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Synthesis and Antimicrobial Activity of 1,2,3-Triazoles Containing Quinoline Moiety

V. Sumangala[1,2], Boja Poojary[1], N. Chidananda[1,2], Jennifer Fernandes[3], and N. Suchetha Kumari[4]

[1]Department of Chemistry, Mangalore University, Mangalagangothri-574199, Karnataka, India, [2]SeQuent Scientific Limited, Mangalore-575011, Karnataka, India, [3]Department of Pharmachemistry, N G S M College of Pharmacy, Panner, Deralakatte-574 160, Karnataka, India, and [4]Department of Biochemistry, K.S.Hegde Medical Academy, Deralakatte-574 162, Karnataka, India

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A new series of substituted 1,2,3-triazoles (**4a-n**) were synthesized from 4-azido-2,8-bistrifluoromethylquinoline **2**. The 1,3-dipolar cycloaddition reaction of **2** with ethyl acetoacetate afforded 1-(2,8-Bistrifluoromethylquinolin-4-yl)-5-methyl-1,2,3-triazole-4-carboxylic acid **3**, which was then converted into its corresponding acid hydrazide **3a**. Condensation of this hydrazide with different aromatic aldehydes resulted in the formation of Schiff's bases, N-[1-Arylmethylene]-1-[2,8-bistrifluoromethylquinoline-4-yl]-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides (**4a-n**). These newly synthesized 1,2,3-triazole derivatives were characterized by analytical and spectral data. All the synthesized compounds were evaluated *in vitro* for their antibacterial and antifungal activity. A brief investigation of the structure activity relationships revealed that the nature of the substituent on position 4 of the triazole ring influences the antimicrobial activity. Among the newly synthesized compounds, the most active compound was **4n**, which contained the 3-methylthien-2-yl moiety and showed a broad spectrum of antimicrobial activity against all the strains used for testing. Compounds **4b**, **4c**, **4e**, **4f**, **4h** and **4l** showed significant antimicrobial activity at the concentration of 6.25 μg/mL.

Key words: 1,3-Dipolar cycloaddition, 4-Bromo-2,8-bistrifluoromethylquinoline, 4-Azido-2,8-bistrifluoromethylquinoline, Ethyl acetoacetate, Antimicrobial activity

INTRODUCTION

The increased prevalence of diseases caused by microorganisms has become a worldwide problem. The gradual development of pathogen resistance to routinely used pesticides demands a renewed effort to seek antimicrobial agents effective against pathogenic microbes (Vinaya et al., 2009). Furthermore, the use of combinations of pesticides with different modes of activity can delay the process of resistance development among these pathogens. Fungicide mixtures may also interact synergistically so that the amount of active ingredient used can be reduced. The control of routine disease caused by bacterial and fungal pathogens has become challenging due to the rapid spread of these pathogens as well as the lack of economic feasibility. In this context, development of new chemicals with dual activity against bacterial and fungal pathogens is required to overcome this problem.

Functionalized 1,2,3-triazoles constitute one of the common fragments in biologically active compounds (Bohm and Karow, 1981). This has resulted in a wealth of synthetic methodologies for their preparation and incorporation in more complex structures. Interest in these compounds continues to be expressed in the pharmaceutical community, and the biological properties of these agents have been the subject of ongoing investigations (Campos et al., 2009).

The triazole scaffold has a wide range of therapeutic uses as it is found ubiquitously in drugs. The derivatives of 1,2,3-triazoles constitute an important family of heterocyclic compounds due to their chemotherapeu-

Correspondence to: Boja Poojary, Department of Chemistry, Mangalore University, Mangalagangothri, Karnataka 574 199, India
Tel: 91-824-2287262, Fax: 91-824-2284367
E-mail: bojapoojary@gmail.com

tic values (Sanghvi et al., 1990). Some 1,2,3-triazoles are used as DNA cleaving agents and potassium channel activators (Biagi et al., 2004). Since many of them have remarkable antimicrobial (Karimkulov et al., 1991; Sherement et al., 2004), analgesic (Savini et al., 1994), anti-inflammatory (Savini et al., 1994), local anesthetic (Banu et al., 1999), antimalarial (Julino and Malcolm, 1998), antiviral (Diana and Nitz, 1993), antiproliferating (Manfredini et al., 2000), anticonvulsant (Meier, 1986), antineoplastic (Passannanti et al., 1998) and anticancer activity (Deng et al., 2008), their synthesis and transformations have received particular interest for some time.

Fluorinated compounds exhibit dramatically improved potency compared to their non-fluorinated analogues (Secor and DeBardeleben, 1971) since the incorporation of fluorine alters the electronic, lipophilic and steric parameters and can critically increase the intrinsic activity and the chemical and metabolic stability. In particular, the introduction of the CF_3 group into organic molecules immensely increases both the pharmacological activity and the lipophilicity (Barnard et al., 1993).

