 (1H, s, thiazole–H), 6.97 (2H, d, J = 8.83 Hz, Ar–H), 7.02 (2H, d, J = 8.71 Hz, Ar–H), 7.08 (2H, d, J = 8.37 Hz, Ar–H), 7.17 (1H, d, J = 6.52 Hz, Ar–H), 7.70–7.74 (2H, m, Ar–H), 7.75 (2H, d, J = 8.71 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 23.16 ($2CH_2$), 29.15 (CH_2), 29.48 (CH_2), 43.71 (pyrazoline C_4), 55.41 ($O\underline{C}H_3$), 64.43 (pyrazoline C_5), 102.46 (thiazole C_5), 114.17, 115.32, 128.09, 129.44 (2CH, Ar–C), 124.14, 130.89, 136.79, 137.40, 138.65 (Ar–C), 123.80 127.61, 127.72 (Ar–CH), 157.37 (thiazole C_2), 161.10 (pyrazoline C_3), 161.39 ($\underline{C}$–OCH_3), 163.35 (C–F), 165.27 (S–C=N).

For $C_{29}H_{26}FN_3OS$ calculated: (%) C 72.03, H 5.42, N 8.69; found: (%) C 72.08, H 5.38, N 8.56.

MS $[M+1]^+$: m/z 484

4.2.2.2. 4-(4'-Chlorophenyl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7b)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.75–1.86 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.73–2.81 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.35 (1H, dd, J = 17.37 Hz, J = 6.42 Hz, pyrazoline C_4–H), 3.72–3.76 (1H, dd, J = 16.75 Hz, J = 12.90 Hz, pyrazoline C_4–H), 3.90 (3H, s, OCH_3), 5.62–5.73 (1H, br, pyrazoline C_5–H), 6.79 (1H, s, thiazole–H), 6.97 (2H, d, J = 8.83 Hz, Ar–H), 7.07 (1H, d, J = 8.38 Hz, Ar–H), 7.18 (2H, br, Ar–H), 7.32 (2H, d, J = 8.55 Hz, Ar–H), 7.68 (2H, d, J = 8.54 Hz, Ar–H), 7.74 (2H, d, J = 8.89 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 23.16 ($2CH_2$), 29.16 (CH_2), 29.49 (CH_2), 43.67 (pyrazoline C_4), 55.41 ($O\underline{C}H_3$), 64.43 (pyrazoline C_5), 103.35 (thiazole C_5), 114.17, 127.26, 128.07, 128.52 (2CH, Ar–C),

123.82, 127.68, 129.28 (Ar–CH), 124.16, 133.07, 136.79, 137.38, 138.69 (Ar–C), 149.94 (thiazole C_2), 152.15 (pyrazoline C_3), 161.08 ($\underline{C}$-OCH_3), 165.27 (S–C=N).

MS $[M+1]^+$: m/z 500

4.2.2.3. 4-(4'-Bromophenyl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7c)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.76–1.85 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.73–2.82 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.36 (1H, dd, J = 17.39 Hz, J = 6.57 Hz, pyrazoline C_4–H), 3.87 (1H, dd, J = 17.39 Hz, J = 11.89 Hz, pyrazoline C_4–H), 3.88 (3H, s, OCH_3), 5.58–5.67 (1H, br, pyrazoline C_5–H), 6.80 (1H, s, thiazole–H), 6.97 (2H, d, J = 8.91 Hz, Ar–H), 7.06 (1H, d, J = 8.39 Hz, Ar–H), 7.17 (2H, m, Ar–H), 7.47 (2H, d, J = 8.60 Hz, Ar–H), 7.62 (2H, d, J = 8.54 Hz, Ar–H), 7.74 (2H, d, J = 8.87 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 23.16 ($2CH_2$), 29.16 (CH_2), 29.49 (CH_2), 43.67 (pyrazoline C_4), 55.41 ($O\underline{C}H_3$), 64.44 (pyrazoline C_5), 103.48 (thiazole C_5), 114.17, 127.57, 128.07, 131.46 (2CH, Ar-C), 121.29, 133.76, 136.79, 137.38, 138.66 (Ar–C), 123.82,127.68, 129.45 (Ar–CH), 149.90 (thiazole C_2), 152.23 (pyrazoline C_3), 161.08 ($\underline{C}$–OCH_3), 165.27 (S–C=N).

