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Synthesis and Antibacterial Activity of N-substituted-1-benzyl-*1H*-1,2,3-triazole-carbohydrazide derivatives

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ABSTRACT

*The synthesis of 1-benzyl-1H-1,2,3-triazole-4-carbohydrazides **6a-j** data was prepared in four steps from commercially available benzyl bromide **1**. Reaction of benzylbromide **1** with sodium azide gave 1-(azidomethyl)benzene **2,** which upon **c**ondensation with ethyl(triphenylphosphine phosphoranyldene)pyruvate **3** in xylene in sealed tube gave ethyl 1-benzyl-1H-1,2,3-triazole-4-carboxylate **4**. Hydrazinolysis of ethylester **4** followed by the condensation with various aromatic / heteroaromatic aldehydes **a-j** in ethanol at reflux produced 1-benzyl-1H-1,2,3-triazole-N-substituted hydrazone derivatives **6a-j** in quantitative yields. The structural assignment of the synthesized hydrazone derivatives **6a-j** was determined by the spectroscopic techniques like ^{1}H NMR, IR and mass spectral. Compounds **6a-j** were screened in vitro at a concentration of 250 µg/mL for antibacterial activity against two Gram-positive (Staphylococcus aureus and Staphylococcus pyogenes) and two Gram-negative strains (Escherichia coli and Pseudomonas aeruginosa) with reference to the standard antibacterial drug ciprofloxacin (250 µg/disc). Compounds **6c** (Quinaxaline ring), **6d** (Imidazole ring), **6e** (Pyridine ring) and **6f** (Quinoline ring) exhibited significant antibacterial activity.*

Keywords: Synthesis, 1,2,3-Triazole, Carbohydrazides, Antibacterial activity

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INTRODUCTION

The chemical structure diversity of the 1,2,3-triazole family and their biological activities made these compounds to become attractive targets in synthetic organic chemistry [1]. 1,2,3-triazole moiety does not occur in nature, although synthetic molecules containing 1,2,3-triazole have shown several biological activities including antibacterial, herbicidal, fungicidal, antiallergic and anti-HIV [2,3]. Literature survey has recently reported the preparation of triazole derivatives linked to carbohydrates and biological evaluation of their glicoconjugates, have been shown to stand out as HIV reverse transcriptase (HIV-RT) inhibitors1, anti-trypanosomal agents [2,4] inhibitors of α-glucosidases [3,5] antitubercular activity [6,7] and antitumor agents [4,8]. 1,2,3-Triazoles have found widespread use in pharmaceuticals and agrochemicals [9-11]. Several drugs like Carboxyamidotriazole, Cefatrizine, Rufinamide and Tazobactam bear 1,2,3-triazole in their structure [12-13]. The synthesis of substituted 1,2,3- triazoles strongly relies on Huisgen's 1,3-dipolar cycloaddition between organic azides and substituted alkynes [14].

In spite of the introduction of a variety of antibacterial agents in multiple unrelated drug classes, resistance continues to emerge. The pharmaceutical field (including academic) must respond to these clinical challenges by bringing forward a stream of new agents with promising antibacterial activity against bacteria, Advantages of these agents include their higher predictability for success, well-defined biomarkers, shorter clinical trials, and shorter duration of

therapy leading to fewer long-term safety concerns [15]. Due to the rapid development of bacterial resistance to antibacterial agents, it is vital to discover novel scaffolds for the design and synthesis of new antibacterial agents to help in the battle against pathogenic microorganisms. A number of these compounds are today's blockbusters of the antibacterial market due to their therapeutic efficacy having tolerable side-effects and thus, challenging the predominance of well established β-lactum antibiotics which are becoming more prone to the resistant pathogenic bacteria. The present paper reports the synthesis, characterization and antibacterial activity of some new N-substituted-1-benzyl-1H-1,2,3-triazole-carbohydrazide derivatives **6a-6j**.

