



Synthesis and Biological activities of New Pyrazoline Incorporated Pyridine-Triazole Derivatives

AJAYKUMAR RATHOD^{1*}, AKHIL HADIYA² and SHRUTI BARMEDA¹

¹Department of Chemistry, KSKV Kachchh University, Bhuj (370001) Gujarat, India.

²Department of Chemistry, Saurashtra University, Rajkot (360005), Gujarat, India.

*Corresponding author E-mail: ajayrathod.chem@gmail.com

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ABSTARCT

The potential of pyridine, triazole and pyrazoline scaffolds as anticancer, anticonvulsant, antidiabetic, antioxidant, analgesic, antibacterial, neuroprotective, etc. makes them essential intermediates for new drug discovery. Framing pyrazoline scaffolds with triazole and pyridine is a novel approach to design effective drugs. We have prepared a series of pyrazoline incorporated pyridine-triazole (6a-o) from Isoiniazid. FT-IR, ¹H NMR, ¹³C NMR, and LCMS spectra were used to describe all produced compounds. The antibacterial and radial scavenging screening were carried out through disc diffusion and DPPH, respectively.

Keywords: Pyridine, Triazole, Pyrazoline, Antibacterial, Antifungal, Antioxidant.

INTRODUCTION

The emergence of bacterial resistance to conventional antibiotics has grown to be a worldwide issue, necessitating the continuous seeking out novel antimicrobial agents. In this regard, heterocycle's diverse biological activities and structural versatility have drawn significant interest.¹⁻³ Among various heterocyclic scaffolds, pyridine, 1,2,4-triazole, and 4,5-dihydro-pyrazole have demonstrated remarkable potential in medicinal chemistry,^{4,5} particularly in the development of antimicrobial agents.⁶

Pyridine is a fundamental nitrogen-containing heterocycle widely found in bioactive molecules. Its unique electronic properties and ability to participate in hydrogen bonding make it

a crucial pharmacophore in drug design. Pyridine derivatives possess antibacterial activity,⁷⁻⁹ making them promising candidates for the development of new antimicrobial agents.

Similarly, 1,2,4-triazole is an important five-membered heterocyclic ring known for its therapeutic potential.¹⁰⁻¹² The presence of nitrogen atoms in the triazole ring enhances its binding interactions with biological targets, improving its pharmacokinetic and pharmacodynamic properties.¹³⁻¹⁵ Triazole derivatives have been extensively studied for their role in inhibiting bacterial growth and overcoming resistance mechanisms.

Furthermore, the 4,5-dihydro-pyrazole moiety has been widely explored in medicinal



chemistry due to its significant biological activities. Dihydro-pyrazole derivatives are known for their antimicrobial, anti-inflammatory, and anticancer properties.¹⁶⁻¹⁸ Their ability to interact with bacterial enzymes and disrupt essential metabolic pathways makes them promising leads in the development of antibacterial agents.

Currently, our team have designed and prepared novel compounds incorporating pyridine, 1,2,4-triazole, and 4,5-dihydro-pyrazole scaffolds to evaluate their antibacterial potential. The efficacy of the newly developed derivatives was assessed by antimicrobial screening. Our findings contribute to the ongoing efforts in developing new antimicrobial agents to combat resistant bacterial strains.

MATERIALS AND METHODS

Sigma Aldrich is the source of all Initial materials. The Perkin-Elmer RX1 spectrophotometer was used to record the infrared spectra. Using a Bruker 500 MHz spectrometer, the ¹HNMR and ¹³CNMR spectra were captured in CDCl₃ solutions. The ElementarVario EL III elemental analyzer was used to perform the elemental analysis. Progress of reaction was recorded by TLC.

