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Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 1,2,3-TRIAZOLE-TETHERED NITROGUAIACOL ETHERS

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ABSTRACT

Objective: Nitroaromatic/nitrophenols have been widely distributed in nature and are mostly isolated from marine microorganisms and had shown a broad spectrum of antimicrobial activities against a wide range of microbial pathogens**.** The objective of the present work is to synthesize some new 1,2,3-triazole-tethered nitroguaiacol ethers and evaluated of their antibacterial and antifungal activities.

Methods: A focused library of 1,2,3-triazole-tethered nitroguaiacol ethers was prepared by employing Cu (I) catalyzed click chemistry reaction and evaluated for their antimicrobial activities by broth microdilution method.

Results: Among the tested compounds, compounds **8e, 8f, 8g,** and **8i** exhibited broad-spectrum activity against selected pathogenic strains, with the MIC of 8 µg/mL for Gram-positive bacteria (*Staphylococcus aureus*), 16 µg/mL for *Pseudomonas aeruginosa* (Gram-negative bacteria), and *Candida* species, respectively.

Conclusion: Future investigations with this class of compounds may lead to the development of potential candidates for antimicrobial drug discovery.

Keywords: Nitroguaiacol, 1,2,3-triazoles, Antibacterial, Antifungal.

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INTRODUCTION

The escalating resistance of currently available antibiotics demands the development of new molecular entities to counterpart infectious deceases [1]. Nitroaromatic/nitrophenols have been widely distributed in nature and are mostly isolated from marine microorganisms. They had shown a broad spectrum of antimicrobial activities against a wide range of microbial pathogens [2]. Nitrophenol derivatives have been known for their antimicrobial activities over the years [3]. 4-Nitrocatechol and other derivatives of catechol were found to be active against a panel of intestinal bacteria [4]. 4,6-Dinitroguaiacol and isomeric 3,5-dinitroguaiacol isolated from the red algae *Marginisporum aberrans* showed antimicrobial activity against *Bacillus subtilis* [3,5]. Guaiacol is a naturally occurring phenolic compound which has gained attention due to their various synthetic applications. Nitroguaiacol ether derivatives have been found to display potential antibacterial activities [6].

On the other hand, 1,2,3-triazoles attained great attention due to their broad spectrum of biological activities including antimicrobial [7,8], anti-inflammatory [9,10], analgesic [10], anticancer [7,11-16], antitubercular [17-19], local anesthetic [9], immunomodulatory [20], antimalarial [8,21,22], anti-HIV [7,23-25], antileishmanial [26], and antiviral [7] activities. It has become an adjuvant ligand in drug discovery, which has been conjugated to improve the biological properties of molecules. 1,2,3-Triazole moieties are the versatile connecting units which are stable to metabolic degradation conditions and capable of making hydrogen bonds, which can be favorable in the binding of bimolecular targets and can improve the solubility [27,28]. Thus, 1,2,3-triazoles have emerged as powerful pharmacophores on their own right [29,30].

After the extensive work on triazole chemistry with respect to the antimicrobial drug development, 1,2,3-triazole-containing β-lactam antibiotics tazobactam (**4**) and cephalosporins cefatrizine (**5**) are in the market and some are in pipeline [8]. 1,2,3-Triazole-containing fluconazole analogs (**6** and **7**) were found to be potential antifungal agents against *Candida* fungal pathogens than control drugs fluconazole and amphotericin B [31].

Keeping in view the antimicrobial properties of nitroguaiacol and 1,2,3-triazole moieties, a focused library of 1,2,3-triazole-tethered nitroguaiacol ethers (**8a-8l**) has been designed by applying "hybrid conjugation of bioactive ligands" strategy and synthesized by employing Cu (I) catalyzed 1,3-dipolar cycloaddition reactions between O-propargyl nitroguaiacol and substituted aromatic azides.

