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Design And Biological Screening Of Novel 1,2,3-Triazole-Tethered Tetrazole Scaffolds Bearing Methylthio Moieties

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Abstract:
*A new series of 1,2,3-triazole-tetrazole hybrids (**5a–l**) was synthesized via a Cu(I)-catalyzed azide-alkyne cycloaddition reaction and structurally confirmed using FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis. The synthesized compounds were evaluated for their in vitro antioxidant, antibacterial, and antifungal activities. Among them, compounds 5d, 5j, and 5l exhibited notable antioxidant potential in DPPH and HRS assays, comparable to ascorbic acid. These compounds also demonstrated excellent antibacterial and antifungal activity, surpassing standard drugs against several strains. The results suggest that these triazole-tetrazole hybrids hold promise as potential therapeutic agents with broad-spectrum bioactivity.*

Keywords: *1,2,3-triazole-tetrazole hybrids; CuAAC reaction; antioxidant studies; antimicrobial investigations.*

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I. Introduction

Heterocyclic organic chemistry is one of the most important and extensively studied fields in medical chemistry [**1,2**]. Because they contain various atoms, such as nitrogen, sulfur, and oxygen, heterocyclic bioactive compounds are significant [**3, 4**]. The compounds' pharmacokinetic and metabolic profiles, biological activity, interactions with biological targets, and core skeleton reactivity are all directly impacted by these heteroatoms [**5-7**].

Azole is a five-membered class of nitrogen-containing heterocycles that has attracted a lot of interest in medical research. [**8**, **9**] One of the most promising heterocyclic scaffolds is 1,2,3-triazoles. Composed of two carbons and three nitrogen atoms arranged in a five-membered ring, this core is essential for controlling biological activity due to its electrical characteristics and metabolic stability [**10**,**11**].Individual azole types, including imidazole, pyrazole, triazole, tetrazole, and pentazole, were the most commonly investigated 1,2,3-triazole motifs, indicating their relevance in current medicinal chemistry research, according to a bibliometric search of the Scopus database using these keywords [**12-14**].

The emergence of click chemistry, particularly the copper (I) - catalyzed azide-alkyne cycloaddition (CuAAC) reaction, is primarily responsible for this heightened interest. The synthesis of 1,2,3-triazole scaffolds has been facilitated by this efficient and regioselective method, resulting in numerous derivatives, hybrids, and conjugates with enhanced pharmacological profiles. [**15-18**].