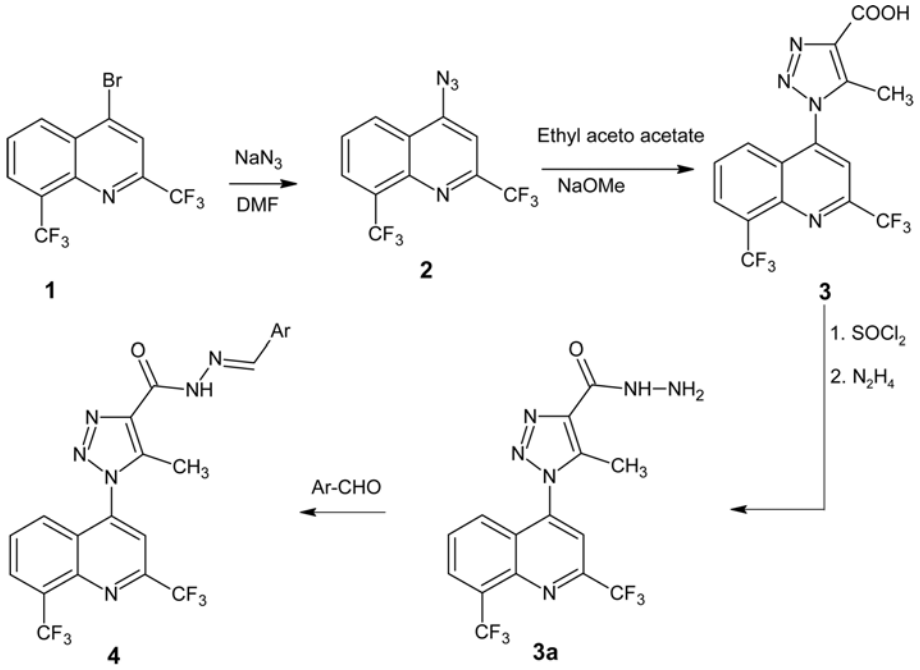
Recently, we reported the synthesis and antimicrobial studies of bioactive 1,2,3-triazoles attached to a quinoline moiety with a CF_3 group (Holla et al., 2005). In a continuation of our research on bioactive heterocycles and their biological evaluation, we synthesized some 1,2,3-triazoles containing bistrifluoromethyl-substituted quinoline moiety and evaluated the *in vitro* antimicrobial activity.

MATERIALS AND METHODS

Chemistry

Melting points were determined in open capillaries using Sewell Instruments, Inc. melting point apparatus and were uncorrected. The purity of the compounds was checked by thin layer chromatography on a silica-coated aluminum sheet (silica gel F_{254}). The Infrared (IR) spectra (KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Bruker Avance II 400 (400 MHz) spectrometer using $CDCl_3$/CD_3OD as a solvent and TMS as an internal standard (chemical shift in δ ppm). The spin multiplets are given as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra were recorded on a Jeol JMS-D 300 mass spectrometer operating at 70eV. Elemental analysis was carried out using FlashEA 1112 Series, CHNSO Analyzer (Thermo). Mass spectra were determined on a Jeol SX 102/Da-600 mass spectrometer/Data System using Argon/Xenon (6 kV, 10 mA) as the FAB gas. Elemental analyses were obtained on a CHNS elemental analyzer. Solvents and reagents of the appropriate grade were purchased from commercial vendors and were used without purification.

The new derivatives of 1,2,3-triazoles (**4a-n**) were prepared by the method outlined in Scheme 1. Initially, 4-bromo-2,8-bistrifluoromethylquinoline **1** was



Scheme 1

prepared in good yield as described (Guenter and Vogt, 1977), and subsequently treated with sodium azide in DMF to afford 4-azido-2,8-bistrifluoromethylquinoline **2** (Holla et al., 2005). 1,3-Dipolar cycloaddition reaction of compound **2** with ethyl acetoacetate in the presence of sodium methoxide in methanol at 0°C yielded 1-(2,8-Bistrifluoromethylquinolin-4-yl)-5-methyl-1,2,3-triazole-4-carboxylic acid **3**. The triazole carboxylic acid **3** was treated with thionyl chloride in dichloromethane to afford acid chloride; subsequent treatment with hydrazine hydride at 0°C gave the corresponding 1-[2,8-bistrifluoromethylquinolin-4-yl]-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide **3a** in good yield. Finally, the acid hydrazide was treated with different aromatic aldehydes to yield the corresponding Schiff's bases, N-[1-arylmethylene]-1-[2,8-bistrifluoromethylquinolin-4-yl]-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides (**4a-n**).