MATERIALS AND METHODS

Standard operating procedures was implemented for the purification of solvents before being utilized for the reactions and work up's. Merck silica gel 60 (230-400 mesh) and Merck pre-coated plates (silica gel 60 F254) was used for the routine column chromatography and visualization of spots (under UV lamp). Mel-temp apparatus was utilized for the determination of melting point (m.p). Agilent ion trap MS was utilized for recording the mass spectra. Perkin Elmer FT-IR spectrometer was used for recording the IR data. Varian NMR-300MHz and 500 MHz instrument was used to record ^{1}H NMR spectra. Chemical shifts values are measured in terms of δ ppm (parts per million) with reference to tetramethylsilane (TMS) as internal standard. The following notations *viz.*, singlet-s, doublet-d, double doublet-dd, and multiplet-m was used for the signals that has appeared in the proton NMR spectrum and the coupling constant value was measured in terms of Hz.

Experimental methods

Preparation of 1-(azidomethyl)benzene (2)

To a stirred solution of benzylbromide **1** (1g, 5.84 mmol) in DMF (10 mL) was added NaN_3 (460 mg, 7.0 mmol). The reaction mixture was heated to 90^{o}C for 2h. The reaction mixture was cooled to room temperature and diluted with ethylacetate (25 mL). The organic layer was washed with water (2 x 15 mL) followed by brine solution (25 mL). The organic layer was separated, dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to obtain the crude compound **2**. The crude compound was taken to next step without further purification. Yellow liquid; Yield**:** 0.7 g, 88%.

Preparation of ethyl 1-benzyl-1H-1,2,3-triazole-4-carboxylate (4)

To a stirred solution of ethyl(triphenylphosphine phosphoranyldene)pyruvate **3** (10.15g, 26.994 mmol) in xylene (30 mL) was added 1-(azidomethyl)benzene **2** (3g, 22.556 mmol). The reaction mixture was refluxed for 16 h in a closed vessel. The reaction mixture was concentrated and the residue was extracted with ethyl acetate. The organic layer was washed with water followed by brine solution, dried (over Na_2SO_4, filtered and evaporated under reduced pressure to obtain crude compound **4**. The crude compound was purified by column chromatography (using silica gel 60 – 120 mesh , 6% ethyl acetate in pet ether); Pale yellow liquid; Yield: 1.8 g, 35 %; FT-IR (KBr pellet): ν_{max} 3124, 3064, 3035, 2980, 2937, 1718, 1539, 1494, 1460, 1392, 1367, 1301, 1228, 1136, 1095, 1043, 1020, 993, 862, 779, 717, 690, 576, 451 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.82 (s, 1H), 7.38-7.34 (m, 5H), 5.62 (s, 2H), 4.30 (q, J = 5.6 Hz, 2H), 1.30 (t, J = 5.6 Hz, 3H); ESIMS: m/z 232.0 $(M+H)^+$.

Preparation of 1-benzyl-1H-1,2,3-triazole-4-carbohydrazide (5)

To a stirred solution of compound **4** (1.0g, 4.32 mmol) in ethanol (10 mL) was added hydrazine- hydrate (1.50 g, 30.25 mmol) and refluxed for 2 h. The reaction mixture was cooled to room temperature and the precipitated solid was filtered and washed with pet-ether and dried under vacuum to obtain 1-benzyl-1H-1,2,3-triazole-4-carbohydrazide **5**. White solid; Yield: 0.84 g, 90%; M.p: 116-167 ^{o}C; FT-IR (KBr pellet): ν_{max} 3331, 3275, 3009, 3039, 2954, 1762, 1660, 1606, 1564, 1492, 1452, 1429, 1344, 1263, 1228, 1205, 1095, 1045, 1020, 937, 889, 860, 821, 717, 673, 630, 572, 476 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 9.70 (brs, 1H), 8.58 (s, 1H0, 7.38-7.34 (m, 5H), 5.60 (s, 2H), 4.40 (s, 2H); ESIMS: m/z 218.0 $(M+H)^+$.

General Experimental Procedure for the Synthesis of Hydrazone derivatives (6a-j)

To a stirred solution of compound **5** (0.23 mmol) in ethanol was added aromatic and hetero aromatic aldehydes (**a-j**, 0.23 mmol) and refluxed for 1 h. The reaction mixture was cooled to room temperature and filtered the precipitated solids and washed with pet-ether, to obtain the pure compounds **6a-j.** Yields of the products varied between 90 and 98%.