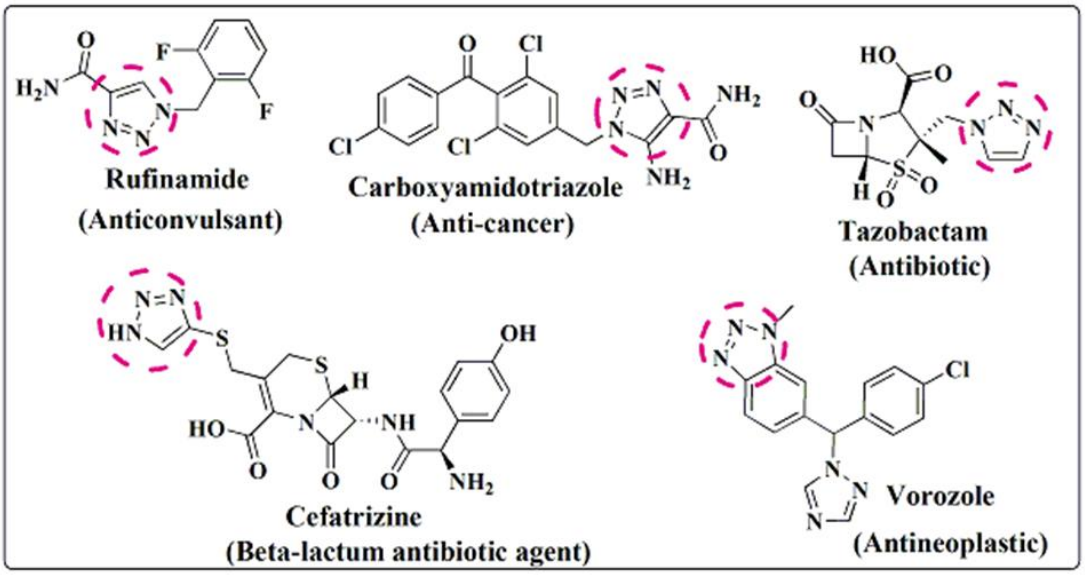


Fig. 1. Bioactive Molecules with 1,2,3-Triazole Scaffolds

The broad range of biological effects of compounds containing 1,2,3-triazoles, such as their antibacterial [**20**], anti-infective [**21**], antioxidant [**19**], and anticancer [**22**] properties, have been shown in numerous studies. Additionally, these heterocycles have shown promise in affecting the biology of ADP-ribosylation, a crucial post-translational modification associated with repair and signaling in cells [**23**, **24**]. To increase membrane permeability, resistance to enzyme degradation, and structural rigidity, triazole units are added to peptide frameworks [**25**, **26**]. These modifications must be managed carefully, though, as they may inadvertently change the biological efficacy and functional structure of the parent peptides [**27**, **28**].The nitrogen-rich structure of the triazole ring, which also contributes to its remarkable hydrogen-bonding properties, dipole interactions, and general biological compatibility, makes compounds containing triazoles promising candidates for use as lead molecules in drug development [**29**–**32**].

In light of this scaffold's increasing importance, the current study focuses on the synthesis and biological assessment of 5-(methylthio)-1-(4-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-tetrazole derivatives, which contain both tetrazole and triazole moieties. These alterations are anticipated to boost their therapeutic potential and further strengthen their interaction with biological targets.

II. Results And Discussions

Biological studies

Antioxidant assays

The antioxidant ability of the novel developed **5a–l** derivatives was tested using two common methods: DPPH and HRS assays at 25 and 50 mM.

Significant antioxidant activity was demonstrated by compounds **5d**, **5j**, and **5l** among the developed compounds. With inhibition values closely comparable to that of the standard, ascorbic acid. The moderate activity of compounds **5a**, **5c**, **5e**, **5h**, **5i**, and **5k** suggested some antioxidant potential that could be increased with additional structural optimization. Compounds **5b**, **5f**, and **5g**, on the other hand, showed less activity than other newly created ones.

Table 2: In vitro antioxidant activity of **5a-l** derivatives using DPPH and HRS assay

Entry	Concentration (mM)			
	DPPH method (%)		HRS method (%)	
	25	50	25	50
5a	30.78	61.34	31.89	64.12
5b	18.58	41.66	20.52	44.34
5c	24.45	52.09	25.29	57.23
5d	**43.27**	**80.37**	**44.56**	**82.05**
5e	**33.18**	**63.21**	**34.56**	**67.39**
5f	20.43	46.89	21.76	48.12
5g	22.56	49.78	23.37	52.05
5h	26.66	57.71	28.43	60.54
5i	25.89	56.91	27.13	58.42
5j	**38.92**	**73.22**	**39.89**	**75.67**
5k	25.31	55.55	26.67	58.06

5l	**35.98**	**67.72**	**36.47**	**70.66**
Ascorbic acid	44.57	81.23	45.34	83.56

Antibacterial assays

The antibacterial activity of synthetic triazole compounds **5a–l** against a panel of six bacterial strains is shown in **table 2**. Using the agar well diffusion method at a concentration of 100µg/mL, the effectiveness was determined by measuring the diameter of the inhibition zone (in mm). For comparison, ciprofloxacin, a common antibiotic, was employed as a positive control.

According to the findings, the majority of the synthetic compounds demonstrated moderate to good antibacterial activity against all tested strains. Compound **5d** showed the strongest inhibitory activity among them; its MZI values, which ranged from 22 to 28 mm, were very close to those of ciprofloxacin (MZI: 23–26 mm). Compounds **5j** and **5l** also demonstrated significant activity, especially against *B. sphaericus, S. aureus, and B. subtilis*. The compounds **5f**, **5g**, and **5k**, on the other hand, showed the least antibacterial effect; their inhibition zones ranged from 10 to 14 mm.

