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Synthesis, characterization and antimicrobial studies of some new trifluoromethyl quinoline-3-carbohydrazide and 1,3,4-oxadiazoles

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Abstract

Present paper describes the synthesis of two new series of 7-(trifluoromethyl)-4-hydroxy substituted quinoline carbohydrazide derivatives (**6a-e** and **7a-g**) and N-alkyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl) quinolin-4-amine derivatives (**9a-f**). Newly synthesized compounds were characterized by spectral studies. Structure of **9a** was evidenced by X-ray crystallographic study. Synthesized compounds were screened for their antibacterial studies against *Mycobacterium smegmatis* and *Pseudomonas aeruginosa*. Antifungal activity was also carried out on the fungal stains *Candida albicans* and *Penicillium chrysogenum*. Compounds **7a** and **9c** showed significant antimicrobial activity against all the tested microorganisms. Among all the compounds, **6d** and **6e** showed lowest MIC value of 6.25 µg/ml against *Mycobacterium smegmatis* indicating these compounds can be a possible future antituberculosis agents.

Keywords: 7-trifluoromethyl Quinoline-3-carbohydrazide, 1,3,4-oxadiazole, antimicrobial activity.

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1. Introduction

Heterocyclic compounds play an important role in medicinal chemistry. They are well known to possess diverse pharmacological properties, viz. antimicrobial, anti-inflammatory, anticonvulsant, antiviral, antimalarial, antituberculosis and anticancer.[1-4] Pathogenic microorganisms developing resistance to drugs is a serious problem in last few decades. Different structural modification has been made to enhance the antimicrobial activity by introducing the different functional groups around the quinoline nucleus. Among the heterocyclic compounds, substituted quinolines are more important because of their wide spectrum of biological activities. A large variety of quinoline derivatives have been used as anticancer,[5] antiviral,[6] anti-inflammatory,[7] antimicrobial,[8,9] antioxidant,[10] antimalarial,[11] anti-tuberculosis,[12] agents. Mefloquine (Antimalarial) and Bedaquiline or TMC207 (Anti-tuberculosis) are well-known drugs which contain quinoline core moiety (Fig. 1).

Figure 1

It has been well established that fluorinated quinolines, in particular, CF_3 substituted quinolines have got a significant place in modern medicinal chemistry. Introduction of trifluoromethyl group provides better electronic effect at neighboring carbon centers, as well as having a substantial effect on the molecule's dipole moment, acidity and basicity of neighboring groups.[13] Their biological studies clearly indicated that, the presence of trifluoromethyl group in position seven and eight of the quinoline ring is responsible for their enhanced biological activity[14-16] and are the subject of considerable growing interest. Further, various types of hydrazones have attracted continued interest in the field of medicine owing to their varied biological activities as antimicrobial,[17] antimalarial,[18] and antitubercular properties.[19] On the other hand, compounds containing 1,3,4-oxadizole rings are very well known to exhibit powerful antimicrobial,[20, 21] analgesic,[22] cannabinoid receptor 2 (CB2) agonist,[23] VEGFR-2 and Tublin Inhibitor[24] properties. Therefore there is great importance for the synthesis of oxadiazoles as target structures and evaluation of their biological activities.

In our previous studies, we reported the synthesis and antimicrobial activity of some fluorophenyl and trifluoromethyl quinoline derivatives. It was found that, fluorinated compounds are good antimicrobial agents. Encouraged by these results and in continuation of the synthesis of new heterocyclic compounds,[25, 26, 4] the present study was focused on the synthesis of new trifluoromethylquinoline derivatives, their characterization and antimicrobial activity.

2. Results and discussion

2.1. Chemistry

The targeted compounds (**6a-e, 7a-g** and **9a-f**) were synthesized by employing sequential reactions, which are presented in **Scheme 1**. The quinolone skeletons were built up by the Gould-Jacobs procedure starting from 3-(trifluoromethyl) aniline **1**. Condensation of **1** with diethyl ethoxymethylene malonate and subsequent thermal cyclization in dowtherm (Biphenyl:biphenyloxide (3:7)) yielded the 4-hydroxy-quinoline-3-carboxylic ester **4**[27,16] which on condensation with hydrazine hydrate in alcoholic medium resulted 4-hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid hydrazide **5**[28]. Further, the key intermediates, pyrazole-4-carbaldehydes were prepared by the Vilsemeier-Haack reaction of the corresponding hydrazones.[29]