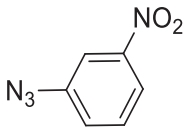
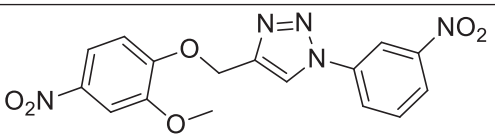
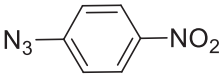
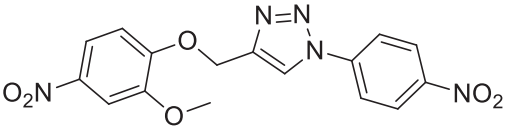
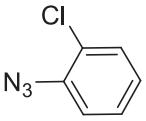
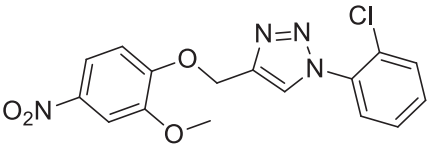
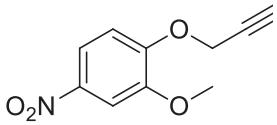
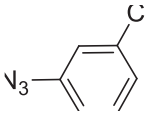
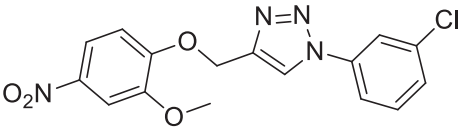
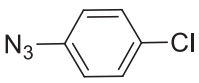
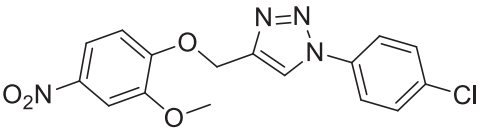
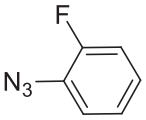
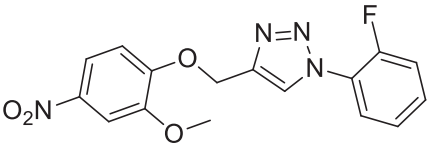
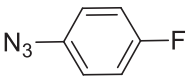
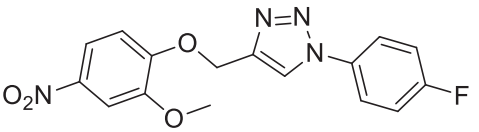
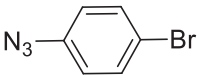
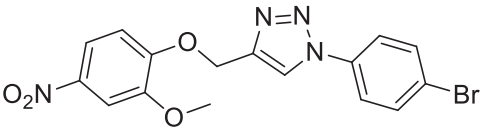
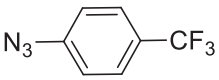
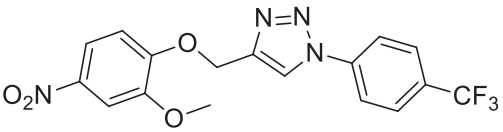
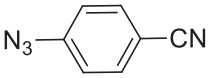
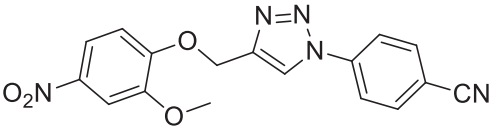
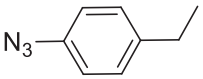
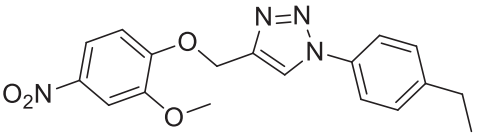
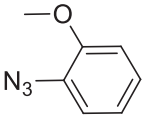
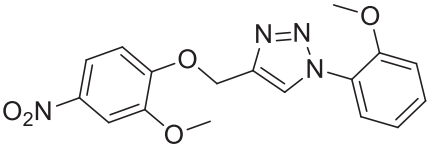
RESULTS AND DISCUSSION

Chemistry

1,2,3-Triazole-tethered nitroguaiacol ethers were synthesized and evaluated for their antibacterial and antifungal activities. As illustrated in Scheme 1, 1,2,3-triazole compounds were synthesized by the cycloaddition reaction of 2-methoxy-4-nitro-1-(prop-2-yn-1-yloxy) benzene **7** with various azides. Toward the synthesis of compound **7**, 2-methoxy-4-nitrophenol **5** was refluxed with propargyl bromide in dry acetone in the presence of potassium carbonate. Various substituted aromatic/sugar azides **8** were reacted with propargylated nitroguaiacol ether **7** under click chemistry reaction conditions to obtain the novel 1,2,3-triazole-tethered nitroguaiacol ethers 9a–9l in quantitative yields. Aromatic azides with varying substitutions including electron-withdrawing and electron-donating groups were reacted with compound **7**.

A focused library of 12 compounds was synthesized (Table 1) by varying nature and cite of substitution on the aromatic ring attached to triazole moiety. All the synthesized triazoles were screened for their

Table 1: 1,2,3-Triazole-tethered nitroguaiacol ethers

S. No.	Terminal alkyne (11)	Azide (12)	Triazole (8)	Yields (%)	MP (°C)
a				95	195.7
b				93	226.7
c				98	120.4
d				99	142.6
e				94	152.4
f				93	130.6
g				95	120.5
h				97	166.4
i				97	128.3
j				99	179.6
k				98	131.7
l				99	154.4

[a]Isolated yields

antimicrobial activity. All the products were characterized by ^{1}H NMR, ^{13}C NMR, IR, and ESI-MS. In the ^{1}H NMR spectra, the formation of triazole was confirmed by the resonance of H–C of the triazole ring in the aromatic region (8.05–8.25 parts per million [ppm]). The structure was

further supported by the ^{13}C NMR spectra, which showed the C–atom signals corresponding to triazole derivatives at δ 142–148.