Table 4. In Vitro Antibacterial studies of Compounds 5a–l

Mean zone inhibition (MZI)[a] in 100 *µ*g/mL						
Compound	***B. subtilis***	***B. sphaericus***	***S. aureus***	***P. aeruginosa***	***K. aerogenes***	***C. violaceum***
5a	**22**	**17**	**18**	**17**	**16**	**18**
55b	15	14	15	12	12	14
5c	16	15	16	14	15	16
5d	**28**	**26**	**26**	**23**	**22**	**24**
5e	**23**	**18**	**19**	**18**	**17**	**19**
5f	14	13	13	12	11	13
5g	13	12	12	10	11	12
5h	15	14	15	13	12	14
5i	17	14	16	13	15	15
5j	**26**	**23**	**24**	**22**	**20**	**22**
5k	12	11	12	10	10	13
5l	**24**	**20**	**21**	**19**	**18**	**20**
Ciprofloxacin	26	25	26	24	23	25

In Vitro Antifungal Screening

The antifungal activity of synthetic compounds **5a–l** against four pertinent fungus strains is compiled in **table 3**. The agar well diffusion method was used to assess the antifungal potential, and the inhibition zones (measured in millimeter) were noted. The reference control was the commonly used antifungal medication Amphotericin-B.

Compound **5d** demonstrated the greatest inhibition across all tested strains (MZI: 27–31 mm), even marginally outperforming Amphotericin B against *A. fumigatus*, according to the data, which also showed that other compounds exhibited varied degrees of antifungal activity. Additionally, compounds **5j** and **5l** showed noteworthy efficacy that was on par with the standard, particularly against *T. mentagrophytes and C. albicans*. In contrast, compounds 5f, 5g, and 5k had MZI values between 12 and 16 mm, indicating comparatively modest antifungal activity. According to these findings, the kind and location of substituents on the core scaffold significantly influence the antifungal activity.

Table 5. In Vitro Antifungal Screening of Compounds **5a–l**

Mean zone inhibition (MZI)[a] in 100 *µ*g/mL				
Compound	***A. fumigatus***	***C. albicans***	***T. mentagropytes***	***T. rubrum***
5a	**24**	**21**	**23**	**22**
5b	17	15	16	17
5c	19	17	18	17
5d	**31**	**28**	**27**	**27**
5e	**26**	**24**	**25**	**25**
5f	16	14	15	16
5g	15	13	14	14
5h	18	16	17	17
5i	20	18	19	20
5j	**30**	**27**	**26**	**25**
5k	14	12	13	13
5l	**28**	**26**	**27**	**26**
Amphotericin B	**29**	**27**	**28**	**28**

III. Conclusions

A series of novel 1,2,3-triazole–tetrazole hybrids (**5a–l**) was synthesized via a click chemistry approach, involving the propargylation of 4-(5-(methylthio)-1*H*-tetrazol-1-yl)phenol followed by Cu(I)-catalyzed azide-alkyne cycloaddition with aryl azides. The final products were evaluated for antioxidant, antibacterial, and antifungal properties. Compounds 5d, 5j, and 5l exhibited significant antioxidant activity, comparable to ascorbic acid, while others showed moderate potential. Antibacterial studies revealed 5d as the most active, with inhibition zones close to that of ciprofloxacin. Similarly, 5d showed potent antifungal activity, outperforming *amphotericin B* against *A. fumigatus*, followed by 5j and 5l. Compounds 5f, 5g, and 5k displayed comparatively lower bioactivity in all assays. These findings suggest that the nature and position of substituents play a crucial role in modulating biological activity. Overall, compounds 5d, 5j, and 5l emerged as promising multifunctional agents warranting further development as potential therapeutic candidates.