Scheme 1

The final compounds 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide (**6a-e** and **7a-g**) were obtained by reacting quinoline hydrazide **5** with various substituted aldehydes in ethanolic media (**Scheme 1**). Reaction of **5** with benzoic acid in $POCl_3$ yielded 4-chloro-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinoline **8**. Finally chlorine in **8** were replaced with various aliphatic and aromatic amines to obtain the targeted N-alkyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolin-4-amine derivatives **9a-f**. The crude products were purified by column chromatography using pet ether and ethyl acetate (7:3) as the eluent. All the synthesized compounds were characterized by IR, NMR, mass spectral and C, H, N elemental analysis.

Formation of 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide derivatives (**6a-e** and **7a-g**) were confirmed by recording their IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra. The FT-IR spectrum of compound **6a** showed two bands at 3410 cm^{-1} and 3138 cm^{-1}, which are due

to the hydroxyl and amide groups respectively. Band at 1668 cm^{-1} is due to C=O stretch of carbonyl group. The ^{1}H-NMR spectrum of **6a** showed a singlet at δ 8.07 ppm which is due to the imine proton (N=CH). Hydroxyl and amide protons appeared as singlet at δ 12.90 and 13.10 ppm respectively further confirmed the structure of the compound. The mass spectrum of **7a** showed molecular ion peak at m/z = 502 (M+1), which is in agreement with the molecular formula $C_{27}H_{18}F_3N_5O_2$. Similarly the spectral values for all the compounds and C, H, N analyses are presented in the experimental part and the characterization data are provided in Table 1 and Table 2.

Table 1

Table 2

2.2. Antimicrobial studies

Antibacterial studies of newly synthesized compounds (**6a-e, 7a-g** and **9a-f**) were carried out against two pathogenic bacteria *Mycobacterium smegmatis* (MTCC 943) and *Pseudomonas aeruginosa* (MTCC4676) by well diffusion method using nutrient agar media[30, 31]. Antifungal activity was carried out against two fungi *Candida albicans* (MTCC 183) and *Penicillium chrysogenum* (MTCC 6795)[32, 33]. All the compounds were dissolved in dimethylsulfoxide (DMSO) and used for testing at 25 and 50 µg/mL concentrations. Antimicrobial activities were determined by measuring the diameter of inhibition zone in millimetre. A minimum inhibition concentration (MIC) was also determined for the test compounds at concentration ranging from 1.6-50 µg/mL against three bacteria *M. smegmatis, P. aeruginosa* and *Staphylococcus aureus* and one fungi *C. albicans*. Ciprofloxacin was used as standard antimicrobial compound for antibacterial studies, while Fluconazole was used as standard for the antifungal studies. All the experiments were performed in triplicates and average value was taken. The details of the results of the antimicrobial analysis are furnished in **Table 3** and **Table 4**.

Table 3

Table 4

2.3. Acute Toxicity and Gross Behavioral Studies

The acute oral toxicity study for the newly synthesized organic compounds **6a-e, 7a-g** and **9a-f** was carried out by the following OECD guidelines No. 420 (OECD Guidelines, 2008).[34-36] Each group consisting of 6 mice (overnight fasted) and kept in colony cage at 25±2°C with 55% relative humidity and 12 hours of light and dark cycle. A specified dose of 100, 250, 500, 750, 1000, 1500 and 2000 mg/kg body weight of mice was administered orally as a single dose as a fine suspension prepared in saline using gum acacia powder. The acute toxic symptoms and the behavioral changes produced by the test compounds were observed continuously for 4 h periods at 8th, 12th and 24th h on set of toxic symptoms and the gross behavioral changes were also recorded. These animals were maintained for further 10 days with observation made daily.