The structures of the synthesized compounds were established on the basis of spectral and analytical data. The C, H, N analysis of these compounds is in agreement with the calculated values within the limits of experimental error. The characterization data of the synthesized compounds are presented in Table I.

Synthesis of 4-Azido-2, 8-bistrifluoromethylquinoline (2)

4-Bromo-2,8-bistrifluoromethylquinoline (10 g, 0.03 mol) was treated with sodium azide (2.08 g, 0.03 mol) in 25 mL of DMF. The reaction mixture was heated to 90°C for 1 h. The progress of the reaction was monitored by TLC using hexane:methanol (9.9:0.1, v/v) as the mobile phase. After the completion of the reaction, the reaction mixture was cooled to 20°C and quenched with ice water, the precipitated yellow 4-azido-2,8-bistrifluoromethyl-quinoline was isolated by filtration. Recrystallization of the crude product from methanol gave pure product (7.6 g, 85%).

IR (KBr, γ/cm^{-1}): 3060 (Ar-H), 2130 (N_3), 980 (C-F); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.51 (s, Ar-H, 1H), 7.65 (t, 1H, Ar-H, J = 9.0 Hz), 8.18 (d, 1H, Ar-H, J = 6.0 Hz), 8.33 (d, 1H, Ar-H, J = 6.00 Hz). Anal. Calcd. for $C_{11}H_4F_6N_4$ (in %) : C, 43.15; H, 1.32; N, 18.30. Found: C, 43.15; H, 1.30; N, 18.10.

Synthesis of 1-(2, 8-Bistrifluoromethylquinolin-4-yl)-5-methyl-1,2,3-triazole-4-carboxylic acid (3)

To a solution of 4-azido-2,8-bistrifluoromethylquinoline (**2**, 5 g, 0.016 mol) in 25 mL of methanol, ethyl acetoacetate (2.125 g, 0.016 mol) was added and the mixture was cooled to 0°C. Sodium methoxide (0.88 g, 0.016 mol) was then added to the above mixture under a nitrogen atmosphere over a period of 30 min with stirring. Stirring was continued for 8 h. The progress of the reaction was monitored by TLC using ethyl acetate: hexane (1.5:4.5, v/v) as the mobile phase. After completion of the reaction, the reaction mass was quenched with ice water and neutralized with acetic acid. The compound **3** was isolated by filtration as a yellow solid. The crude product was purified by converting it into its corresponding sodium salt and then neutralizing it with acetic acid. The precipitated compound was filtered, washed and dried. The isolated product was recrystallized by ethyl acetate (3.18 g, 50%).

Table I. Characterization data of compounds **4a-n**

Compounds	Ar	Mol. formula	Mol. weight	m.p. (°C)	Yield (%)
2	-	$C_{11}H_4F_6N_4$	306.16	63-65	86
3	-	$C_{15}H_8F_6N_4O_2$	390.24	118-12	50
3a	-	$C_{15}H_{10}F_6N_6O$	404.27	189-193	88
4a	C_6H_5	$C_{22}H_{14}F_6N_6O$	492.37	180-182	60
4b	4-OCH_3-C_6H_4	$C_{23}H_{16}F_6N_6O_2$	522.4	138-142	65
4c	3-OCH_3-C_6H_4	$C_{23}H_{16}F_6N_6O_2$	522.4	186-188	64
4d	3,4-$(OCH_3)_2$-C_6H_3	$C_{24}H_{18}F_6N_6O_3$	552.42	214-216	64
4e	2-OH-C_6H_4	$C_{22}H_{14}F_6N_6O_2$	508.37	176-178	70
4f	4-OH-C_6H_4	$C_{22}H_{14}F_6N_6O_2$	508.37	105-107	62
4g	2,3,4-$(OH)_3$-C_6H_2	$C_{22}H_{14}F_6N_6O_4$	540.37	210-212	70
4h	2-OH-3-OCH_3-C_6H_3	$C_{23}H_{16}F_6N_6O_3$	538.4	198-200	68
4i	4-NO_2-C_6H_4	$C_{22}H_{13}F_6N_7O_3$	537.4	200-202	74
4j	2-NO_2-3,4-$(OCH_3)_2$-C_6H_2	$C_{24}H_{17}F_6N_7O_5$	597.42	>280	66
4k	4-C_6H_5-C_6H_4	$C_{28}H_{18}F_6N_6O$	568.47	140-148	60
4l	6-OCH_3-2-Naphthyl	$C_{27}H_{18}F_6N_6O_2$	572.46	202-204	75
4m	3-Pyridyl	$C_{21}H_{13}F_6N_7O$	493.34	234-236	68
4n	3-Methylthien-2-yl	$C_{21}H_{14}F_6N_6OS$	512.43	105-106	72