Biology

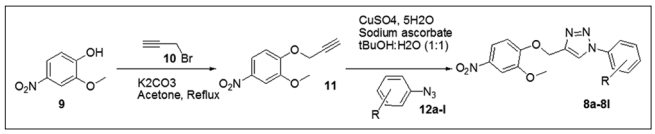
All the above-synthesized nitroguaiacols were tested for their *in vitro* antibacterial as well as antifungal activities against one reference of strains of each (total four strains) selected Gram-positive bacteria, Gram-negative bacteria, and *Candida* species by broth microdilution method following Clinical and Laboratory Standards Institute (CLSI) guidelines. The results are tabulated in Table 2.

Ciprofloxacin and amphotericin B served as drug control for bacterial strains and fungal strains, respectively. The results are representative of two separate experiments performed in duplicate and similar results were observed each time.

Compounds **8e, 8f, 8g, and 8i** exhibited broad-spectrum activity against a variety of selected pathogenic strains, with the minimum inhibitory concentration (MIC) of 8 μg/mL for Gram-positive bacteria (*Staphylococcus aureus*), 16 μg/mL for *Pseudomonas aeruginosa* (Gram-negative bacteria), and *Candida* species, respectively. They have demonstrated significantly improved activities when compared to parent nitroguaiacol **9 (MICs ≥**128 μg/mL). Among the compounds synthesized, halogen-substituted compounds were found to be active against *S. aureus* and moderately active against *P. aeruginosa* and fungal strains. Among the halogen-substituted compounds, -chloro, -fluoro, and trifluoromethyl-substituted compounds demonstrated good activities. Regarding the position of substitution, para-position has mostly shown good activity. Compounds substituted with strong electron-withdrawing groups such as $–NO_2$ and –CN groups and electronic-donating groups such as $–OCH_3$ and $–C_2H_5$ are found to be either moderately active or inactive.

CONCLUSION

A focused library of 1,2,3-triazole-linked nitroguaiacol ethers was prepared by employing Cu (I) catalyzed click chemistry reaction and all the synthesized compounds were evaluated for their antimicrobial activities. Among the synthesized compounds, halogen-substituted compounds were demonstrated good-to-moderate activities against both Gram-positive and Gram-negative bacteria and fungal strains. These compounds may be potential candidates for the further antimicrobial drug discovery.



Scheme 1: Synthesis of 1,2,3-triazole-tethered nitroguaiacol ethers

Experimental section

Chemistry

All commercial chemicals used as starting materials and reagents in this study were purchased from Merck (India), Spectrochem, and Sigma-Aldrich which were of reagent grade. All melting points were uncorrected and measured using Electrothermal IA 9100 apparatus (Shimadzu, Japan); infrared (IR) spectra were recorded on Bruker ALPHA Fourier transform IR spectrometer (Germany), ^1H-NMR spectra were determined on an Agilent (400 MHz) spectrometer and chemical shifts were expressed as ppm against TMS as internal reference. Mass spectra were recorded on 70 eV (EI Ms–QP 1000 EX, Shimadzu, Japan), column chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm).

Synthesis of *2-methoxy-4-nitro-1-(prop-2-yn-1-yloxy)benzene* (11)

In a dry two neck round-bottomed flask equipped with a condenser, to the acetone solution of nitroguaiacol **9 (**4.83 mmol), was added activated K_2CO_3 (24.14 mmol) and stirred for 10 min at room temperature under anhydrous condition. Then, propargyl bromide **10 (**5.79 mmol) was added to the reaction mixture and the reaction mixture was heated to reflux and continued to stir for 12 h. After the completion of the reaction monitored by thin-layer chromatography (TLC), the reaction mixture was quenched with excess of water (50 mL) and the product was extracted with ethyl acetate (50 mL×2). The combined organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford the crude product which was recrystallized with ethyl acetate/hexane to afford the pure compound **11** in quantitative yields.