2.4. Biological results

The antimicrobial screening in well diffusion method revealed that, few of the tested compounds showed excellent inhibition against tested microbial strains. Among the synthesized compounds, **7a** and **9c** showed significant antimicrobial activity against all the tested microorganisms. The enhanced activity may be due to presence of trifluoromethyl functional group at seventh position of the quinoline core moiety and electron donating groups at third (**7a**: 4-methoxyphenyl) and fourth (**9c**: ethanolamine) position of quinoline ring. The compounds **6c**, **6d**, **6e**, **7b**, **7c**, **7f**, **9a**, **9b** and **9d** are exhibited excellent antimicrobial activity against all the microorganisms except the filamentous fungi *Penicillium chrysogenum*. Among all the compounds, **6d** is inhibiting *Mycobacterium smegmatis* to the maximum of 16 mm diameter may be due to the presence of strong electron withdrawing group (NO_2) on pyrazole derivative at third position of 7-trifluoromethy-4-hydroxyquinoline. The presence of 1-phenyl-3-p-tolyl-*1H*-pyrazole carbohydrazide at third position of the 7-trifluoromethy-4-hydroxyquinoline may be the reason for the enhanced activity of **6e** against Gram-negative bacteria *Pseudomonas aeruginosa* (maximum extent of 19 mm). Six of these compounds inhibited the filamentous fungi *Penicillium chrysogenum* at 25 µg/mL concentration. Except **6b**, **7e**, **9e** and **9f,** all compounds inhibited Gram-negative bacteria *P. aeruginosa*. MIC of **6d** and **6e** showed that are most active compounds against *M. smigmatis* with value of 6.25 µg/mL each. Nine compounds are having better MIC values (12.5 µg/mL) for *M. smigmatis* compared to other microorganisms. All the compounds are showing lower activity against Gram positive bacteria *S. aureus* except few of the 6 series compounds having 25 µg/mL MIC. Results of antimicrobial studies have been

presented in Table 3 and Table 4.

Table 3

Table 4

3. Conclusion

Two series of new 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide (**6a-e** and **7a-g**) derivatives and N-alkyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolin-4-amine derivatives (**9a-f**) were synthesized in reasonably good yields. They were characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry, IR studies and elemental analyses. The structure of **9a** has also been confirmed by X-ray crystallographic study. All the newly synthesized compounds were screened for *in-vitro* antimicrobial activity by well plate method and MIC was determined using serial dilution method. Among the screened samples, **7a** and **9c** are showed significant antimicrobial activity against all the tested microbial strains in well diffusion method. Whereas, from the MIC studies **6d** and **6e** found to be the most active compounds among all others.

The compound **9c** has showed significant inhibition against all the tested micro organisms as compared to other synthesized compounds, which may be due to the presence of 7-trifluoromethy, 3-(5-phenyl-1,3,4-oxadiazol) and biologically active amines (ethanolamine) at fourth position of quinoline ring. Compound **7b** has also shown good inhibition against both bacterial and fungal strains. This is possibly due to the presence of 3-(3,4-dimethoxy phenyl) carbohydrazide, 4-hydroxy and 7-trifluoromethyl groups on quinoline ring. The compounds containing pyrazole carbohydrazide derivatives and methoxyphenyl, N-diethylphenyl carbohydrazides at position 3 of 4-hydroxy-(7-trifluoromethy)quinoline accounted for the enhanced activity of *Mycobacterium smegmatis* up to 12-16 mm zone of inhibition of the compounds **6a**, **6b**, **6d**, **7a**, **7b**, and **7f**. In conclusion, antibacterial activity increases with increase of electron withdrawing group on pyrazole carbohydrazide and electron donating groups on phenylcarbohydrazide at third position of quinoline. In oxadiazole series (**9a-f**), antibacterial activity increases with introducing aliphatic amines at fourth position of the 7-(trifluoromethyl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)quinoline instead of aromatic amine.

As regards the relationship between the structure of the heterocyclic scaffold and the detected antimicrobial properties, it can be concluded that the combination of two different heterocyclic moieties namely quinoline and heterocyclic carbohydrazides, 1,3,4-oxadiazoles has enhanced biological activity and hence they are ideally suited for further notification to obtain more efficient antimicrobial compounds.

The acute oral toxicity study for the newly synthesized organic compounds **6a-e, 7a-g** and **9a-f** was performed and there were no mortality and significant behavioral changes observed for first 24 h for all newly synthesized compounds at all concentrations. But, the compounds **6b**, **6d**, **7a**, **9b** and **9f** showed mortality at 750 mg/kg and above concentrations after 24 h. The remaining compounds are not showing any behavioral changes at all concentration throughout the experiment.