IR (KBr, γ/cm^{-1}): 3400 (O-H), 3060 (Ar-H), 2940 (C-H), 1690 (C=O), 980 (C-F); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.53 (s, CH_3, 3H), 7.83 (d, 1H, Ar-H, *J* = 8.48 Hz), 7.96 (t, 1H, Ar-H, *J* = 8.16 Hz), 8.37 (s, Ar-H, 1H), 8.43 (d, 1H, Ar-H, *J* = 7.2 Hz), 9.8 (s,1H, -OH); MS (*m/z*, %): 390 (M^+, 5). Anal. calcd for $C_{15}H_8F_6N_4O_2$ (in %): C, 46.17; H, 2.07; N, 14.36. Found: C, 46.15; H, 2.05; N, 14.33.

Synthesis of 1-(2,8-Bistrifluoromethylquinolin-4-yl)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (3a)

Compound **3** (3.0 g, 0.0077 mol) was taken in 10 mL of dry chloroform containing one 0.1 mL of DMF and cooled to 0-5°C. The resulting mixture was treated with thionyl chloride (0.011 mol) in drops. The temperature was slowly increased and the reaction mass was refluxed for 5 h to yield the acid chloride. Excess thionyl chloride and solvent were removed by distillation under vacuum. In another flask, the acid chloride was added dropwise to 5 mL of methanol. The resulting methyl ester was isolated after removing the solvent by vacuum. The ester was then refluxed with hydrazine hydrate (0.011 mol) in ethanol for 8 h at 80°C. The solvent was removed under vacuum and the residue was quenched with ice water to obtain the white solid. The solid was filtered, washed, dried and recrystallized from aqueous methanol to get the pure compound **3a** (2.75 g, 88%).

IR (KBr, γ/cm^{-1}): 3360 (N-H), 3060 (Ar-H), 2955 (C-H), 1695 (C=O), 980 (C-F).

General procedure for the synthesis of 1-(2,8-bistrifluoromethylquinolin-4-yl)-N'-(arylmethylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides (4a-n)

Compound **3a** was treated with an equimolar quantity of aromatic aldehyde in 10 volumes of methanol and 1 volume of acetic acid and refluxed for 1 h. Completion of the reaction was checked by TLC using ethyl acetate:hexane (1:4, v/v) as the mobile phase. The reaction was cooled to 0°C, and the resulting solid was filtered and washed with cold methanol to yield compound **4a-n**. Recrystallization in methanol yielded 60-70% of pure product.

1-(2,8-Bistrifluoromethylquinolin-4-yl)-N'-(4-benzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4a)

IR (KBr, γ/cm^{-1}): 3345 (N-H), 3070 (Ar-H), 1699 (C=O), 985 (C-F); ^{1}H-NMR (CD_3OD, 300 MHz): δ 2.53 (s, 3H, CH_3), 7.40-7.38 (m, 2H, phenyl), 7.45-7.42 (m, 1H, phenyl), 7.47 (s, 1H, trifluoromethylquinolinyl), 7.67 (t, 1H, trifluoromethyl quinolinyl, *J* = 7.65 Hz), 7.79-7.77 (m, 2H, phenyl), 8.13 (d, 1H, trifluoromethylquinolinyl, *J* = 6.0 Hz), 8.32 (s, 1H, =CH), 8.50 (s, 1H, N-H), 8.51 (d, 1H, trifluoromethylquinolinyl, *J* = 6.0 Hz); MS (*m/z*, %): 492 (M^+, 25). Anal. calcd for $C_{22}H_{14}F_6N_6O$ (in%): C, 53.67; H, 2.87; N, 17.07. Found: C, 53.65; H, 2.83; N, 17.09.

1-(2,8-Bistrifluoromethylquinolin-4-yl)-N'-(4-methoxybenzylidene)-5-methyl-1 *H*-1,2,3-triazole-4-carbohydrazide (4b)

IR (KBr, γ/cm^{-1}): 3343 (N-H), 3075 (Ar-H), 1692 (C=O), 985 (C-F); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.59 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 6.99 (d, 1H, trifluoromethylquinolinyl, *J* = 8.0 Hz), 7.130 (s, 1H, trifluoromethylquinolinyl), 7.59 (t, 1H, trifluoromethylquinolinyl, *J* = 8.0 Hz), 7.72 (d, 1H, trifluoromethylquinolinyl, *J* = 8.0 Hz), 8.00 (d, 1H, 4-methoxy phenyl, *J* = 8.0 Hz), 8.09 (d, 1H, 4-methoxy phenyl, *J* = 8.0 Hz), 8.12 (s, 1H, =CH), 8.50 (s, 1H, N-H); MS (*m/z*, %): 522 (M^+, 25). Anal. calcd for $C_{23}H_{16}F_6N_6O_2$ (in %): C, 52.88; H, 3.09; N, 16.09. Found: C, 52.84; H, 3.13; N, 16.06.