2-methoxy-4-nitro-1-(prop-2-yn-1-yloxy)benzene (**11**)

M.P. **118.6**°C; IR (KBr) (cm^{-1}): 3116, 2366, 2348, 1827, 1730, 1713, 1694, 1680, 1669, 1633, 1613, 1588 1569, 1553, 1534, 1515, 1504, 1468, 1453, 1377, 1340, 1280, 1232, 1136, 1082, 1016, 872, 806, 752; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.56 (s, 1H), 3.93 (s, 3H), 4.84 (s, 2H), 7.05 (d, 1H, *J*=8.80 Hz), 7.73 (s, 1H), 7.87 (d, 1H, *J*=8.80 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.31, 56.84, 106.83, 112.19, 117.29, 142.22, 149.36, 152.10; ESIMS: 207 (M^+), 208 (M^++1).

General procedure for the synthesis of 1,2,3-triazoles (Click Chemistry)

Compound 2 was dissolved in 20 mL of *tert*-Butanol:water (1:1) solvent at ambient temperature and then was charged $CuSO_4.5H_2O$ and the reaction mixture was stirred for 5 min. Reaction mixture was

Table 2: *In vitro* antimicrobial activity of nitroguaiacol

Test compounds	Antibacterial (MIC in *μg/mL*)		Antifungal (MIC in μg/mL)	
	Staphylococcus aureus **ATCC 29213**	***Pseudomonas aeruginosa*** **ATCC 27853**	***Candida albicans*** **ATCC 90028**	***Candida parapsilosis*** **ATCC 22019**
9	>128	>128	>128	>128
11	>128	>128	>128	>128
8a	32	128	128	128
8b	32	128	128	128
8c	16	64	64	32
8d	16	64	64	32
8e	8	16	16	16
8f	8	16	16	16
8g	8	16	16	16
8h	32	>128	128	128
8i	8	16	16	16
8j	16	64	32	32
8k	>128	>128	>128	>128
8l	64	>128	>128	>128
Ciprofloxacin	0.25	0.25	NT	NT
Amphotericin B	NT	NT	0.5	0.5

NT: Not tested, MIC: Minimum inhibitory concentration, ATCC: American Type Culture Collection

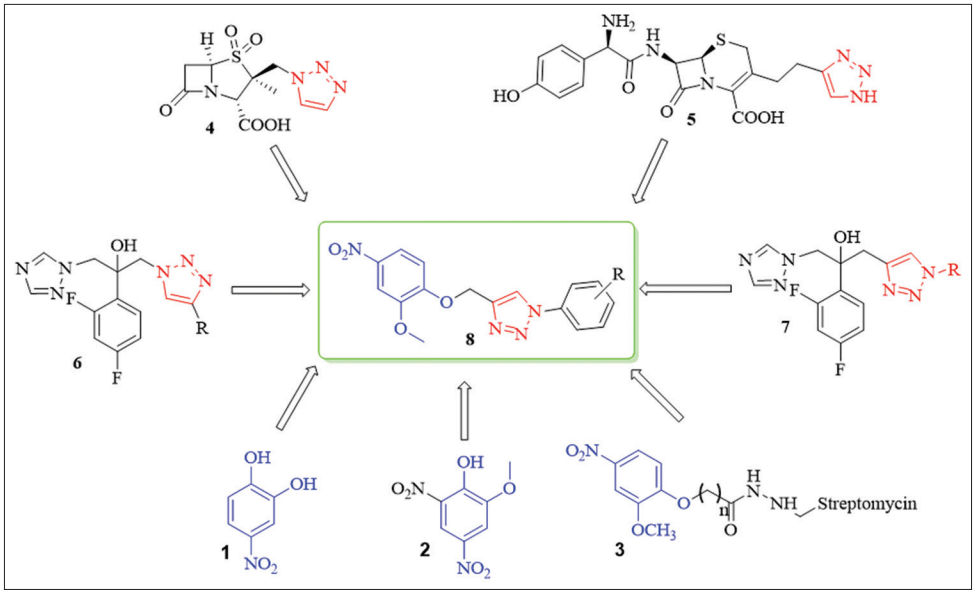


Fig. 1: Hybrid conjugation of bioactive ligand

light blue in color. Then, sodium ascorbate was added at once to the reaction mixture and allowed to stir for 15 min. Reaction mixture color was changed to dark yellow. After 15 min, azide was added at once. The reaction mixture was allowed to stir for further 8 h at ambient temperature. After the completion of the reaction, monitored by TLC, reaction mixture was quenched with water and extracted with ethyl acetate. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the final product.