General procedure

Synthesis of 1,2,4-triazol-3-thiol derivative(3):

1,2,4-triazol-3-thiol was prepared as per reported method.¹⁹ Isoniazid (20 mmol) and ethyl isothiocyanate (20 mmol) in methanol were reflux for 2 hours. The resultant reaction mixture was poured into cold water to isolate compound 2. Compound 2 was solubilized in 20% NaOH and cyclized through reflux for 3 hours. The reaction mixture was acidified by dil HCl to yield 4-ethyl-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (3). Solids were filtered, dried and recrystallized using ethanol. Yield-72%

Synthesis of Chalcones derivatives(4a-o)

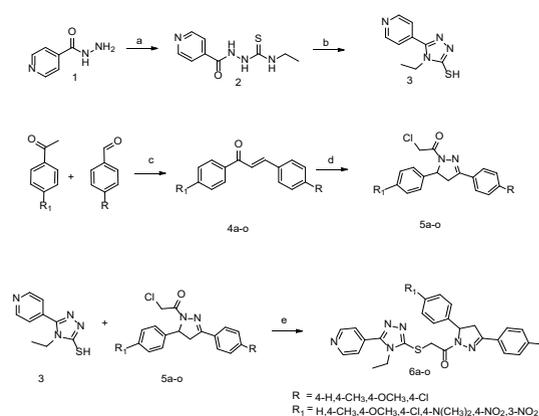
Pyrazolines were prepared as per reported method.^{20,21} acetophenone (50 mmol), various aldehydes (50 mmol), and NaOH (50 mmol) were triturated in a mortar at 300C for 30 minutes. The solid was rinsed with water to remove excess NaOH. The resultant chalcones (4a-4o) were filtered and recrystallized using ethanol. Yield between 80% - 86%.

Synthesis of chloro acetyl pyrazoline derivatives(5a-o)

Compounds (4a-4o) (15 mmol) and hydrazine hydrate (20 mmol) were solubilized in ethanol in FBF. The reaction mixture was subjected to reflux for 11 hours. The solvent was eliminated via distillation to obtain pyrazole. Pyrazoles were subsequently dissolved in dichloromethane. 15 millimoles of chloroacetyl chloride were introduced at 5°C and agitated for seven to eight hours at ambient temperature. Compounds (5a-5o) were obtained via distillation and recrystallized using ethanol.

Synthesis of pyrazoline incorporated pyridine-triazole (6a-o)

4-ethyl-5-(4-pyridinyl)-4H-1,2,4-triazole-3-thiol (0.005 mol) 2-chloro-(5-aryl-3-arylpyrazol-1-yl) ethane-one (0.005 mol) and anhydrous K₂CO₃ were dissolved in 100 mL DMF. Reaction mass was stirred for 8-9 h at ambient temperature. The resultant content was then added in the cold water. Solids were filtered off as well as recrystallized with ethyl alcohol. Yield between 62%-71%.



Reaction conditions

(a) CH₃OH, Ethyl isothiocyanate (b) 20% NaOH (c) Ethanol, aq. NaOH
(d) (1)NH₂NH₂·H₂O,(2) chloro acetyl chloride (f) DMF , K₂CO₃

Scheme 1. Preparation route of derivatives 6a-o

RESULTS AND DISCUSSION

Biological Screening

Compounds 6a–6o have been screened against *S. aureus*, *B. subtilis*, *E. coli*, *E.aerogenes*, *C. albicans* and *A. niger* using disc diffusion method (Fig. 1).²² All new compound exhibit moderate to good potential towards bacterial and fungal strains (Table 1). When compared to conventional antibiotics, several of these substances exhibit superior inhibitory zones, including compound 6l is

quite efficient against *B. subtilis* and *E. coli* and it has the maximum activity against *S. aureus* (36 mm).

Another potent chemical is compound 6e, which exhibits excellent suppression against a variety of pathogens, including a 32 mm zone for *S. aureus*. Compound 6h has displayed antifungal potential with 22 and 20 mm inhibition against *C. albicans* and *A. niger* respectively.

The type of substitution at positions R and R markedly changed the studied compounds' antimicrobial properties. Substitution at R with electron-withdrawing groups like 4-nitro (6e) was greatly increased activity, particularly against *S. aureus* and *E. coli*, as compared to the unsubstituted compound 6a. While to a lesser degree, activity was also enhanced by electron-

donating groups such as 4-methoxy (6d). In general, adding a methyl group at R (6f–6j) increased potency overall, especially when paired with substituents like 3-nitro or 4-chloro at R, as compounds 6g and 6j demonstrated, which showed broad-spectrum action. Strong antibacterial effects were produced by methyl substitution at R (6k–6l), with 6l (R = 4-OCH₃, R = 4-NO₂) showing the greatest activity among all tested bacteria and even coming close to conventional medicines like amoxicillin. Likewise, chloro substitution at R (6m–6o) in conjunction with groups like 3,4-dimethoxy or 4-dimethylamino improved the antifungal and antibacterial properties even further. The antimicrobial profile was often enhanced by the combination of electron-donating and withdrawing groups, with compounds such as 6j, 6l, and 6o exhibiting particularly strong and wide-ranging action.