Experimental

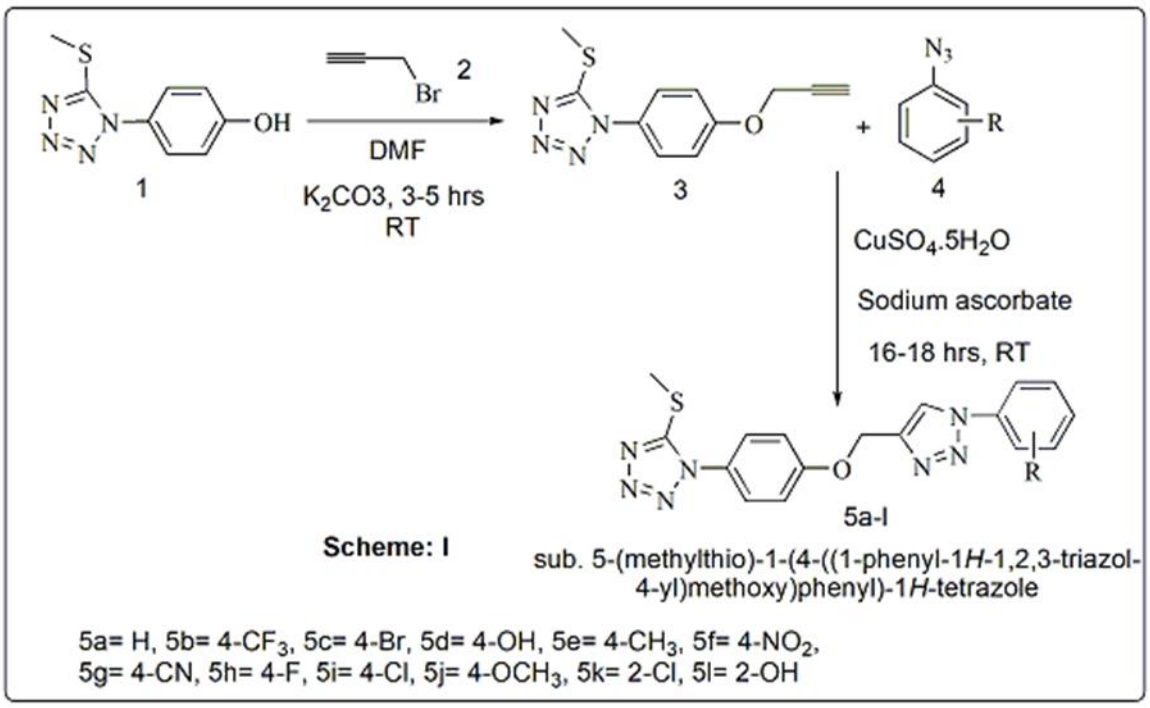
Synthesis

Synthesis of 5-(methylthio)-1-(4-(prop-2-ynyloxy)phenyl)-1H-tetrazole (3)

4-(5-(methylthio)-1H-tetrazol-1-yl)phenol(2 g, 1.0 eq,) was dissolved in DMF (5 volumes), followed by the addition of K_2CO_3 (1.99 g, 2.5 eq). Subsequently, propargyl bromide (1.37 g, 1.2 eq) was added drop wise, and the reaction was stirred for 3–5 hours at 35 ^oC. Progress was monitored by TLC. Upon completion, the reaction mass was poured into crushed ice. The resulting white solid was filtered, washed with cold water, and dried under vacuum to afford the desired compound in 93% yield. Compound weight: 2.2 gr.

Synthesis of aryl azides (4)

Combine aqueous $CuSO_4.5H_2O$ (10 % 3mL), aqueous sodium ascorbate (10% 3mL), aromatic substituted azide (1.2eq, 12mmols), and compound 3 (250 mg, 1eq and 10mmols) in DMF (3 mL). Stir for overnightat RT. Using TLC; track the reaction's development until all of the starting material has been used. Filter the solid products after using broken ice to quench the reaction. Using hexane and cold water wash the filtered solid to obtain sub. **5-(methylthio).the compounds of -1-(4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-tetrazole** (**5a-l**) in yields between 69 and 78%.



Scheme: I. Synthesis of 1,2,3- Triazole derivatives

Spectral data:

5a compound: A. data: M.F: $C_{17}H_{15}N_7OS$.Colour: Off white, Yield (%): 69, M.P (^oC): 239-241. IR (cm^{-1}): 3037 (C-H, Ar), 2936 (C-H, Ali), 1610 (C=N), 1536 (N=N), 1504 (C=C, Ar), 1161 (C-O-C), 675 (C-S).^{1}H NMR (DMSO, 400 MHz, δ ppm): 8.19 (1H, s, Triazole ring-CH), 7.787.80 (d, 2H, *J*= 8.4 MHz), 7.61-7.65(m, 5H), 7.38-7.40 (d, 2H, *J*= 8.4 MHz), 5.22 (s, 2H, CH_2), 2.624 (s, 3H, S-CH_3). ^{13}C NMR (DMSO, 400 MHz, δ ppm):

162.011, 155.92, 145.63, 136.78, 130.52, 128.36, 126.25, 124.59, 123.70, 119.58, 119.19, 114.71, 112.76, 72.31, 17.52. Mass: 366 $[M+1]^+$ E.analysis: Cal %: C, 55.88; H, 4.14; N, 26.83; O, 4.38; S, 8.78. Found %: C, 55.83; H, 4.11; N, 26.86; O, 4.35; S, 8.81.