1-(2,8-Bistrifluoromethylquinolin-4-yl)-N'-(3-methoxybenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4c)

IR (KBr, γ/cm^{-1}): 3340 (N-H), 3075 (Ar-H), 1695 (C=O), 980 (C-F); ^{1}H-NMR (CD_3OD, 400 MHz): δ 2.50 (s, 3H, CH_3), 4.87 (s, 3H, OCH_3), 6.90 (d, 2H, 3-methoxy phenyl, *J* = 8.0 Hz), 7.28 (t, 2H, 3-methoxy phenyl, *J* = 6.0 Hz), 7.31 (s, 1H, trifluoromethyl quinolinyl), 7.59 (t, 1H, trifluoromethylquinolinyl, *J* = 8.0 Hz), 7.72 (s, 1H, 3-methoxy phenyl), 8.07 (d, 1H, trifluoromethyl quinolinyl, *J* = 8.0 Hz), 8.18 (s, 1H, =CH), 8.39 (d, 1H, trifluoromethyl-quinolinyl, *J* = 8.0 Hz), 8.50 (s, 1H, N-H); MS (*m/z*, %): 522 (M^+, 25). Anal. calcd for $C_{23}H_{16}F_6N_6O_2$ (in %): C, 52.88; H, 3.09; N, 16.09. Found: C, 52.85; H, 3.05; N, 16.05.

1-(2,8-Bistrifluoromethylquinolin-4-yl)-N'-(3,4-dimethoxybenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4d)

IR (KBr, γ/cm^{-1}): 3340 (N-H), 3072 (Ar-H), 1690 (C=O), 980 (C-F); ^{1}H-NMR (CD_3OD, 300 MHz): δ 2.30 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 7.04 (d, 1H, 3,4-di-methoxyphenyl, *J* = 6.0 Hz), 7.28 (t, 1H, trifluoromethylquinoline, *J* = 9.0 Hz), 7.46 (s, 1H, trifluoromethylquinolinyl), 7.65 (d, 1H, 3,4-dimethoxyphenyl, *J* = 6.0 Hz), 8.12 (d, 1H, trifluoromethylquinolinyl, *J* = 9.0 Hz), 8.26 (s, 1H, =CH), 8.26 (s, 1H, 3,4-dimethoxyphenyl), 8.52 (d, 1H, trifluoromethylquinolinyl, *J* = 9.0 Hz), 8.52 (s, 1H, N-H); MS (*m/z*, %): 552 (M^+, 25). Anal. calcd for $C_{24}H_{18}F_6N_6O_3$ (in %): C, 52.18; H, 3.28; N, 15.21. Found: C, 52.15; H, 3.25; N, 15.20.

1-(2,8-Bistrifluoromethylquinolin-4-yl)-N'-(2-nitro-3,4-dimethoxybenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4j)

IR (KBr, γ/cm^{-1}): 3345 (N-H), 3070 (Ar-H), 1340, 1535 (NO_2, sym and asym str.), 1700 (C=O), 980 (C-F); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.44 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 7.45 (t, 1H, trifluoromethylquinolinyl, *J* = 8.0 Hz), 7.48 (d, 1H, trifluoromethylquinolinyl, *J* = 8.0 Hz), 7.52 (s, 1H, trifluoromethylquinolinyl), 7.66 (d, 1H, trifluoromethylquinolinyl, *J* = 8.0 Hz), 7.97 (d, 1H, nitrodimethoxyphenyl, *J* = 8.0 Hz), 8.50 (d, 1H, nitrodimethoxyphenyl, *J* = 8.0 Hz), 9.05 (s, 1H, =CH), 11.37 (s, 1H, N-H). Anal. calcd for $C_{24}H_{17}F_6N_7O_5$ (in %): C, 48.25; H, 2.87; N, 16.41. Found: C, 48.22; H, 2.86; N, 16.40.