Table 1: 0Antimicrobial activity of 6a-6o

Compound	Inhibition Zone (millimeters)					
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>E. aerogenes</i>	<i>C. albicans</i>	<i>A. niger</i>
6a	22	19	17	15	18	17
6b	21	16	13	16	15	15
6c	20	18	12	16	17	15
6d	26	21	16	17	18	18
6e	32	21	18	19	19	18
6f	24	19	13	16	15	14
6g	27	20	19	14	14	15
6h	26	19	18	19	22	20
6i	27	20	17	17	20	19
6j	30	21	18	18	21	19
6k	28	19	18	19	21	20
6l	36	20	21	20	19	18
6m	25	17	19	17	18	20
6n	28	18	18	18	21	21
6o	28	19	17	19	23	22
Amoxicillin	40	28	22	24	-	-
Ciprofloxacin	40	36	28	30	-	-
Fluconazole	-	-	-	-	28	26



Fig. 1. Antimicrobial activity of 6a-6o

Antioxidant activities

Radical scavenging activities of compound

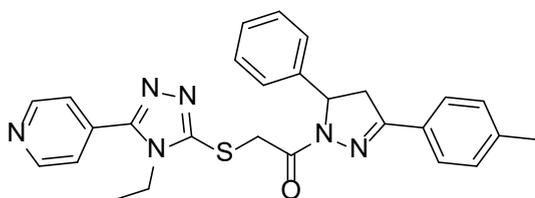
6a-6o have been performed at 50, 100, 150 µg/mL concentration using ascorbic acid by DPPH method.²³ Compound 6l bearing methoxy and nitro substitution has shown highest inhibition with 53% at 150 µg/mL. while comp 6k with methoxy substitution has shown inhibition with 52.46% at 150 µg/mL. all other compound have displayed moderate radical scavenging activities.

Table 2: Antioxidant activities

Conc. (µg/mL)	Compound															
	6a	6b	6c	6d	6e	6f	6g	6h	6i	6j	6k	6l	6m	6n	6o	Ascorbic acid
50	22.5	20.4	21.5	24.5	26.7	24.3	23.5	29.6	24.3	24.7	25.4	25.4	24.6	28.7	26.8	83.4
100	34.6	30.4	34.5	35.6	37.7	30.7	32.1	36.6	37.5	37.7	40.2	40.1	34.9	40.4	38.8	87.28
150	39.7	41.3	44	47.1	50.6	38.8	40.2	49.9	51.3	50.2	52.5	53	45.2	50.5	49.5	88.6

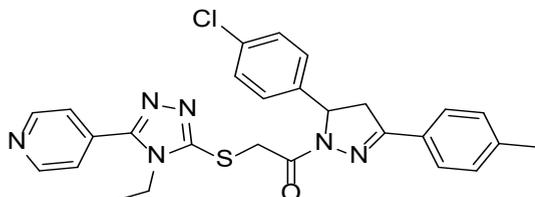
Yellow solid, Molecular Formula: $C_{26}H_{23}N_7O_3S$; FT-IR (cm^{-1} , KBr): 2942, 2981, 3033 (C-H), 1669 (C=O), 1606 (C=C Ar), 1419 (C=N-triazole), 699 (str of S-C); 1H NMR : 1.33 (H, CH_3), 3.11, 3.76 (dd, H, Pyrazoline), 4.06, 4.73 (2H, CH_2), 5.56 (dd, H, CH), 7.24-8.76 (13H, Ar-H); ^{13}C NMR (500 MHz, δ ppm $CDCl_3$): 15.61, 37.31, 40.16, 42.44, 60.48, 122.22, 125.69, 126.87, 127.87, 128.85, 128.97, 130.82, 135.04, 141.01, 144.23, 150.57, 152.11, 153.01, 155.45, 164.81; Mass: M+H 514.