5b compound: A. data: M.F: $C_{18}H_{14}F_3N_7OS$.Colour: Cream, Yield (%): 72, M.P (^oC): 226-228. IR (cm^{-1}): 3041 (C-H, Ar), 2932 (C-H, Ali), 1601 (C=N), 1532 (N=N), 1501 (C=C, Ar), 1145 (C-O-C), 664 (C-S).^1H NMR (DMSO, 400 MHz, δ ppm): 8.22 (1H, s, Triazole ring-CH), 7.797.81 (d, 2H, *J*= 7.18 MHz), 7.58-7.61(m, 4H), 7.24-7.26 (d, 2H, *J*= 8.9 MHz), 5.29 (s, 2H, CH_2), 2.79 (s, 3H, S-CH_3).^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.20, 156.24, 145.91, 136.09, 133.05, 131.41, 130.04, 127.07, 126.74, 126.26, 126.07, 119.23, 115.69, 114.60, 72.63, 17.02.Mass: 434 $[M+1]^+$ E.analysis: Cal %: C, 49.88; H, 3.26; F, 13.15; N, 22.62; O, 3.69; S, 7.40. Found %: C, 49.84; H, 3.29; F, 13.11; N, 22.65; O, 3.66; S, 7.42.

5c compound: A. data: M.F: $C_{17}H_{14}BrN_7OS$.Colour: Pale yellow, Yield (%): 75, M.P (^oC): 233 235. IR (cm^{-1}): 3055 (C-H, Ar), 2961 (C-H, Ali), 1620 (C=N), 1558 (N=N), 1503 (C=C, Ar), 1152 (C-O-C), 663 (C-S).^1H NMR (DMSO, 400 MHz, δ ppm): 8.20 (1H, s, Triazole ring-CH), 7.617.63 (d, 2H, *J*= 8.79 MHz), 7.38-7.40(m, 4H), 7.12-7.14 (m, 2H), 5.23 (s, 2H, CH_2), 2.78 (s, 3H, S-CH_3).^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.01, 159.91, 144.89, 136.07, 132.89, 131.70, 131.37, 129.89, 127.01, 125.60, 119.12, 114.02, 112.01, 72.07, 17.39. Mass: 446 $[M+2]^+$ E.analysis: Cal %: C, 45.95; H, 3.18; Br, 17.98; N, 22.07; O, 3.60; S, 7.22. Found %: C, 45.92; H, 3.22; Br, 17.95; N, 22.04; O, 3.63; S, 7.20.

5d compound: A. data: M.F: $C_{17}H_{15}N_7O_2S$.Colour: Off white, Yield (%): 71, M.P (^oC): 236 238. IR (cm^{-1}): 3452 (OH), 3049 (C-H, Ar), 2966 (C-H, Ali), 1614 (C=N), 1553 (N=N), 1508 (C=C, Ar), 1147(C-O-C), 668 (C-S).^1H NMR (DMSO, 400 MHz, δ ppm): 9.28 (s, 1H, OH), 8.25 (1H, s, Triazole ring-CH), 7.667.68 (d, 2H, *J*= 8.79 MHz), 7.45-7.47(m, 4H), 7.18-7.20 (m, 2H), 5.25 (s, 2H, CH_2), 2.81 (s, 3H, S-CH_3).^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.23, 156.88, 144.56, 136.22, 133.09, 130.98, 128.11, 129.72, 127.23, 125.72, 119.18, 114.5, 112.08, 72, 23.58. Mass: 382 $[M+1]^+$ E.analysis: Cal %: C, 53.53; H, 3.96; N, 25.71; O, 8.39; S, 8.41. Found %: C, 53.50; H, 3.92; N, 25.74; O, 8.37; S, 8.43.