MS $[M+1]^+$: m/z 546

4.2.2.4. 4-(4'-Methylphenyl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7d)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.75–1.85 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.38 (3H, s, CH_3), 2.72–2.84 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.35 (1H, dd, J = 17.36 Hz, J = 6.55 Hz, pyrazoline C_4–H), 3.86 (1H, dd, J = 17.35 Hz, i=11.91 Hz, pyrazoline C_4–H), 3.88 (3H, s, OCH_3), 5.59–5.68 (1H, br, pyrazoline C_5–H), 6.76 (1H, s, thiazole–H), 6.97 (2H, d, J = 8.91 Hz, Ar–H), 7.06 (1H, d, J = 8.45 Hz, Ar–H), 7.17 (2H, d, J = 8.14 Hz, Ar–H), 7.18 (1H, s, Ar–H), 7.19 (1H, d, J = 6.52 Hz, Ar–H), 7.65 (2H, d, J = 8.11 Hz, Ar–H), 7.74 (2H, d, J = 8.88 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 21.25 (CH_3), 23.18 ($2CH_2$), 29.47 (CH_2), 29.73 (CH_2), 43.61 (pyrazoline C_4), 55.40 ($O\underline{C}H_3$), 64.42 (pyrazoline C_5), 102.19 (thiazole C_5), 114.15, 123.86, 125.93, 129.41 (2CH, Ar–C), 124.27, 132.06, 136.69, 137.33, 138.76 (Ar–C), 127.72, 128.04, 129.41 (Ar–CH), 151.05 (thiazole C_2), 152.02 (pyrazoline C_3), 161.01 ($\underline{C}$-OCH_3), 165.14 (S–C=N).

MS $[M+1]^+$: m/z 480

4.2.2.5. 4-(4'-Methoxyphenyl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7e)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.74–1.85 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.70–2.84 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.35 (1H, dd, J = 17.37 Hz, J = 6.36 Hz, pyrazoline C_4–H), 3.84 (3H, s, OCH_3), 3.87 (1H, dd, J = 17.50 Hz, J = 10.00 Hz, pyrazoline C_4–H), 3.8 (3H, s, OCH_3), 5.60–5.83 (1H, br, pyrazoline C_5–H), 6.67 (1H, s, thiazole–H), 6.90 (2H, d, J = 8.80 Hz, Ar–H), 6.97 (2H, d, J = 8.84 Hz, Ar–H), 7.06 (1H, d, J = 8.20 Hz, Ar–H), 7.18 (1H, s, Ar–H), 7.19 (1H, d, J = 7.00 Hz, Ar–H), 7.70 (2H, d, J = 8.79 Hz, Ar–H), 7.74 (2H, d, J = 8.84 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 23.15 ($2CH_2$), 29.16 (CH_2), 29.47 (CH_2), 43.76 (pyrazoline C_4), 55.30 ($O\underline{C}H_3$), 55.41 ($O\underline{C}H_3$), 64.43 (pyrazoline C_5), 101.00 (thiazole C_5), 113.84, 114.18, 123.84, 129.45 (2CH, Ar–C), 127.41, 127.57, 128.18 (Ar–CH), 124.06, 129.36, 136.81, 137.44, 138.55 (Ar–C), 156.41 (thiazole C_2), 159.32 (pyrazoline C_3), 161.08 ($\underline{C}$–OCH_3), 161.18 ($\underline{C}$–OCH_3), 165.09 (S–C=N).

MS $[M+1]^+$: m/z 496

4.2.2.6. 4-(4'-Nitrophenyl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7f)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.77–1.84 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.74–2.82 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.40 (1H, dd, J = 17.43 Hz, J = 6.15 Hz, pyrazoline C_4–H), 3.89 (3H, s, OCH_3), 3.93 (1H, dd, J = 17.43 Hz, J = 11.76 Hz, pyrazoline C_4–H), 5.76–5.85 (1H, br, pyrazoline C_5–H), 6.98 (2H, d, J = 8.87 Hz, Ar–H), 7.01 (1H, s, thiazole–H), 7.08 (1H, d, J = 8.35 Hz, Ar–H), 7.18 (1H, s, Ar–H), 7.19 (1H, d, J = 6.86 Hz, Ar–H), 7.76 (2H, d, J = 8.88 Hz, Ar–H), 7.89 (2H, d, J = 8.91 Hz, Ar–H), 8.22 (2H, d, J = 8.95 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 23.11 ($2CH_2$), 29.15 (CH_2), 29.50 (CH_2), 43.90 (pyrazoline C_4), 55.44 ($O\underline{C}H_3$), 64.47 (pyrazoline C_5), 106.91 (thiazole C_5), 114.25, 123.93, 126.55, 128.27 (2CH, Ar–C), 123.76, 127.55, 129.56 (Ar–CH), 123.40, 137.09, 137.57, 138.12, 140.04 (Ar–C), 146.86 (thiazole C_2), 148.20 (pyrazoline C_3), 161.38 ($\underline{C}$–OCH_3), 165.37 (S–C=N).