Compound 6f



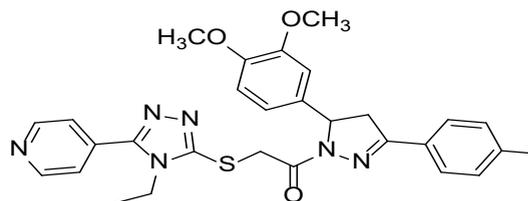
White solid, Molecular Formula: $C_{27}H_{26}N_6OS$; FT-IR (cm^{-1} , KBr): 3032, 2978, 2931 (C-H), 1666 (C=O), 1597 (C=C Ar), 1435 (C=N-triazole), 709 (str of S-C); 1H NMR: 1.33 (H, CH_3), 2.39 (H, CH_3), 3.16, 3.78 (dd, H, Pyrazoline), 4.05, 4.70 (2H, CH_2), 5.55 (dd, H, CH), 7.21-8.75 (13H, Ar-H); ^{13}C NMR (500 MHz, δ ppm $CDCl_3$): 15.62, 21.54, 37.38, 40.15, 42.48, 60.50, 122.27, 125.72, 126.86, 127.85, 128.05, 128.96, 129.52, 135.03, 141.13, 141.25, 150.56, 152.17, 153.02, 155.51, 164.75; Mass: M+H 483.

Compound 6g



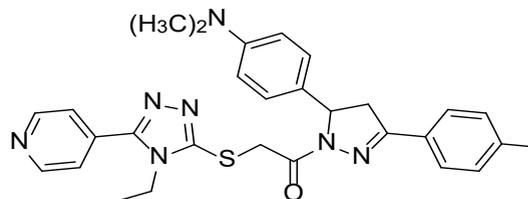
White solid, Molecular Formula: $C_{27}H_{25}ClN_6OS$; FT-IR (cm^{-1} , KBr): 2953, 2977, 3044 (C-H), 1668 (C=O), 1608 (C=C Ar), 1431 (C=N-triazole), 707 (str of S-C); 1H NMR : 1.32 (H, CH_3), 2.40 (H, CH_3), 3.14, 3.79 (dd, H, Pyrazoline), 4.06, 4.71 (2H, CH_2), 5.55 (dd, H, CH), 7.23-8.78 (12H, Ar-H); ^{13}C NMR (500 MHz, δ ppm $CDCl_3$): 15.63, 37.32, 40.15, 42.42, 60.56, 122.25, 125.74, 126.84, 127.84, 128.78, 128.93, 135.06, 141.44, 141.43, 150.56, 152.11, 153.08, 155.46 164.70; Mass: M+H 517.

Compound 6h



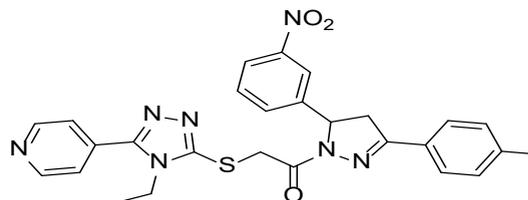
White solid, Molecular Formula: $C_{29}H_{30}N_6O_3S$; FTIR (cm^{-1} , KBr): 2942, 2968, 3024 (C-H) 1675 (C=O amide), 1602 (C=C - aromatic), 1421 (C=N str-triazole), 702 (str of S-C); 1.33 (H, CH_3), 2.41 (H, CH_3) 2.96 (H, OCH_3) 3.12, 3.82 (dd, H, Pyrazoline), 4.04, 4.71 (2H, CH_2), 5.56 (dd, H, CH), 7.23-8.79 (11H, Ar-H); ^{13}C NMR (500 MHz, δ ppm $CDCl_3$): 15.62, 21.59, 37.34, 40.17, 42.46, 55.86, 60.35, 122.28, 125.74, 126.82, 127.88, 128.01, 128.95, 129.49, 135.09, 141.14, 141.38, 150.58, 152.14, 153.04, 155.54, 164.73; Mass: M+H 543