1-benzyl-*N*'-[(*E*)-(5-nitrothiophen-3-yl)methylidene]-1*H*-1,2,3-triazole-4-carbohydrazide (6a)
Pale yellow solid; Yield: 94%; M.p: 100-102 ^{o}C; FT-IR (KBr pellet): ν_{max} 3232, 3057, 1668, 1608, 1568, 1535, 1500, 1450, 1344, 1222, 1068, 1045, 945, 879, 864, 815, 719, 669, 650, 621, 574 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.22 (s, 1H), 8.82 (s, 1H), 8.26 (s, 1H), 7.38-7.24 (m, 5H), 5.70 (s, 2H); ESIMS: m/z 336.1 $(M+H)^{+}$.

1-benzyl-*N*'-[(*E*)-(5-nitrothiophen-2-yl)methylidene]-1*H*-1,2,3-triazole-4-carbohydrazide (6b)
Pale yellow solid; Yield: 94%; M.p: 131-132 ^{o}C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.50 (s, 1H), 8.90 (s, 1H), 8.70 (s, 1H), 8.08 (d, J = 3.6 Hz, 1H), 7.58 (d, J = 3.6 Hz, 1H), 7.38-7.26 (m, 5H), 5.70 (s, 2H); ESIMS: m/z 357.0 $(M+H)^{+}$.

1-benzyl-*N*'-[(*E*)-quinoxalin-2-ylmethylidene]-1*H*-1,2,3-triazole-4-carbohydrazide (6c)
Yellow solid; Yield: 96%; M.p: 139-140^{o}C; FT-IR (KBr pellet): ν_{max} 3255, 3134, 3059, 2989, 1670, 1568, 1512, 1460, 1359, 1323, 1230, 1215, 1124, 1078, 954, 869, 759, 715, 655, 626, 574, 480, 412 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.70 (s, 1H), 9.42 (s, 1H), 8.98 (s, 1H), 8.78 (s, 1H), 8.18 (d, J = 4.6 Hz, 2H), 7.90 (d, J = 4.6 Hz, 2H), 7.44-7.38 (m, 5H), 5.68 (s, 2H); ESIMS: m/z 358.0 $(M+H)^{+}$.

1-benzyl-*N*'-[(*E*)-(4-phenyl-2*H*-imidazol-2-yl)methylidene]-1*H*-1,2,3-triazole-4-carbohydrazide (6d)
Pale yellow; Yield: 96%; M.p: 125-126^{o}C; FT-IR (KBr pellet): ν_{max} 3265, 3115, 3059, 1668, 1604, 1558, 1506, 1485, 1369, 1300, 1244, 1213, 1149, 1049, 968, 900, 873, 819, 713, 690, 651, 621, 578, 532 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.16 (s, 1H), 8.82 (s, 1H), 8.58 (s, 1H), 8.28 (s, 1H), 7.82-7.74 (m, 5H), 7.44-7.38 (m, 5H), 7.18 (s, 1H), 5.64 (s, 2H); ESIMS: m/z 372.0 $(M+H)^{+}$.

1-benzyl-*N*'-[(*E*)-(4-phenylpyridin-2-yl)methylidene]-1*H*-1,2,3-triazole-4-carbohydrazide (6e)
Off-white solid; Yield: 94%; M.p: 80-81^{o}C; FT-IR (KBr pellet): ν_{max} 3290, 3109, 3055, 3008, 1670, 1604, 1562, 1512, 1460, 1431, 1369, 1311, 1242, 1211, 1147, 1055, 1022, 962, 900, 877, 844, 781, 715, 646, 597, 570, 482, 405 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.18 (s, 1H), 8.90 (s, 1H), 8.70 (d, J = 7.2 Hz, 1H), 8.58 (s, 1H), 8.20 (t, J = 7.6 Hz, 2H), 8.06 (t, J =6.8 Hz, 1H), 7.96-7.94 (m, 11H), 7.80 (t, J = 7.6 Hz, 2H), 7.38-7.28 (m, 6H), 5.70 (s, 2H); ESIMS: m/z 383.0 $(M+H)^{+}$.