MS $[M+1]^+$: m/z 511

4.2.2.7. 4-(3',4'-Dichlorophenyl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7g)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.75–1.86 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.70–2.80 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.36 (1H, dd, J = 17.44 Hz, J = 6.77 Hz, pyrazoline C_4–H), 3.87 (1H, dd, J = 17.50 Hz, J = 10.00 Hz, pyrazoline C_4–H), 3.88 (3H, s, OCH_3), 5.19–5.36 (1H, br, pyrazoline C_5–H), 6.79 (1H, s, thiazole–H), 6.97 (2H, d, J = 8.84 Hz, Ar–H), 7.07 (1H, d, J = 8.00 Hz, Ar–H), 7.15 (1H, dd, J = 9.5 Hz, J = 1.62 Hz, Ar–H), 7.19 (1H, s, Ar–H), 7.38 (1H, d, J = 8.40 Hz, Ar–H), 7.52 (1H, dd, J = 8.38 Hz, J = 2.02 Hz, Ar–H), 7.73 (2H, d, J = 8.85 Hz, Ar–H), 7.81 (1H, d, J = 1.98 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 23.14 ($2CH_2$), 29.15 (CH_2), 29.45 (CH_2), 43.67 (pyrazoline C_4), 55.40 ($O\underline{C}H_3$), 64.54 (pyrazoline C_5), 104.31 (thiazole C_5), 114.18, 128.08 (2CH, Ar–C), 123.73, 124.96, 127.89, 128.04, 129.53, 130.24 (Ar–CH), 124.06, 129.57, 131.01, 132.46, 136.89, 137.41, 138.47 (Ar–C), 148.66 (thiazole C_2), 152.47 (pyrazoline C_3), 161.13 ($\underline{C}$–OCH_3), 165.28 (S–C=N).

MS $[M+1]^+$: m/z 534

4.2.2.8. 4-(Benzo[1,3]dioxol-5-yl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7h)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.71–1.81 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.68–2.80 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.45 (1H, dd, J = 17.55 Hz, J = 3.55 Hz, pyrazoline C_4–H), 3.90 (3H, s, OCH_3), 4.01 (1H, dd, J = 17.57 Hz, J = 6.45 Hz, pyrazoline C_4–H), 5.95–6.02 (1H, br, pyrazoline C_5–H),

6.00 (2H, s, dioxolane), 6.53 (1H, s, thiazole–H), 6.87 (1H, d, J = 8.15 Hz, Ar–H), 7.00 (2H, d, J = 8.85 Hz, Ar–H), 7.05 (1H, d, J = 7.93 Hz, Ar–H), 7.22 (1H, s, Ar–H), 7.25 (1H, s, Ar–H), 7.41 (1H, d, J = 8.12 Hz, Ar–H), 7.67 (1H, d, J = 8.81 Hz, Ar–H), 7.78 (2H, d, J = 9.23 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 22.99 ($2CH_2$), 29.17 (CH_2), 29.43 (CH_2), 44.62 (pyrazoline C_4), 55.51 ($O\underline{C}H_3$), 64.57 (pyrazoline C_5), 101.43 (thiazole C_5), 107.16 (CH_2–dioxolane), 114.42, 123.74 (2CH, Ar–C), 108.62, 113.99, 123.64, 127.22, 129.18, 129.88 (Ar–CH), 121.64, 128.46, 134.37, 136.91, 138.07 (Ar–C), 144.34 (thiazole C_2), 147.98, 148.73 (2C–dioxolane), 149.84 (pyrazoline C_3), 162.37 ($\underline{C}$–OCH_3), 164.64 (S–C=N).