1-(2,8-Bistrifluoromethylquinolin-4-yl)-N'-(4-phenylbenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4k)

IR (KBr, γ/cm^{-1}): 3345 (N-H), 3070 (Ar-H), 1699 (C=O), 985 (C-F); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.54 (s, 3H, CH_3), 7.41 (d, 2H, phenyl, *J* = 6.0 Hz), 7.47 (d, 2H, phenyl, *J* = 6.0 Hz), 7.50 (s, 1H, trifluoromethylquinolinyl), 7.66-7.64 (m, 2H, phenyl), 7.72-7.67 (m, 3H, phenyl), 7.87 (d, 1H, trifluoromethylquinolinyl, *J* = 6.0 Hz), 7.90 (s, 1H, =CH), 8.08-8.06 (d, 1H, trifluoromethylquinolinyl, *J* = 6.0 Hz), 8.14-8.12 (t, 1H, trifluoromethylquinolinyl), 8.63 (s, 1H, NH). Anal. calcd for $C_{28}H_{18}F_6N_6O$ (in %): C, 59.16; H, 3.19; N, 14.78. Found: C, 59.13; H, 3.17; N, 14.75.

1-(2,8-Bistrifluoromethylquinolin-4-yl)-N'-(6-methoxy-2-naphthylmethylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4l)

IR (KBr, γ/cm^{-1}): 3350 (N-H), 3073 (Ar-H), 1700 (C=O), 980 (C-F); ^{1}H-NMR (CD_3OD, 300 MHz): δ 2.23 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 7.14 (d, 1H, naphthyl, *J* = 9.0 Hz), 7.17 (d, 1H, naphthyl, *J* = 9.0 Hz), 7.24 (s, 1H, naphthyl), 7.66 (t, 1H, trifluoromethylquinolinyl, *J* = 7.5 Hz), 7.88 (s, 1H, naphthyl), 7.80 (d, 1H, naphthyl, *J* = 9.0 Hz), 7.90 (s, 1H, trifluoromethylquinolinyl), 8.02 (d, 1H, naphthyl, *J* = 9.0 Hz), 8.14-8.11 (d, 1H, trifluoromethylquinolinyl, *J* = 9.0 Hz), 8.42 (s, 1H, =CH), 8.51 (s, 1H, N-H), 8.52 (d, 1H, trifluoromethylquinolinyl, *J* = 9.0 Hz). Anal. calcd for $C_{27}H_{18}F_6N_6O_2$ (in %): C, 56.65; H, 3.17; N, 14.68. Found: C, 56.65; H, 3.16; N, 14.63.

1-(2,8-Bistrifluoromethylquinolin-4-yl)-N'-(3-pyridylmethylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4m)

IR (KBr, γ/cm^{-1}): 3333 (N-H), 3075 (Ar-H), 1700 (C=O), 977 (C-F); ^{1}H-NMR (CD_3OD, 300 MHz): δ 2.30 (s, 3H, CH_3), 7.77 (t, 1H, pyridyl, *J* = 9.0 Hz), 7.82 (s, 1H, pyridyl), 8.10 (s, 1H, trifluoromethylquinolinyl), 8.16 (d, 1H, pyridyl, *J* = 6.0 Hz), 8.31 (d, 1H, pyridyl, *J* = 6.0 Hz), 8.37 (s, 1H, =CH), 8.53 (d, 1H, trifluoromethylquinolinyl, *J* = 9.0 Hz), 8.85 (t, 1H, trifluoromethylquinolinyl, *J* = 7.5 Hz), 8.90 (d, 1H, trifluoromethylquinolinyl, *J* = 9.0Hz), 9.04 (s, 1H, NH); MS (*m*/*z*, %): 493 (M^+, 25). Anal. calcd for $C_{21}H_{13}F_6N_7O$ (in%): C, 51.12; H, 2.66; N, 19.87. Found: C, 51.10; H, 2.66; N, 19.85.

1-(2,8-Bistrifluoromethylquinolin-4-yl)-5-methyl-N'-(3-[3-methylthien-2-yl]methylidene)-1*H*-1,2,3-triazole-4-carbohydrazide (4n)

IR (KBr, γ/cm^{-1}): 3333 (N-H), 3055 (Ar-H), 2940 (C-H), 1697 (C=O), 1077 (C-F); ^{1}H-NMR (CD_3OD, 300 MHz): δ 2.18 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 6.89 (d, 1H, thienyl, *J* = 4.8 Hz), 7.02 (d, 1H, thienyl, *J* = 4.8 Hz), 8.12 (s, 1H, trifluoromethylquinolinyl), 8.27 (s, 1H, =CH), 8.52-8.50 (d, 1H, trifluoromethylquinolinyl, *J* = 9.0 Hz), 8.84-8.81 (t, 1H, trifluoromethylquinolinyl, *J* = 7.5 Hz), 8.88-8.86 (d, 1H, trifluoromethylquinolinyl, *J* = 9.0 Hz), 9.01 (s, 1H, NH); MS (*m*/*z*, %): 512 (M^+, 5). Anal. calcd for $C_{21}H_{14}F_6N_6OS$ (in%): C, 49.22; H, 2.75; N, 16.40. Found: C, 49.19; H, 2.78; N, 16.38.