1-benzyl-*N*'-[(*E*)-quinolin-4-ylmethylidene]-1*H*-1,2,3-triazole-4-carbohydrazide (6f)
Yellow solid; Yield: 92%; M.p: 151-152^{o}C; FT-IR (KBr pellet): ν_{max} 3253, 3130, 3059, 1666, 1588, 1502, 1458, 1392, 1365, 1222, 1068, 1049, 958, 902, 852, 761, 711, 655, 628, 574, 534, 433, 368 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.42 (s, 1H), 9.24 (s, 1H), 8.96 (s, 1H), 8.62 (t, J = 5.8 Hz, 1H), 8.10 (d, J = 5.6 Hz, 2H), 7.82 (t, J = 5.4 Hz, 2H), 7.78-7.74 (m, 1H), 7.38-7.28 (m, 5H), 5.70 (s, 2H); ESIMS: m/z 357.0 $(M+H)^{+}$.

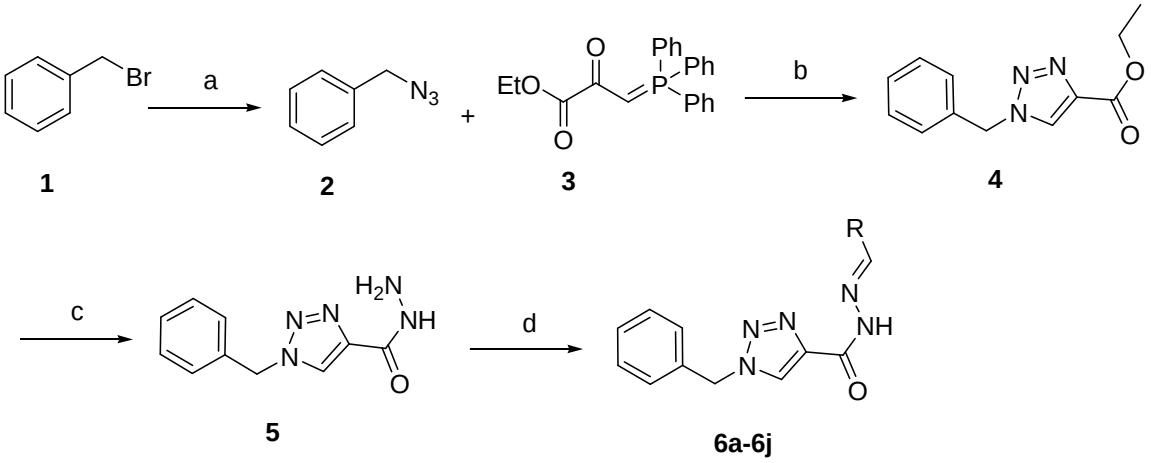
(*E*)-N'-(4-(methylsulfonyl)benzylidene)-1-benzyl-1H-1,2,3-triazole-4-carbohydrazide (6g)
White solid; Yield:95%; M.p: 126-127 ^{o}C; FT-IR (KBr pellet): ν_{max} 3277, 3115, 1730, 1676, 1606, 1566, 1517, 1409, 1458, 1406, 1375, 1301, 1290, 1242, 1217, 1147, 1082, 1053, 1022, 974, 900, 867, 839, 775, 713, 694, 650, 619, 572, 543, 511 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.38 (s, 1H, -CO-NH-), 8.90 (s, 1H, -N=CH-), 8.62 (s, 1H, Triazole ring proton), 7.98 (d, J = 7.8 Hz, 2H, p-SO_2CH_3-Phenyl ring), 7.94 (d, J = 7.8 Hz, 2H, p-SO_2CH_3-Phenyl ring), 7.42-7.38 (m, 5H, Phenyl ring), 5.70 (s, 2H, -CH_2-), 3.20 (s, 3H, -SO_2-CH_3); ESIMS: m/z 383.9 $(M+H)^{+}$.