4-((2-methoxy-4-nitrophenoxy)methyl)-1-(3-nitrophenyl)-1H-1,2,3-triazole (8a)

M.P. **195.7°C**; IR (KBr) (cm^{-1}): 3116, 2366, 2348, 1827, 1730, 1713, 1694, 1680, 1669, 1633, 1613, 1588 1569, 1553, 1534, 1515, 1504, 1468, 1453, 1377, 1340, 1280, 1232, 1136, 1082, 1016, 872, 806, 752; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.95 (s, 3H), 5.46 (s, 2H), 7.17 (d, 1H, *J*=9.20 Hz), 7.73–7.77 (m, 2H), 7.88–7.90 (dd, 1H, *J*=9.20 and 2.80 Hz), 8.16–8.18 (dd, 1H, *J*=8.00 and 1.20 Hz), 8.23 (s, 1H), 8.30–8.32 (dd, 1H, *J*=8.40 and 1.20 Hz), 8.58 (t, 1H, *J*=1.60 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.33, 62.88, 106.91, 112.11, 115.34, 117.54, 121.29, 123.46, 125.99, 131.07, 137.51, 142.21, 148.97, 149.32, 152.76; ESIMS: 372 (M^++1), 394 (M^++Na); Anal. Calcd. for $C_{16}H_{13}N_5O_6$: C, 51.76; H, 3.53; N, 18.86%, Found: C, 51.77; H, 3.54; N, 18.82%.

4-((2-methoxy-4-nitrophenoxy)methyl)-1-(4-nitrophenyl)-1H-1,2,3-triazole (8b)

M.P. **226.7°C**; IR (KBr) (cm^{-1}): 3132, 2359, 2338, 1729, 1712, 1693, 1679, 1668, 1644, 1631, 1594, 1569, 1552, 1513, 1467, 1374, 1341, 1275, 1256, 1097, 1046, 863, 737, 685, 635; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.95 (s, 3H), 5.46 (s, 2H), 7.18 (d, 1H, *J*=8.80 Hz), 7.76 (s, 1H), 7.90 (d, 1H, *J*=7.60 Hz), 7.97 (d, 2H, *J*=8.00 Hz), 8.21 (s, 1H), 8.41 (d, 2H, *J*=8.00 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.33, 62.85, 106.93, 112.14, 117.55, 120.62, 121.21, 125.57, 131.91, 140.88, 147.47, 152.71, 152.88; ESIMS: 372 (M^++1), 394 (M^++Na); Anal. Calcd. for $C_{16}H_{13}N_5O_6$: C, 51.76; H, 3.53; N, 18.86%, Found: C, 51.79; H, 3.50; N, 18.88%.

1-(2-chlorophenyl)-4-((2-methoxy-4-nitrophenoxy)methyl)-1H-1,2,3-triazole (8c)

M.P. **120.4°C**; IR (KBr) (cm^{-1}): 3055, 2918, 2353, 2199, 1865, 1823, 1789, 1763, 1730, 1714, 1694, 1644, 1585, 1568, 1518, 1453, 1415, 1339, 1289, 1231, 1171, 1093,1032, 865, 795, 753, 711,642, 544, 447; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.93 (s, 3H), 5.47 (s, 2H), 7.21 (d, 1H, *J*=8.80 Hz), 7.41–7.47 (m, 2H), 7.55–7.61 (m, 2H), 7.74 (d, 1H, *J*=2.40 Hz), 7.87–7.90 (dd, 1H, *J*=8.80 and 2.00 Hz), 8.10 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.31, 63.06, 106.88, 112.38, 117.58, 125.33, 127.74, 127.97, 130.81, 130.96, 134.65, 142.06, 142.69, 149.36, 153.01; ESIMS: 361 (M^+), 362 (M^++1), 363 (M^++2), 383 (M^++Na); Anal. Calcd.for $C_{16}H_{13}ClN_4O_4$: C, 53.27; H, 3.63; Cl, 9.83; N, 15.53 %, Found: C, 53.26; H, 3.65; Cl, 9.81; N, 15.54%.