Biology

Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (recultured) bacterial strains by serial plate dilution method (MacLowry et al., 1970; Barry, 1991). Serial dilutions of the drug in Muller-Hinton broth were taken in tubes, and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated into each tube and incubated for 16-18 h at 37°C. The minimum inhibitory concentration (MIC) was the lowest concentration of the drug at which there was no visible growth.

A number of antibacterial discs were placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Twenty milliliters of agar media were poured into each Petri dish. The excess suspension was decanted and plates were dried in an incubator at 37°C for an hour. Using a punch, wells were made on these seeds agar plates, and minimum inhibitory concentrations of the test compounds in dimethyl sulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using DMSO as a solvent. The Petri dishes were prepared in triplicate and maintained at 37°C for 3-4 days. Antibacterial activity was determin-

ed by measuring the diameter of the inhibition zone. The activity of each compound was compared with that of ciprofloxacin as a standard (Fenlon et al., 1986; Davis et al., 1996). The zone of inhibition was determined for **4a-n**.

Antifungal activity

Newly synthesized compounds were also screened for their antifungal activity against *Aspergillus flavus* (NCIM No. 524), *Aspergillus fumigatus* (NCIM No. 902), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method (Verma et al., 1998; Arthington-Skaggs et al., 2000). Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make suspensions of spores of fungal strains for lawning. A loopful of a particular fungal strain was transferred to 3 mL of saline to prepare a suspension of the corresponding species. Twenty milliliters of agar media was poured into each Petri dish. The excess suspension was decanted and plates were dried in an incubator at 37°C for 1 h. Using a punch, wells were made on these seeded agar plates. Minimum inhibitory concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using DMSO as solvent. The Petri dishes were prepared in triplicate and maintained at 37°C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The activity of each compound was compared with cyclopiroxolamine as a standard. Zones of inhibition were determined for **4a-n**.

RESULTS

Several new derivatives of 1,2,3-triazoles containing quinoline moiety **4a-n** were synthesized by 1,3-dipolar addition in normal to good yield. All the test compounds **4a-n** were evaluated by the serial plate dilution method for antimicrobial activity against four bacterial strains, *E. coli,* S. *aureus*, *P. aeruginosa* and *K. pneumoniae*, and against four fungal strains, *A. flavus*, *A. fumigatus*, *P. marneffei* and *T. mentagrophytes*. All the test compounds showed moderate to good antibacterial and antifungal activities against pathogenic strains. Compound **4n** showed the highest activity against the microbial strains used for screening. Significant activity against bacterial and fungal pathogens was also provided by compounds **4b**, **4c**, **4e**, **4f**, **4h** and **4l** at the concentration of 6.25 μg/mL. Compounds **4a** exhibited significant activity against *P. aeruginosa*, *K. pneumoniae*, *A. flavus* and *T. mentagrophytes*. However, it showed moderate activity against the remaining four bacterial and fungal strains. On the other hand, compounds **4d**, **4g**, **4i**, **4j**, **4k** and **4m** exhibited marginal antimicrobial activity. The minimum inhibitory concentrations and zones of inhibition of the test compounds are presented in Tables II and III.

Table II. Antibacterial activity of compounds **4a-n**

Compounds	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
4a	12.5 (11-15)	12.5 (11-16)	6.25 (16-20)	6.25 (16-22)
4b	6.25 (20-26)	6.25 (19-25)	6.25 (20-25)	6.25 (20-27)
4c	6.25 (16-20)	6.25 (17-22)	6.25 (15-20)	6.25 (17-20)
4d	25 (13-17)	12.5 (11-15)	12.5 (12-18)	25 (13-18)
4e	6.25 (15-20)	12.5 (11-18)	6.25 (16-20)	6.25 (18-24)
4f	6.25 (16-20)	12.5 (12-17)	6.25 (18-24)	6.25 (19-23)
4g	12.5 (12-18)	12.5 (12-18)	12.5 (14-22)	12.5 (13-18)
4h	6.25 (16-19)	6.25 (16-20)	12.5 (15-20)	6.25 (16-20)
4i	12.5 (11-15)	12.5 (11-15)	25 (13-17)	25 (16-20)
4j	25 (13-17)	25 (13-17)	25 (13-17)	25 (13-17)
4k	25 (13-17)	25 (15-19)	25 (13-18)	25 (15-19)
4l	6.25 (16-21)	12.5 (11-15)	6.25 (16-20)	6.25 (16-20)
4m	25 (13-17)	25 (13-17)	25 (13-17)	25 (13-17)
4n	3.125 (18-24)	6.25 (20-25)	6.25 (16-20)	6.25 (16-20)
Standard (ciprofloxacin)	1.56 (22-30)	6.25 (30-40)	6.25 (25-33)	6.25 (23-27)

The MIC values were evaluated over the concentration range, 1.56-25 μg/mL. The figures in the table show the MIC values in μg/mL and the corresponding zone of inhibition in mm.