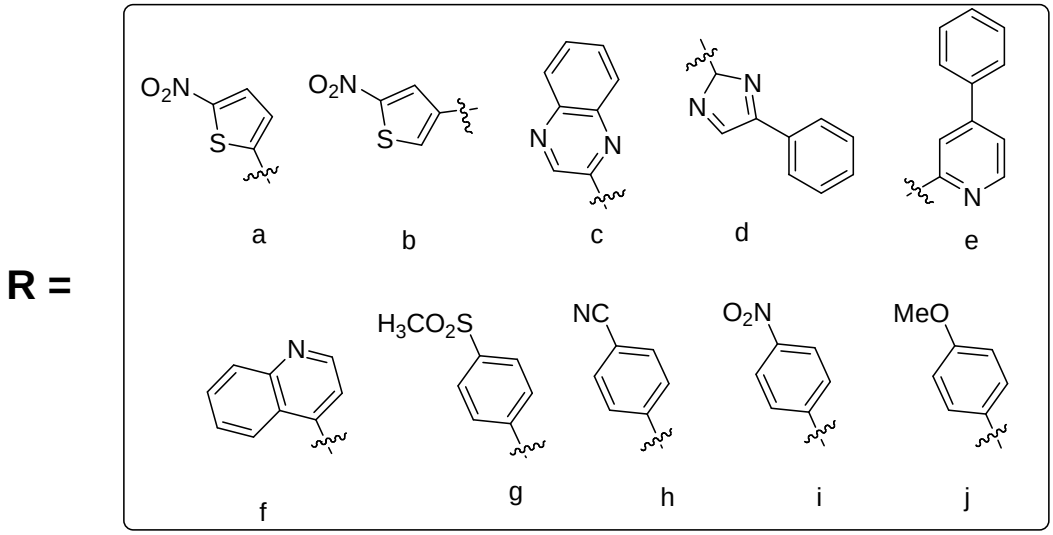
(*E*)-N'-(4-(cyano)benzylidene)-1-benzyl-1H-1,2,3-triazole-4-carbohydrazide (6h)
White solid; Yield:94%; M.p: 111-112 ^{o}C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.36 (s, 1H), 8.88 (s, 1H), 8.60 (s, 1H), 7.96 (d, J = 7.8 Hz, 2H), 7.92 (d, J = 7.8 Hz, 2H), 7.44-7.40 (m, 5H), 5.68 (s, 2H), 3.20 (s, 3H); ESIMS: m/z 331.2 $(M+H)^{+}$.

(*E*)-N'-(4-nitrobenzylidene)-1-benzyl-1H-1,2,3-triazole-4-carbohydrazide (6i)
White solid; Yield: 98%; M.p: 119-120 ^{o}C; FT-IR (KBr pellet): ν_{max} 3263, 3118, 3061, 1670, 1587, 1558, 1516, 1458, 1371, 1342, 1240, 1209, 1068, 1049, 1022, 966, 900, 852, 823, 715, 690, 653, 623, 578, 505 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.40 (s, 1H), 8.90 (s, 1H), 8.62 (s, 1H), 8.30 (d, J = 8.2 Hz, 2H), 7.92 (d, J = 8.2 Hz, 2H), 7.40-7.36 (m, 5H), 5.70 (s, 2H); ESIMS: m/z 350.9 $(M+H)^{+}$.

(*E*)-N'-(4-methoxybenzylidene)-1-benzyl-1H-1,2,3-triazole-4-carbohydrazide (6j)
Off-White solid; Yield: 92%; M.p: 139-140 ^{o}C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.28 (s, 1H), 8.78 (s, 1H), 8.60 (s, 1H), 8.26 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.2 Hz, 2H), 7.38-7.36 (m, 5H), 5.70 (s, 2H); ESIMS: m/z 350.9 $(M+H)^{+}$.

Antibacterial Screening
The synthesized compounds **6a-j** was screened *in-vitro* at a concentration of 250µg/mL for antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus pyogenes) and* Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa).* The antibacterial activity was determined using disc diffusion method by measuring zone of inhibition in mm [16] and tabulated in **Table-1.** Each experiment was done in triplicate and the average reading was taken. Standard antibacterial drug ciprofloxacin (250 µg/disc) was also tested under similar conditions against these organisms Compounds (**6a-j)** were dissolved in dimethyl sulphoxide at 250 µg/mL concentration. Growth inhibition was calculated with reference to positive control. The inhibition zones were measured in millimeters at the end of an incubation period of 48 hours at (35±2) °C. DMSO alone showed no inhibition. The composition of nutrient agar medium was, Bactotryptone (10 g), yeast extract (5 g), NaCl (10 g), final pH 7.4.