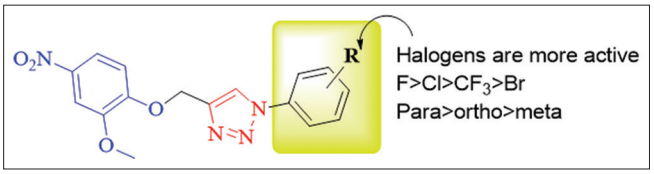


Fig. 2: Structure–activity relationship of 1,2,3-triazole-tethered nitroguaiacol ethers

1-(3-chlorophenyl)-4-((2-methoxy-4-nitrophenoxy)methyl)-1H-1,2,3-triazole (8d)

M.P. **142.6°C**; IR (KBr) (cm^{-1}): 3147, 3091, 2941, 2937, 2179, 1843, 1819, 1788, 1730, 1713, 1694, 1681, 1593, 1551, 1517, 1465, 1402, 1385, 1371, 1337, 1279, 1231, 1140, 989, 873, 849, 806, 788, 745, 678, 641; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.94 (s, 3H), 5.44 (s, 2H), 7.18 (d, 1H, *J*=8.80 Hz), 7.41–7.48 (m, 2H), 7.62 (d, 1H, *J*=7.60 Hz), 7.74–7.76 (m, 2H), 7.87–7.90 (dd, 1H, *J*=8.80 and 2.40 Hz), 8.10 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.31, 62.94, 106.86, 112.11, 117.58, 118.56, 120.87, 121.39, 129.13, 130.89, 135.69, 137.63, 142.10, 149.27, 152.86; ESIMS: 361 (M^+), 362 (M^++1), 363 (M^++2), 383 (M^++Na); Anal. Calcd. for $C_{16}H_{13}ClN_4O_4$: C, 53.27; H, 3.63; Cl, 9.83; N, 15.53 %, Found: C, 53.28; H, 3.61; Cl, 9.85; N, 15.57%.

1-(4-chlorophenyl)-4-((2-methoxy-4-nitrophenoxy)methyl)-1H-1,2,3-triazole (8e)

M.P. **152.4°C**; IR (KBr) (cm^{-1}): 3138, 3092, 2919, 2354, 2198, 1864, 1823, 1789, 1746, 1729, 1713, 1694, 1665, 1644, 1587, 1551, 1514, 1457, 1408, 1345, 1278, 1278, 1227, 1136, 1094, 1025, 774, 739, 628; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.93 (s, 3H), 5.43 (s, 2H), 7.18 (d, 1H, *J*=8.80 Hz), 7.49 (d, 2H, *J*=8.80 Hz), 7.66 (d, 2H, *J*=8.80 Hz), 7.74 (d, 1H, *J*=2.40 Hz), 7.87–7.89 (dd, 1H, *J*=8.80 and 2.40 Hz), 8.08 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.31, 63.06, 106.88, 112.38, 117.58, 125.33, 127.74, 127.97, 130.81, 130.96, 134.65, 142.06, 142.69, 149.36, 153.01; ESIMS: 361 (M^+), 362 (M^++1), 363 (M^++2), 383 (M^++Na); Anal. Calcd. for $C_{16}H_{13}ClN_4O_4$: C, 53.27; H, 3.63; Cl, 9.83; N, 15.53 %, Found: C, 53.24; H, 3.66; Cl, 9.84; N, 15.54%.

1-(2-fluorophenyl)-4-((2-methoxy-4-nitrophenoxy)methyl)-1H-1,2,3-triazoletriazole (8f)

M.P. **130.6**°C; IR (KBr) (cm^{-1}): 3166, 2949, 2843, 2333, 2198, 1864, 1842, 1823, 1789, 1769, 1748, 1731, 1714, 1699, 1644, 1634, 1590, 1550, 1518, 1472, 1410, 1370, 1338, 1282, 1229, 1141, 1098, 864, 804, 758, 711, 635; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.93 (s, 3H), 5.47 (s, 2H), 7.21 (d, 1H, *J*=8.80 Hz), 7.34–7.47 (m, 2H), 7.58–7.63 (m, 2H), 7.74 (d, 1H, *J*=2.40 Hz), 7.87–7.90 (dd, 1H, *J*=8.80 and 2.00 Hz), 8.12 (s, 1H); ESIMS: 345 (M^++1), 367 (M^++Na); Anal. Calcd.for $C_{16}H_{13}FN_4O_4$: C, 55.82; H, 3.81; F, 5.52; N, 16.27 %, Found: C, 55.79; H, 3.83; F, 5.51, N, 16.29%.