5e compound: A. data: M.F: $C_{18}H_{17}N_7OS$.Colour: White, Yield (%): 77, M.P (^oC): 229 231. IR (cm^{-1}): 3042 (C-H, Ar), 2960 (C-H, Ali), 1611 (C=N), 1547 (N=N), 1506 (C=C, Ar), 1152(C-O C), 662 (C-S). ^1H NMR (DMSO, 400 MHz, δ ppm): 8.18 (1H, s, Triazole ring-CH), 7.807.82 (d, 2H, *J*= 8.79 MHz), 7.56-7.60(m, 4H), 7.32-7.34 (d, 2H, *J*= 8.79 MHz), 5.23 (s, 2H, CH_2), 2.72(s, 3H, S-CH_3), 2.23 (s, 3H, CH_3).^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.02, 156.11, 144.81, 130.21, 128.58, 126.23, 123.30, 122.11, 119.20, 116.04, 114.57, 113.55, 112.06, 72.11, 24.35, 17.13. Mass: 380 $[M+1]^+$ E.analysis: Cal %: C, 56.98; H, 4.52; N, 25.84; O, 4.22; S, 8.45.Found %: C, 56.95; H, 4.50; N, 25.87; O, 4.18; S, 8.47.

5f compound: A. data: M.F: $C_{17}H_{14}N_8O_3S$.Colour: Orange, Yield (%): 70, M.P (^oC): 221 222. IR (cm^{-1}): 3035 (C-H, Ar), 2953 (C-H, Ali), 1615 (C=N), 1542 (N=N), 1514 (C=C, Ar), 1158 (C-O C), 669 (C-S).^1H NMR (DMSO, 400 MHz, δ ppm): 8.36-8.34 (d, 2H, *J*= 9.2 MHz), 8.07 (1H, s, Triazole ring-CH), 7.767.78 (d, 3H, *J*= 8.79 MHz), 7.52-7.54(d, 3H, *J*= 7.19 MHz), 7.32-7.34 (d, 2H, *J*= 9.6 MHz), 5.28 (s, 2H, CH_2), 2.81(s, 3H, S-CH_3).^{13}C NMR (DMSO, 400 MHz, δ ppm): 163.91, 156.83, 145.56, 136.11, 133.00, 131.70, 131.37, 129.89, 127.01, 125.98, 125.49, 119.11, 114.83, 112.56, 72.37, 17.51. Mass: 411 $[M+1]^+$ E.analysis: Cal %: C, 49.75; H, 3.44; N, 27.30; O, 11.70; S, 7.81. Found %: C, 49.71; H, 3.40; N, 27.33; O, 11.68; S, 7.84.

5g compound: A. data: M.F: $C_{18}H_{14}N_8OS$. Colour: Pale yellow, Yield (%): 72, M.P (^oC): 237 239. IR (cm^{-1}): 3038 (C-H, Ar), 2948 (C-H, Ali), 2235 (CN), 1612 (C=N), 1549 (N=N), 1509 (C=C, Ar), 1151 (C-O C), 661 (C-S). ^1H NMR (DMSO, 400 MHz, δ ppm): 8.18 (1H, s, Triazole ring-CH), 7.79 7.80 (d, 2H, *J*= 5.6MHz), 7.41-7.42 (d, 2H, *J*= 5.2 MHz), 7.07-7.09 (d, 2H, *J*= 8.79 MHz), 7.02-7.04 (d, 2H, *J*= 9.2 MHz) 5.23 (s, 2H, CH_2), 2.79 (s, 3H, S-CH_3).^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.18, 156.19, 145.76, 136.89, 134.26, 130.30, 126.26, 123.24, 120.11, 119.23, 114.71, 113.34, 111.30, 72.56, 17.00.Mass: 391 $[M+1]^+$ E.analysis: Cal %: C, 55.37; H, 3.61; N, 28.70; O, 4.10; S, 8.21. Found %: C, 55.33; H, 3.63; N, 28.67; O, 4.07; S, 8.24.