MS $[M+1]^+$: m/z 510

4.2.2.9. 4-(3'-Nitrophenyl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7i)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.75–1.85 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.73–2.89 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.39 (1H, dd, J = 17.43 Hz, J = 6.69 Hz, pyrazoline C_4–H), 3.90 (1H, dd, J = 17.37 Hz, J = 11.94 Hz, pyrazoline C_4–H), 3.89 (3H, s, OCH_3), 5.57–5.67 (1H, br, pyrazoline C_5–H), 6.94 (1H, s, thiazole–H), 6.99 (2H, d, J = 8.94 Hz, Ar–H), 7.10 (1H, d, J = 8.37 Hz, Ar–H), 7.21 (1H, d, J = 6.76 Hz, Ar–H), 7.22 (1H, s, Ar–H), 7.50 (1H, t, J = 7.97 Hz, Ar–H), 7.75 (2H, d, J = 8.84 Hz, Ar–H), 8.03 (1H, d, J = 7.82 Hz, Ar–H), 7.74 (1H, dd, J = 8.15 Hz, J = 1.4 Hz, Ar–H), 8.59 (1H, s, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 23.14 ($2CH_2$), 29.17 (CH_2), 29.43 (CH_2), 43.75 (pyrazoline C_4), 55.42 ($O\underline{C}H_3$), 64.56 (pyrazoline C_5), 105.13 (thiazole C_5), 114.20, 128.10 (2CH, Ar–C), 121.05, 121.92, 123.93, 127.71, 129.21, 129.57, 131.43 (Ar–CH), 124.04, 136.49, 136.96, 137.57, 138.52 (Ar–C), 148.59 (C–NO_2), 148.72 (thiazole C_2), 152.53 (pyrazoline C_3), 161.17 ($\underline{C}$–OCH_3), 165.44 (S–C=N).

MS $[M+1]^+$: m/z 511

4.2.2.10. 4-(2',5'-Dimethoxyphenyl)-2-[3-(4'-methoxyphenyl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]thiazole (7j)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.74–1.85 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.69–2.82 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.33 (1H, dd, J = 17.43 Hz, J = 7.06 Hz, pyrazoline C_4–H), 3.80 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.88 (1H, dd, J = 15.00 Hz, J = 10.00 Hz, pyrazoline C_4–H), 3.89 (3H, s, OCH_3), 5.57–5.81 (1H, br, pyrazoline C_5–H), 6.79 (1H, dd, J = 8.87 Hz, J = 3.03 Hz, Ar–H), 6.85 (1H, s, thiazole–H), 6.97 (2H, d, J = 8.94 Hz, Ar–H), 7.04 (1H, d, J = 7.83 Hz, Ar–H), 7.19 (1H, s, Ar–H), 7.21 (1H, d, J = 8.00 Hz, Ar–H), 7.35 (1H, m, Ar–H), 7.58 (1H, d, J = 3.03 Hz, Ar–H), 7.74 (2H, d, J = 8.60 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 23.16 ($2CH_2$), 29.17 (CH_2), 29.42 (CH_2), 43.92 (pyrazoline C_4), 55.41 ($O\underline{C}H_3$), 55.69 ($O\underline{C}H_3$), 56.03 ($O\underline{C}H_3$), 64.83 (pyrazoline C_5), 108.21 (thiazole C_5), 114.16, 129.35 (2CH, Ar–C), 112.42, 114.44, 123.92, 127.33, 128.06 (Ar–CH), 124.20, 136.61, 137.44, 138.95 (Ar–C), 151.33 (thiazole C_2), 153.53 (pyrazoline C_3), 161.06 ($\underline{C}$–OCH_3), 163.53 (S–C=N).

MS $[M+1]^+$: m/z 526

4.2.3. General procedure for compounds 8a, 8b

A mixture of 3-(4'-methoxyphenyl)-5-phenyl-2-pyrazolin-1-carbothioamide (**5**) (0.001 mol) and 2-bromoacetophenone (0.001 mol) in ethanol (20 mL) was refluxed for 4 h. The reaction mixture was cooled and filtered.