1-(4-fluorophenyl)-4-((2-methoxy-4-nitrophenoxy)methyl)-1H-1,2,3-triazoletriazole (8g)

M.P. **120.5**°C; IR (KBr) (cm^{-1}): 3161, 3093, 2909, 2342, 2202, 1865, 1841, 1823, 1789, 1769, 1730, 1714, 1694, 1644, 1587, 1568, 1550, 1517, 1463, 1391, 1371, 1341, 1281, 1261, 1233, 1048, 1025, 869, 838, 803, 774, 639, 616; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.93 (s, 3H), 5.43 (s, 2H), 7.18–7.22 (m, 3H), 7.67–7.70 (m, 2H), 7.74 (d, 1H, *J*=1.60 Hz), 7.86–7.89 (dd, 1H, *J*=8.80 and 2.00 Hz), 8.05 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.31, 62.95, 106.83, 112.10, 116.68, 116.91, 117.58, 121.61, 122.59, 122.68, 133.09, 142.06, 143.70, 149.25, 152.91, 161.34, 163.82; ESIMS: 345 (M^++1), 346 (M^++2), 367 (M^++Na); Anal. Calcd. for $C_{16}H_{13}FN_4O_4$: C, 55.82; H, 3.81; F, 5.52; N, 16.27 %, Found: C, 55.83; H, 3.78; F, 5.51; N, 16.31%.

1-(4-bromophenyl)-4-((2-methoxy-4-nitrophenoxy)methyl)-1H-1,2,3-triazoletriazole (8h)

M.P. **166.4**°C; IR (KBr) (cm^{-1}): 3141, 3090, 2938, 2360, 2328, 1948, 1729, 1688, 1681, 1643, 1587, 1499, 1453, 1408, 1385, 1369, 1283, 1228, 1173, 1021, 981, 843, 818, 799, 729, 628; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.94 (s, 3H), 5.44 (s, 2H), 7.19 (d, 1H, *J*=8.80 Hz), 7.59–7.66 (m, 4H), 7.74 (d, 1H, *J*=2.40 Hz), 7.87–7.90 (dd, 1H, *J*=8.80 and 2.40 Hz), 8.09 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.32, 62.93, 106.83, 112.10, 117.58, 121.27, 121.98, 122.78, 132.99, 135.75, 142.09, 143.87, 149.23, 152.87; ESIMS: 405 (M^+), 407 (M^++2), 427 (M^++Na), 429 (M^++2+Na); Anal. Calcd. for $C_{16}H_{13}BrN_4O_4$: C, 47.43; H, 3.23; Br, 19.72; N, 13.83 %, Found: C, 47.45; H, 3.24; Br, 19.69; N, 13.84%.

4-((2-methoxy-4-nitrophenoxy)methyl)-1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole (8i)

M.P. **128.3°C**; IR (KBr) (cm^{-1}): 3147, 3093, 2840, 2353, 2198, 1864, 1823, 1789, 1761, 1748, 1730, 1717, 1694, 1644, 1588, 1551, 1519, 1401, 1341, 1282, 1235, 1174, 1101, 1027, 865, 801, 739, 691, 683; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.94 (s, 3H), 5.44 (s, 2H), 7.18 (d, 1H, *J*=8.80 Hz), 7.65–7.75 (m, 3H), 7.87–7.90 (dd, 1H, *J*=8.80 and 2.40 Hz), 7.94 (d, 1H, *J*= 7.60 Hz), 8.00 (s, 1H), 8.17 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.31, 62.92, 106.91, 112.15, 117.56, 121.34, 123.63, 125.65, 130.62, 137.14, 142.15, 144.14, 149.30, 152.85; ESIMS: 395 (M^++1); Anal. Calcd. for $C_{17}H_{13}F_3N_4O_4$: C, 51.78; H, 3.32; F, 14.45; N, 14.21 %, Found: C, 51.81; H, 3.32; F, 14.47; N, 14.19%.

4-(4-((2-methoxy-4-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl) benzonitrile (8j)

M.P. **179.6°C**; IR (KBr) (cm^{-1}): 2927, 2227, 1866, 1838, 1822, 1783, 1730, 1713, 1694, 1644, 1588, 1551, 1505, 1444, 1394, 1370, 1333, 1279, 1222, 1178, 1094, 1017, 839, 800, 739, 639; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.94 (s, 3H), 5.45 (s, 2H), 7.17 (d, 1H, *J*=9.20 Hz), 7.74 (d, 1H, *J*=2.00 Hz), 7.83 (d, 2H, *J*=8.00 Hz), 7.87–7.91 (m, 3H), 8.18 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 51.57, 58.11, 102.18, 107.40, 108.09, 112.79, 115.54, 115.92, 116.33, 124.43, 129.20, 134.81, 137.47, 139.71, 144.56, 148.01; ESIMS: 352.20 (M^++1), 353 (M^++2), 374 (M^++Na), 375 (M^++1+Na); Anal. Calcd. for $C_{17}H_{13}N_5O_4$: C, 58.12; H, 3.73; N, 19.93%, Found: C, 58.09; H, 3.75; N, 19.94%.