5h compound: A. data: M.F: $C_{17}H_{14}FN_7OS$. Colour: Off white, Yield (%): 74, M.P (^oC): 232 234. IR (cm^{-1}): 3030 (C-H, Ar), 2944 (C-H, Ali), 1609 (C=N), 1544 (N=N), 1506 (C=C, Ar), 1145 (C-O C), 664 (C-S).^1H NMR (DMSO, 400 MHz, δ ppm): 8.19 (1H, s, Triazole ring-CH), 7.75 7. 77 (d, 4H, *J*= 8.79 MHz), 7.40-7.42 (d, 2H, *J*= 6.4 MHz), 7.26-7.28 (d, 2H, *J*= 8.4 MHz), 5.23 (s, 2H, CH_2), 2.80 (s, 3H, S-CH_3).^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.15, 155.75, 145.25, 136.11, 133.00, 131.67, 131.41, 129.87, 127.01, 125.89, 125.49, 119.39, 114.72, 112.21, 72.31, 17.79.Mass: 384 $[M+1]^+$. E.analysis: Cal %: C, 53.26; H, 3.68; F, 4.96; N, 25.57; O, 4.17; S, 8.36. Found %: C, 53.21; H, 3.65; F, 4.99; N, 25.54; O, 4.20; S, 8.39.

5i compound: A. data: M.F: $C_{17}H_{14}ClN_7OS$. Colour: Pale cream, Yield (%): 76, M.P (^oC): 232 234. IR (cm^{-1}): 3042 (C-H, Ar), 2940 (C-H, Ali), 1615 (C=N), 1537 (N=N), 1512 (C=C, Ar), 1141 (C-O C), 671 (C-S). ^1H NMR (DMSO, 400 MHz, δ ppm): 8.16 (1H, s, Triazole ring-CH), 7.73 7. 75 (d, 4H, *J*= 8.79 MHz), 7.35-7.37 (d, 2H, *J*= 6.4 MHz), 7.19-7.21 (d, 2H, *J*= 8.4 MHz), 5.21 (s, 2H, CH_2), 2.77 (s, 3H, S-CH_3). ^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.08, 155.45, 144.89, 135.92, 133.11, 131.49, 130.36, 129.54, 127.21, 125.32, 124.72, 119.22, 113.99, 112.08, 72.31, 17.79. Mass: 402 $[M+2]^+$. E.analysis: C, 51.06; H, 3.53; Cl, 8.87; N, 24.52; O, 4.00; S, 8.02. Found %: C, 51.03; H, 3.51; Cl, 8.90; N, 24.49; O, 4.03; S, 7.98.

5j compound: A. data: M.F: $C_{18}H_{17}N_7O_2S$. Colour: Off white, Yield (%): 70, M.P (^oC): 243 245. IR (cm^{-1}): 3039(C-H, Ar), 2943 (C-H, Ali), 1611 (C=N), 1533 (N=N), 1510 (C=C, Ar), 1147 (C-O C), 669 (C-S).^1H NMR (DMSO, 400 MHz, δ ppm): 7.96 (1H, s, Triazole ring-CH), 7.59 7. 57 (d, 4H, *J*= 7.19 MHz), 7.28-7.30 (d, 4H, *J*= 9.2 MHz), 5.27 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 2.78 (s, 3H, S-CH_3).^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.00, 156.83, 144.78, 136.56, 133.31, 130.35, 128.64, 124.98, 124.83, 122.79, 120.06, 119.91, 115.69, 113.12, 72.35, 56.38, 17.24.Mass: 396 $[M+1]^+$. Anal.Data: C, 54.67; H, 4.33; N, 24.79; O, 8.09; S, 8.11. Found %: C, 54.63; H, 4.31; N, 24.82; O, 8.07; S, 8.14.