Table III. Antifungal activity of compounds **4a-n**

Compounds	*A. flavus*	*A. fumigatus*	*P. marneffei*	*T. mentagrophytes*
4a	6.25 (16-20)	12.5 (13-18)	12.5 (12-16)	6.25 (18-22)
4b	6.25 (21-27)	6.25 (19-26)	6.25 (20-29)	6.25 (18-25)
4c	6.25 (17-22)	6.25 (16-20)	6.25 (15-22)	6.25 (16-20)
4d	12.5 (15-19)	25 (13-17)	12.5 (11-15)	25 (15-20)
4e	6.25 (16-20)	6.25 (18-24)	6.25 (15-20)	6.25 (16-21)
4f	6.25 (13-18)	6.25 (15-18)	6.25 (13-19)	6.25 (14-20)
4g	12.5 (12-16)	12.5 (14-19)	12.5 (13-18)	12.5 (15-21)
4h	6.25 (18-24)	6.25 (18-24)	12.5 (19-25)	6.25 (15-20)
4i	12.5 (13-18)	12.5 (12-17)	25 (15-19)	25 (15-20)
4j	25 (13-17)	12.5 (11-15)	25 (13-17)	25 (13-17)
4k	25 (15-19)	25 (13-17)	25 (15-20)	25 (14-19)
4l	6.25 (17-22)	6.25 (18-24)	6.25 (19-24)	6.25 (18-23)
4m	25 (13-17)	25 (15-18)	25 (13-18)	25 (14-17)
4n	3.125 (15-20)	6.25 (16-20)	6.25 (16-20)	6.25 (16-20)
Standard (cyclo-piroxolamine)	3.125 (25-30)	6.25 (25-30)	6.25 (20-27)	3.125 (27-33)

The MIC values were evaluated over the concentration range, 1.56-25 μg/mL. The figures in the table show the MIC values in μg/mL and the corresponding zone of inhibition in mm.

DISCUSSION

From the antimicrobial activity results, the structure activity relationship can be deduced for the test compounds (**4a-n**). The antimicrobial activity of the test compounds was explored by varying only one substituent, viz. N-arylidene carbohydrazide at position 4 of the 1,2,3-triazole moiety. Different electron-donating or electron-withdrawing groups attached to the phenyl ring as the substituent linked to the carbohydrazide group were replaced to explore the antimicrobial potency. Among the tested compounds, **4n** exhibited maximum antimicrobial activity against all of the microbial pathogens, which may be due to the presence of the electron-rich 3-methylthien-2-yl moiety. The compounds **4b**, **4c**, **4e** and **4f** containing one electron-releasing group, such as 4-OMe, 3-OMe, 2-OH, or 4-OH, in the phenyl ring also exhibited enhanced activity. The compound with the 4-OMe group (**4b**) produced maximum inhibition zone compared to 3-OMe, 2-OH and 4-OH substituents. On the other hand, compounds with an unsubstituted phenyl group (**4a**) and with one electron-withdrawing 4-NO_2 substituent (**4i**) showed moderate and marginal antimicrobial activity, respectively. However, the introduction of two electron-releasing substituents, namely 3,4-$(OMe)_2$ (**4d**) and 2-OH-3-OMe (**4h**), did not enhance the activity. But the introduction of three electron-releasing groups in the phenyl ring reduced the activity, as is evident from the activity results of **4g** and **4j** with 2,3,4-$(OH)_3$ and 2-NO_2-3,4-$(OMe)_2$ substituents, respectively. The replacement of the phenyl ring with a naphthyl ring with an electron-releasing substituent (**4l**) did not appreciably change the activity. On the other hand, replacement of the phenyl ring with biphenyl (**4k**) and pyridyl (**4m**) rings dramatically decreased the activity.

Based on the investigation of SAR of the new 1,2,3-triazoles containing different substituents at position 4 of the triazole ring, one or two electron-releasing substituents in the phenyl ring produced relatively significant antimicrobial activity. On the other hand, an electron-withdrawing substituent or addition of more than three substituents marginally reduced the activity. Pyridyl and biphenyl rings dramatically reduced the activity, while the thiophene ring enhanced the activity. These results suggest that the effect of 4-substitution in the triazole moiety on antimicrobial activity is mostly due to the electronic and steric factors of the groups.

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