1-(4-ethylphenyl)-4-((2-methoxy-4-nitrophenoxy)methyl)-1H-1,2,3-triazole (8k)

M.P. **131.7°C**; IR (KBr) (cm^{-1}): 32955, 2923, 2796, 1633, 1614, 1574, 1557, 1455, 1446, 1425, 1377, 1308, 1270, 1227, 1140, 1036, 989, 779, 750; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.25 (t, 3H, *J*=7.60 Hz), 2.70 (q, 2H, *J*=7.60 Hz), 3.94 (s, 3H), 5.43 (s, 2H), 7.22 (d, 1H, *J*=8.40 Hz), 7.33 (d, 1H, *J*=8.00 Hz), 7.61 (d, 2H, *J*= 8.00 Hz), 7.74 (s, 1H), 7.88 (d, 1H, *J*=6.80 Hz), 8.19 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 15.32, 18.43, 56.31, 62.98, 106.83, 112.19, 117.60, 120.71, 129.16, 134.97, 142.02, 145.54, 149.28, 153.01; ESIMS: 355 (M^++1), 377 (M^++Na); Anal. Calcd. for $C_{18}H_{18}N_4O_4$: C, 61.01; H, 5.12; N, 15.81%, Found: C, 61.04; H, 5.09; N, 15.79%.

4-((2-methoxy-4-nitrophenoxy)methyl)-1-(2-methoxyphenyl)-1H-1,2,3-triazole (8l)

M.P. **154.4°C**; IR (KBr) (cm^{-1}): 3144, 2964, 2392, 2187, 1847, 1824, 1789, 1764, 1730, 1713, 1694, 1674, 1644, 1587, 1552, 1516, 1401, 1370, 1343, 1277, 1222, 1096, 1024, 850, 801, 741, 633; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.86 (s, 3H), 3.93 (s, 3H), 5.45 (s, 2H), 7.06–7.10 (m, 2H), 7.25 (d, 1H, *J*=8.40 Hz), 7.41 (t, 1H, *J*=8.00 Hz), 7.75 (t, 2H, *J*=7.60 Hz), 7.88 (d, 1H, *J*=9.20 Hz), 8.24 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.96, 56.31, 62.98, 106.83, 112.30, 117.61, 121.27, 125.38, 125.65, 130.27, 141.95, 149.30, 151.03, 153.19; ESIMS: 357 (M^++1), 379 (M^++Na); Anal. Calcd. for $C_{17}H_{16}N_4O_5$: C, 57.30; H, 4.53; N, 15.72%, Found: C, 57.28; H, 4.53; N, 15.74%.

Biology

Microbial strains

One reference each of the following species was used for their *in vitro* susceptibility to nitroguaiacols in this study: *S. aureus* American Type Culture Collection (ATCC) 29213, *P. aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028, and *Candida parapsilosis* ATCC 22019. These strains were procured from the ATCC, Manassas, VA, USA.

Antimicrobial susceptibility testing assays

The antibacterial and antifungal activities of the nitroguaiacols were performed by broth microdilution methods as per the guidelines of Clinical and Laboratory Standard Institute **(CLSI M07–A8, 2008; CLSI M27–A3, 2008)** [32,33]. Stock solutions were prepared in 100% dimethyl sulfoxide (DMSO); distilled water was used for ciprofloxacin), with a final DMSO concentration of 1% (vol. per vol.) and 2-fold serial dilutions were prepared in media to yield twice the final concentration required for testing, which ranged from 128 to 0.25 µg/mL for nitroguaiacol and 8 to 0.015 µg/mL for ciprofloxacin as well as amphotericin B. The final inoculum concentration of approximately 5×10^5 CFU/mL for bacteria **(CLSI M07–A8, 2008)** and approximately 2.5×10^3 CFU/mL for *Candida* species **(CLSI M27–A3, 2008)**. Ciprofloxacin and amphotericin B served as the standard drug controls for bacterial and fungal strains, respectively. The microtiter plates were incubated at 35°C for 24 h for bacterial strains and 48 h for fungal strains. The plates were read visually and the MIC was defined as the lowest concentration of test sample that prevented visible growth with respect to the growth control. All experiments were conducted twice in duplicates on separate occasions with freshly prepared inoculums and stock solutions.

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CONFLICTS OF INTEREST

None of the authors in this manuscript have conflicts of interest.

STATEMENT OF COMPETING INTEREST

I confirm that none of the authors have any competing interests in the manuscript.

AUTHORS' CONTRIBUTIONS

All authors are contributed in the manuscript preparation.

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