5k compound: A. data: M.F: $C_{17}H_{14}ClN_7OS$. Colour: Pale yellow, Yield (%): 73, M.P (^oC): 236 238. IR (cm^{-1}): 3034(C-H, Ar), 2947 (C-H, Ali), 1612 (C=N), 1529 (N=N), 1508 (C=C, Ar), 1141 (C-O C), 662 (C-S).^1H NMR (DMSO, 400 MHz, δ ppm): 8.16 (1H, s, Triazole ring-CH), 7.81-7.83 (d, 1H, *J*= 9.2 MHz), 7.59-7.63 (m, 4H), 7.42-7.48 (m, 2H), 7.12-7.14 (d, 1H, *J*= 9.2 MHz),5.23 (s, 2H, CH_2), 2.73 (s, 3H, S-CH_3).^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.09, 158.89, 144.76, 134.00, 132.01, 130.06, 128.61, 126.24, 123.27, 122.08, 119.19, 116.04, 114.68, 113.44, 72.24, 16.67.Mass: 396 $[M+1]^+$. E. analysis: C, 51.06; H, 3.53; Cl, 8.87; N, 24.52; O, 4.00; S, 8.02. Found %: C, 51.03; H, 3.57; Cl, 8.85; N, 24.49; O, 4.03; S, 7.99.

5l compound: A. data: M.F: $C_{17}H_{15}N_7O_2S$. Colour: Off white, Yield (%): 78, M.P (^oC): 246-248. IR (cm^{-1}): 3439 (OH), 3097(C-H, Ar), 2934 (C-H, Ali), 1640 (C=N), 1596 (N=N), 1489(C=C, Ar), 1153 (C-O C), 679 (C-S).^1H NMR (DMSO, 400 MHz, δ ppm): 9.58 (1H, s, OH), 7.94 (1H, s, Triazole ring-CH), 7.21- 7.25 (m, 4H), 6.98-7.04 (m, 4H), 5.20 (s, 2H, CH_2), 2.77 (s, 3H, S-CH_3). ^{13}C NMR (DMSO, 400 MHz, δ ppm): 162.16, 159.02, 143.98, 138.51, 134.30, 130.28, 126.24, 123.27, 120.09, 119.19, 114.71, 113.46, 72.27, 16.89. Mass: 382 $[M+1]^+$. E. analysis: C, 53.53; H, 3.96; N, 25.71; O, 8.39; S, 8.41. Found %: C, 53.50; H, 3.94; N, 25.74; O, 8.41; S, 8.38.

Biological assays

Antioxidant investigations

The assessment of free radical neutralization potential for the prepared derivatives was carried out utilizing both DPPH and hydroxyl radical inhibition (HRS) techniques. Solutions containing defined quantities of the synthesized molecules in 0.2 mM DPPH and HRS reagents (2.5 mM 1,10-phenanthroline, phosphate-buffered saline at pH 7.4-, and 2.5-mM ferrous sulfate) were maintained in the absence of light for 30 minutes. Following incubation, the optical density was measured at a wavelength of 517 nm. The percentage of radical scavenging capability was computed using a standard equation as outlined below.

$$\text{Percentage of Inhibition \%}=\frac{(\text{Acontrol}-\text{Asample})}{\text{Acontrol}}x100$$

Where $A_{control}$ denotes the optical absorbance of the untreated reference solution, and A_{sample}corresponds to the absorbance measured for the reaction system containing the investigational compound.

$$\text{HRS Activity (\%)}=\frac{(\text{Acontrol}-\text{Asample})}{\text{Acontrol}}x100$$

Where,$A_{control}$ is the absorbance reading without the added sample, while A_{sample} is measured after including the test compound.

Antibacterial studies

The newly synthesized **5a–l** compounds were evaluated for their antibacterial efficacy against a selection of representative Gram-negative and Gram-positive bacterial strains. The assay was conducted utilizing the disc diffusion technique. A sterile glass spreader was employed to uniformly distribute a standardized microbial suspension ($1–2 \times 10^7$ colony-forming units per milliliter), prepared in accordance with the 0.5 McFarland turbidity standard, over the surface of sterile agar media. Filter paper discs (6.26 mm in diameter), fabricated from Whatman No. 1 paper and sterilized at 140 °C for 1 hour, were subsequently impregnated with known concentrations of the synthesized compounds. These discs were then placed onto the inoculated agar plates, and the antibacterial response was assessed by measuring the diameter of the resulting zones of inhibition following incubation.

Antifungal Activity

The antifungal potential of the synthesized compounds **5a–l** was evaluated using the disc diffusion assay against selected fungal strains. Each compound was solubilized in DMSO, and amphotericin B was employed as the standard reference antifungal agent. The efficacy of the tested compounds was determined by measuring the MZI, with the results presented in Table 4 alongside corresponding control values.

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