4.2.3.1. 2-[3-(4'-Methoxyphenyl)-5-phenyl-2-pyrazolin-1-yl]-4-phenylthiazole (8a)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.36 (1H, dd, J = 17.34 Hz, J = 6.36 Hz, pyrazoline C_4–H), 3.89 (3H, s, OCH_3), 3.93 (1H, dd, J = 17.34 Hz, J = 11.87 Hz, pyrazoline C_4–H), 5.78–5.89 (1H, br, pyrazoline C_5–H), 6.81 (1H, s, thiazole–H), 6.98 (2H, d, J = 8.91 Hz, Ar–H), 7.27–7.42 (6H, m, Ar–H), 7.48 (2H, d, J = 8.37 Hz, Ar–H), 7.71 (2H, d, J = 8.47 Hz, Ar–H), 7.75 (2H, d, J = 8.89 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 43.80 (pyrazoline C_4), 55.42 ($O\underline{C}H_3$), 64.62 (pyrazoline C_5), 103.02 (thiazole C_5), 114.20, 126.01, 126.67, 128.13, 128.43, 128.73 (2CH, Ar–C), 126.55, 127.78 (CH), 124.00, 127.66, 141.54 (C), 145 (thiazole C_2), 150.05 (pyrazoline C_3), 161.18 ($\underline{C}$–OCH_3), 165.12 (S–C=N).

MS $[M+1]^+$: m/z 412

4.2.3.2. 2-[2-(3-(4'-Methoxyphenyl)-5-phenyl-2-pyrazolin-1-yl]thiazol-4-yl]phenol (8b)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.33 (1H, dd, J = 17.47 Hz, J = 7.06 Hz, pyrazoline C_4–H), 3.88 (3H, s, OCH_3), 3.94 (1H, dd, J = 17.47 Hz, J = 11.83 Hz, pyrazoline C_4–H), 5.56 (1H, dd, J = 11.79 Hz, J = 7.00 Hz, pyrazoline C_5–H), 6.80 (1H, two d, J = 7.10 Hz, J = 1.18 Hz, Ar–H), 6.81 (1H, s, thiazole–H), 6.91 (1H, d, J = 8.17 Hz, Ar–H), 6.98 (2H, d, J = 8.89 Hz, Ar–H), 7.15 (1H, two d, J = 7.23 Hz, J = 1.61 Hz, Ar–H), 7.33 (2H, d, J = 8.88 Hz, Ar–H), 7.35 (1H, m, Ar–H), 7.42 (4H, m, Ar–H), 7.49 (1H, dd, J = 7.84 Hz, J = 1.56 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 44.44 (pyrazoline C_4), 55.43 ($O\underline{C}H_3$), 64.88 (pyrazoline C_5), 101.66 (thiazole C_5), 114.27, 126.10, 128.24, 129.31 (2CH, Ar–C), 117.71, 119.12, 125.80, 128.32, 129.54 (CH), 123.59, 125.93, 140.67 (C), 148.78 (thiazole C_2), 153.19 (pyrazoline C_3), 155.73 ($\underline{C}$–OH), 161.40 ($\underline{C}$–OCH_3), 164.82 (S–C=N).

MS $[M+1]^+$: m/z 428

4.2.4. General procedure for compounds 9a, 9b

A mixture 5-phenyl-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-carbothioamide (**6**) and 2-bromoacetophenone (0.001 mol) in ethanol (20 mL) was refluxed for 4 h. The reaction mixture was cooled and filtered.

4.2.4.1. 2-[5-Phenyl-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]-4-(4'-chlorophenyl)] thiazole (9a)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.80–1.91 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.79–2.90 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.36 (1H, dd, J = 17.42 Hz, J = 6.42 Hz, pyrazoline C_4–H), 3.92 (1H, dd, J = 17.42 Hz, J = 11.92 Hz, pyrazoline C_4–H), 5.72–5.84 (1H, br, pyrazoline C_5–H), 6.80 (1H, s, thiazole–H), 7.15 (1H, d, J = 7.97 Hz, Ar–H), 7.29 (1H, m, Ar–H), 7.30 (2H, d, J = 8.59 Hz, Ar–H), 7.38 (2H, t, J = 7.36 Hz, Ar–H), 7.45 (2H, d, J = 7.32 Hz, Ar–H), 7.48 (1H, s, Ar–H), 7.53 (1H, dd, J = 7.85 Hz, J = 1.18 Hz, Ar–H), 7.62 (2H, d, J = 8.52 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 23.02–23.07 ($2CH_2$– tetrahydronaphthalene), 29.43–29.49 ($2CH_2$–tetrahydronaphthalene), 43.76 (pyrazoline C_4), 64.59 (pyrazoline C_5), 103.47 (thiazole C_5), 126.60, 127.22, 128.56, 128.72 (2CH, Ar–C), 123.67, 127.18, 127.78, 129.54 (CH), 128.47, 129.23, 133.18, 137.61, 139.77, 141.61 (C), 149.81 (thiazole C_2), 152.81 (pyrazoline C_3), 165.14 (S–C=N).