Compound 6i



Yellow solid, M. Formula: $C_{29}H_{31}N_7OS$; FTIR (cm^{-1} , KBr): 2942, 2981, 3043 (C-H), 1666.70 (C=O amide), 1597.60 (C=C-aromatic ring), 1426 (C=N-triazole), 693 (S-C); 1.32, 2.40 (H, CH_3) 2.99 (6H, CH_3) 3.14, 3.81 (dd, H, Pyrazoline), 4.06, 4.75 (2H, CH_2), 5.59 (dd, H, CH), 7.20-8.75 (13H, Ar-H); ^{13}C NMR (500 MHz, δ ppm $CDCl_3$): 15.64, 21.56, 37.39, 40.13, 42.42, 42.55, 60.29, 112.24, 122.27, 125.72, 126.86, 127.85, 128.91, 129.69, 135.09, 141.15, 141.28, 150.53, 152.11, 153.11, 155.58, 164.69; Mass: M+H 526.

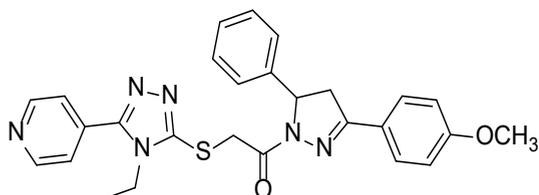
Compound 6j



Yellow solid, M Formula: $C_{27}H_{25}N_7O_3S$; FTIR (cm^{-1} , KBr): 2935, 2965, 3050, (C-H), 1677.40 (C=O amide), 1610 (C=C -Ar), 1431 (C=N-triazole), 709 (S-C); 1.33 (H, CH_3), 2.42 (H, CH_3), 3.11, 3.79 (dd, H, Pyrazoline), 4.05, 4.72 (2H, CH_2), 5.55 (dd,

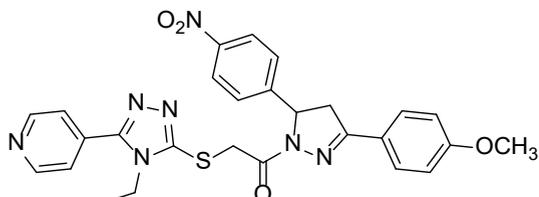
H, CH), 7.23-8.81 (13H, Ar-H); ^{13}C NMR (500 MHz, δ ppm CDCl_3): 15.62, 21.55, 37.32, 40.14, 42.41, 60.46, 122.28, 125.76, 126.84, 127.85, 128.06, 128.89, 129.48, 135.01, 141.09, 141.17, 150.53, 152.15, 153.03, 155.50, 164.72; Mass: M+H 528.

Compound 6k



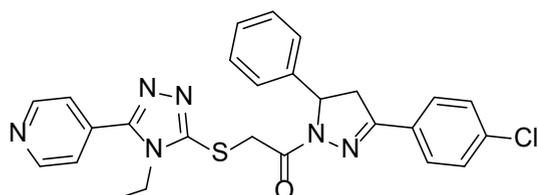
White solid, M. Formula: $\text{C}_{27}\text{H}_{26}\text{N}_6\text{O}_2\text{S}$; FTIR (cm^{-1} , KBr): 2930, 2971, 3022 (C-H), 1667 (C=O amide), 1611 (C=C -aromatic), 1426 (C=N- triazole), 707 (str of S-C); ^1H NMR: 1.33 (H, CH_3), 2.94 (H, OCH_3), 3.11, 3.85 (dd, H, Pyrazoline), 4.04, 4.81 (2H, CH_2), 5.54 (dd, H, CH), 7.26-8.79 (14H, Ar-H); ^{13}C NMR (500 MHz, δ ppm CDCl_3): 15.64, 21.56, 37.30, 40.12, 42.42, 56.12, 59.90, 122.21, 125.68, 126.85, 127.83, 128.01, 128.95, 129.54, 135.01, 141.10, 141.22, 150.52, 152.16, 153.05, 155.56, 164.79; Mass: M+H 499.