SCHEME-1: Synthesis of novel 1,2,3-Triazole carbohydrazides 6a-j
EXPERIMENTAL CONDITIONS: *a) NaN_3, DMF, 90^{o}C, 2h; b) ethyl(triphenylphosphine phosphoranyldene)pyruvate, xylene, reflux, 16h; c) Hydrazine-hydrate, Ethanol, reflux, 2h; d) Aromatic/Hetero aromatic aldehydes **a-j**, Ethanol, reflux, 1h.*

RESULTS AND DISCUSSION

The synthesis of 1-benzyl-1H-1,2,3-triazole-4-carbohydrazides **6a-j** is illustrated in scheme **1**. Reaction of benzylbromide **1** with sodium azide in DMF at 90^{o}C for 2h gave 1-(azidomethyl)benzene **2.** Condensation of azide **2** with ethyl(triphenylphosphine phosphoranyldene)pyruvate **3** in xylene in sealed tube at 16h resulted in ethyl 1-benzyl-1H-1,2,3-triazole-4-carboxylate **4**. Hydrazinolysis of ethylester **4** in presence of hydrazine hydrate at reflux for 2h resulted in 1-benzyl-1H-1,2,3-triazole-4-carbohydrazide **5.** Condensation of carbohydrazide **5** with various aromatic / heteroaromatic aldehydes **a-j** in ethanol at reflux for 1h produced 1-benzyl-1H-1,2,3-triazole-N-substituted hydrazone derivatives **6a-j** in quantitative yields. The structural assignment of the above synthesized hydrazone derivatives **6a-j** was determined by the spectroscopic techniques like ^{1}H NMR, IR and mass spectral data. As a representative example, (*E*)-N'-(4-(methylsulfonyl)benzylidene)-1-benzyl-1H-1,2,3-triazole-4-carbohydrazide is described here, the protons resonating at 12.38 ppm, 8.90 ppm and 8.62 ppm as singlets corresponds to –CONH-, -N=CH- and Triazole ring proton respectively. The proton resonating at 7.98 ppm and 7.94 ppm as doublets is assigned to p-SO_2CH_3 phenyl ring and the multiplet at 7.42-7.38 ppm is assigned to phenyl ring protons. The methylene and methyl protons resonated at 5.70 ppm and 3.20 ppm as singlets respectively. The mass spectral data of the above described compound is in concurrence with the desired molecular formulae (ESIMS: m/z 383.9 $(M+H)^{+}$. Similarly, the ^{1}H NMR, mass and IR spectral data of all the final compounds and the associated intermediates are in agreement with the desired structure.

Table 1: Antibacterial activity data of novel hydrazo-hydrazide derivatives 6a-j

Compound no.	*Gram negative bacteria*		*Gram positive bacteria*	
	***E. coli* MTCC 443**	***P. aeruginosa* MTCC 424**	***S.aureus* MTCC 96**	***S.poygenes* MTCC 442**
	Diameter of Zone of inhibition in mm			
6a	20	20	15	16
6b	20	21	17	16
6c	27	26	21	23
6d	26	25	23	22
6e	25	26	22	21
6f	24	23	22	23
6g	18	19	18	19
6h	16	15	18	18
6i	17	17	16	17
6j	15	16	17	16
Ciprofloxacin (Conc. 250 µg/mL)	28	27	22	22

Antibacterial Activity

The results of antibacterial activity of novel triazole-carbohydrazide derivatives **6a-j** is tabulated in Table 1. The antibacterial activity was measured in terms of zone of inhibition (ZI, in mm). From table-1, it is observed that, in general, compounds **6c** (Quinaxaline ring), **6d** (Imidazole ring), **6e** (Pyridine ring) and **6f** (Quinoline ring) exhibited significant antibacterial activity, while the compounds **6a** and **6b** with thiophene ring displayed good antibacterial activity. The remaining compounds **6g-6i** with aromatic substitution showed moderate antibacterial activity.

CONCLUSION

In conclusion, we have reported the synthesis of novel 1-benzyl-1H-1,2,3-triazole-4-carbohydrazides **6a-j**. The structural determination of all the compounds was determined by IR, ^{1}HNMR and mass spectroscopic techniques. The antibacterial activity test results revealed that 1-benzyl-1H-1,2,3-triazole-4-carbohydrazide derivatives embedded with hetrocyclic rings such as Quinaxaline, Quinoline, Imiddazole and Pyridine ring exhibited significant antibacterial activity.

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