MS $[M+1]^+$: m/z 470

4.2.4.2. 2-[5-Phenyl-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-pyrazolin-1-yl]-4-(4'-methylphenyl)] thiazole (9b)

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.80–1.89 (4H, m, tetrahydronaphthalene $C_{6,7}$–H), 2.36 (3H, s, CH_3), 2.79–2.88 (4H, m, tetrahydronaphthalene $C_{5,8}$–H), 3.36 (1H, dd, J = 17.42 Hz, J = 6.27 Hz, pyrazoline C_4–H), 3.92 (1H, dd, J = 17.40 Hz, J = 11.91 Hz, pyrazoline C_4–H), 5.76–5.92 (1H, br, pyrazoline C_5–H), 6.75 (1H, s, thiazole–H), 7.13 (1H, s, Ar–H) 7.15 (2H, d, J = 8.00 Hz, Ar–H), 7.29 (1H, m, Ar–H), 7.37 (2H, t, J = 7.83 Hz, Ar–H), 7.47 (2H, dd, J = 8.50 Hz, J = 1.15 Hz, Ar–H), 7.48 (1H, s, Ar–H), 7.54 (1H, dd, J = 7.91 Hz, J = 1.6 Hz, Ar–H), 7.60 (2H, d, J= 8.12 Hz, Ar–H).

^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 21.24 (1H, s, CH_3), 23.03–23.07 ($2CH_2$– tetrahydronaphthalene), 29.42–29.49 ($2CH_2$–tetrahydronaphthalene), 43.71 (pyrazoline C_4), 64.58 (pyrazoline C_5), 102.25 (thiazole C_5), 125.92, 126.67, 128.69, 129.11 (2CH, Ar–C), 123.68, 127.19, 127.73, 129.52 (CH), 128.53, 137.39, 137.59, 139.73, 141,70 (C), 149.65 (thiazole C_2), 152.65 (pyrazoline C_3), 164.98 (S–C=N).

MS $[M+1]^+$: m/z 450

4.3. Microbiology

The microbiological assay was carried out according to the CLSI reference M7-A7 broth microdilution method.[26] Chloramphenicol and ketoconazole were used as reference drugs. In the current work, thiazolylpyrazoline derivatives (**7a**–**7j**, **8a**, **8b**, **9a**, and **9b**) were tested for their in vitro antimicrobial activity against *Staphylococcus aureus* (ATCC-25923), *E. faecalis* (ATCC-29212), *E. faecalis* (ATCC-51922), *L. monocytogenes* (ATCC-1911), *K. pneumoniae* (ATCC-700603), *P. aeruginosa* (ATCC-27853), *E. coli* (ATCC-35218), *E. coli* (ATCC-25922), *C. albicans* (ATCC-90028), *C. glabrata* (ATCC-90030), *C. krusei* (ATCC-6258), and *C. parapsilosis* (ATCC-22019) (Table 2).

4.4. Cytotoxicity

Cytotoxicity tests were performed using the MTT assay. The tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] is used to measure the metabolic activity of viable cells. Tetrazolium salts are reduced to formazan by mitochondrial succinate dehydrogenase, an enzyme that is only active in cells with an intact metabolism. The formazan can be quantified photometrically and it is in correlation with the number of viable cells.[27] Cytotoxicity was tested using NIH3T3 (mouse embryonic fibroblast cell line) cells. NIH3T3 cells were incubated in RPMI medium (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) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. NIH3T3 cells were seeded at 10,000 cells into each well of 96-well plates. After 24 h of incubation, the culture media were removed and compounds were added to culture medium in the range between 3.9 and 500 μg mL^{-1} concentrations

with a dilution factor of 2. After 24 h of incubation, 20 μL of MTT solution (5 mg mL^{-1} MTT powder in PBS) was added to each well. After 3 h of incubation at 37 °C, 5% CO_2, contents of the wells were removed and 100 μL of dimethyl sulfoxide (DMSO) was added to each well. Then OD of the plate was read at 540 nm. Inhibition% was calculated for each concentration of the compounds and IC_{50} values were estimated by nonlinear regression analysis. Stock solutions of compounds were prepared in dimethyl sulfoxide (DMSO) and further dilutions were made with fresh culture medium. The final DMSO concentration was under 0.1%. All experiments were performed in triplicate (Table 2).[27]

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