Compound 6l



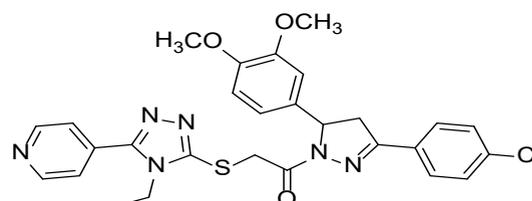
White solid, Molecular Formula: $\text{C}_{27}\text{H}_{25}\text{N}_7\text{O}_4\text{S}$; FTIR (cm^{-1} , KBr): 2946, 2983, 3042 (C-H), 1665 (C=O amide), 1598 (C=C-aromatic ring), 1419 (C=N- triazole), 698 (str of S-C); ^1H NMR: 1.32 (H, CH_3), 3.01 (H, OCH_3), 3.15, 3.86 (dd, H, Pyrazoline), 4.07, 4.78 (2H, CH_2), 5.60 (dd, H, CH), 7.21-8.78 (13H, Ar-H); ^{13}C NMR (500 MHz, δ ppm CDCl_3): 15.62, 21.56, 37.37, 40.20, 42.49, 55.98, 60.30, 122.21, 125.72, 126.86, 127.81, 128.00, 128.91, 129.46, 135.07, 141.18, 141.34, 150.51, 152.14, 153.08, 155.59, 164.76; Mass: M+H 544.

Compound 6m



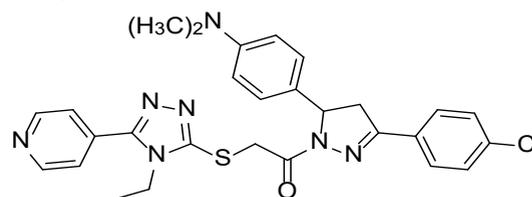
White solid, Molecular Formula: $\text{C}_{26}\text{H}_{23}\text{ClN}_6\text{OS}$; FTIR (cm^{-1} , KBr): 2928, 2971, 3032, 3059 (C-H), 1677.40 (C=O amide), 1606 (C=C Ar), 1421 (C=N- triazole), 709 (S-C); ^1H NMR: 1.32 (H, CH_3), 2.45 (H, CH_3), 3.11, 3.79 (dd, H, Pyrazoline), 4.07, 4.78 (2H, CH_2), 5.55 (dd, H, CH), 7.22-8.83 (12H, Ar-H); ^{13}C NMR (500 MHz, δ ppm CDCl_3): 15.62, 21.54, 37.38, 40.15, 42.48, 60.50, 122.27, 125.72, 126.86, 127.85, 128.05, 128.96, 129.52, 135.03, 141.13, 141.25, 150.56, 152.17, 153.02, 155.51, 164.75; Mass: M+H 504.

Compound 6n



Pale Yellow solid, Molecular Formula: $\text{C}_{28}\text{H}_{27}\text{ClN}_6\text{O}_3\text{S}$; FTIR (cm^{-1} , KBr): 2945, 2984, 3039 (C-H), 1675 (C=O amide), 1612 (C=C- aromatic), 1424 (C=N-triazole), 706 (S-C); ^1H NMR: 1.31 (H, CH_3), 2.96 (6H, OCH_3), 3.12, 3.79 (dd, H, Pyrazoline), 4.05, 4.79 (2H, CH_2), 5.56 (dd, H, CH), 7.19-8.74 (11H, Ar-H); ^{13}C NMR (500 MHz, δ ppm CDCl_3): 15.63, 21.56, 37.38, 40.12, 42.47, 56.81, 60.29, 122.25, 125.75, 126.85, 127.88, 128.04, 128.92, 129.42, 135.03, 141.16, 141.27, 150.56, 152.17, 153.08, 155.56, 164.71; Mass: M+H 564.

Compound 6o



Pale Yellow solid, Molecular Formula: $\text{C}_{28}\text{H}_{28}\text{ClN}_7\text{OS}$; FTIR (cm^{-1} , KBr): 2940, 2979, 3035, 3061 (C-H), 1678.30 (C=O amide), 1612 (C=C-aromatic ring), 1419 (C=N-triazole), 694 (S-C); ^1H NMR: 1.31 (H, CH_3), 2.88 (6H, CH_3), 3.12, 3.77 (dd, H, Pyrazoline), 4.04, 4.77 (2H, CH_2), 5.58 (dd, H, CH), 7.24-8.82 (13H, Ar-H); ^{13}C NMR (500 MHz, δ ppm CDCl_3): 15.61, 21.53, 37.31, 40.12, 42.43, 42.55, 60.29, 122.25, 125.70, 126.81, 127.85, 128.03, 128.88, 129.47, 135.01, 141.16, 141.24, 150.55, 152.18, 153.05, 155.57, 164.69; Mass: M+H 547.

Elemental analysis (EA)

Table 3: Physical data and elemental analysis

Compound	R	R'	Formula	m.p.°C	Yield%	EA							
						Proposed %				Found%			
						C	H	N	S	C	H	N	S
6a	H	H	C ₂₆ H ₂₄ N ₆ O ₃ S	142	65	66.65	5.16	17.94	6.84	66.6	5.08	17.85	6.75
6b	H	4-Cl	C ₂₆ H ₂₃ ClN ₆ O ₃ S	146	71	62.08	4.61	16.71	6.37	62.0	4.50	16.62	6.31
6c	H	4-CH ₃	C ₂₇ H ₂₆ N ₆ O ₃ S	140	62	67.20	5.43	17.41	6.64	67.2	5.35	17.33	6.54
6d	H	4-OCH ₃	C ₂₇ H ₂₆ N ₆ O ₄ S	154	68	65.04	5.26	16.86	6.43	65.0	5.20	16.80	6.30
6e	H	4-NO ₂	C ₂₆ H ₂₃ N ₇ O ₃ S	174	65	60.81	4.51	19.09	6.24	60.8	4.41	19.01	6.15
6f	4-CH ₃	H	C ₂₇ H ₂₆ N ₆ O ₃ S	160	69	67.20	5.43	17.41	6.64	67.2	5.4	17.30	6.59
6g	4-CH ₃	4-Cl	C ₂₇ H ₂₅ ClN ₆ O ₃ S	158	63	62.72	4.87	16.25	6.20	62.7	4.75	16.17	6.20
6h	4-CH ₃	3,4-di OCH ₃	C ₂₉ H ₃₀ N ₆ O ₃ S	146	62	64.19	5.57	15.49	5.91	64.1	5.50	15.42	5.80
6i	4-CH ₃	4-N(CH ₃) ₂	C ₂₉ H ₃₁ N ₇ O ₃ S	136	65	66.26	5.94	18.65	6.10	66.2	5.80	18.59	6.00
6j	4-CH ₃	3-NO ₂	C ₂₇ H ₂₅ N ₇ O ₃ S	144	68	61.47	4.78	18.58	6.08	61.4	4.71	18.50	6.00
6k	4-OCH ₃	H	C ₂₇ H ₂₆ N ₆ O ₄ S	128	71	65.04	5.26	16.86	6.43	65.0	5.18	16.80	6.30
6l	4-OCH ₃	4-NO ₂	C ₂₇ H ₂₅ N ₇ O ₄ S	158	70	59.66	4.64	18.04	5.90	59.6	4.53	17.92	5.80
6m	4-Cl	H	C ₂₆ H ₂₃ ClN ₆ O ₃ S	144	64	62.08	4.61	16.71	6.37	62.0	4.51	16.65	6.25
6n	4-Cl	3,4-di OCH ₃	C ₂₈ H ₂₇ ClN ₆ O ₃ S	134	69	59.73	4.83	14.93	5.69	59.7	4.75	14.82	5.60
6o	4-Cl	4-N(CH ₃) ₂	C ₂₈ H ₂₈ ClN ₇ O ₃ S	130	66	61.58	5.17	17.95	5.87	61.5	5.05	17.90	5.81

CONCLUSION

Potent triazole-pyridine fused pyrazoline derivatives 6a-6o were synthesized from pyrazoline and 3-mercapto triazole. Potential as antimicrobial agents was demonstrated by screening against bacterial and fungal strains. Methoxy and nitro functionalized compound 6l has shown highest inhibition towards *S. aureus*, *E. coli*, *E. aerogenes*. All compounds have demonstrated modest scavenging

capabilities where methoxy substitution promotes antioxidant activities.

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Conflict of interest

None

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