4. Experimental

4.1. Analysis and instruments

All the chemicals were purchased from Sigma Aldrich, Merck and S. D. Fine chemicals-India. Commercial grade solvents were used and were distilled before use. Melting points were determined by open capillary method and were uncorrected. The IR spectra (Neat) were recorded on a JASCO FT/IR-4100 spectrophotometer and Bruker (400 MHz) spectrometer was used to record ^{1}H-NMR and ^{13}C-NMR spectra (DMSO-d_6, $CDCl_3$) using TMS as internal standard. Chemical shift values were given in δ (ppm) scales. The mass spectra were recorded on LC-MS-Agilent 1100 series and elemental analysis was performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reactions was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254). The names of the structures were given as per chemdraw.

4.2. Synthesis of diethyl ({[3-(trifluoromethyl)phenyl]amino}methylidene)propanedioate (3)

3-(Trifluoromethyl) aniline **1** (10.0 g, 0.062 mol) and diethyl ethoxymethylene malonate **2** (18.61 mL, 0.093 mol) were heated to 110 °C for 6 h. The reaction mixture was cooled to room temperature, the solid thus formed was taken in pet ether and stirred for 20 min and filtered to get compound **3** as a white crystalline solid. Yield: 19.0 g, 92 %; m.p: 44-46 °C; IR (Neat, ν_{max}

cm^{-1}): 3252 (N-H), 3118, 2979 (C-H-str), 1708 and 1616 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.35 (t, -CH_3, 3H, J=5.3 Hz), 1.38 (t, -CH_3, 3H, J=5.3 Hz), 4.25 (q, -CH_2, 2H), 4.34 (q, -CH_2, 2H), 7.25 (m, -CH, 1H), 7.36 (d, -CH, 1H, J= 6.1 Hz), 7.62 (m, -CH, 2H), 8.45 (d, -NCH=C-, 1H, J=9.6 Hz), 11.45 (brd, -NH, 1H, J=13.8). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 14.97, 59.61, 111.32, 119.81, 119.87, 122.61, 123.10, 130.92, 131.55; Anal. calcd. For $C_{15}H_{16}F_3NO_4$; Calcd: C, 54.38; H, 4.80; N, 4.23; found: C, 54.35; H, 4.80; N, 4.20%.[16]

4.3. Synthesis of 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (4)

Diethyl ({[3-(trifluoromethyl)phenyl]amino}methylidene)propanedioate **3** (10.0 g, 0.030 mol) and Dowtherm (100 mL) were heated to 250 °C for 5 h. The reaction mixture was then cooled to 25 °C and stirred in 150 mL hexane for 10 min. The solid product obtained was filtered and dried. The crude product obtained was purified by column chromatography using pet ether and ethyl acetate (5:5) as the eluent. Yield: 7.2 g, 84 %; m.p: 298-300 °C; IR (Neat, ν_{max} cm^{-1}): 3322 (-OH), 3029, 2970 (C-H-str), 1706 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.23 (t. 3H, J = 8.0 Hz, -CH_3), 4.18 (q, 2H, -CH_2), 7.53 (t, 1H, ArH, J = 8.0 Hz), 8.07 (d, 1H, ArH, 7.8 Hz), 8.41 (d, 2H, ArH, J = 8.0 Hz), 11.62 (s, 1H, -OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 14.71, 60.49, 111.38, 119.10, 124.61, 125.35, 130.99, 131.55, 146.43. MS: m/z = 286 (M+1); Anal. calcd. For $C_{13}H_{10}F_3NO_3$; Calcd: C, 54.74; H, 3.53; N, 4.91; found: C, 54.77; H, 3.50; N, 4.95%.[16]

4.4. Synthesis of of 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid hydrazide (5)

A mixture of ethyl 4-hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester **4** (5.0 g, 0.017 mol) and hydrazine hydrate (4.1 mL, 0.085 mol) in ethanol (50 mL) were refluxed for 4 h. After the completion of the reaction, the reaction mixture was concentrated and allowed to cool. The solid product obtained was filtered, washed with water and recrystallized from ethanol to give **5** as a white solid. Yield: 4.25 g, 89 %; m.p: 255-257 °C; IR (Neat, ν_{max} cm^{-1}): 3442 (-OH), 3296 and 3244 (N-H), 3088, 2963 (C-H-str), 1649 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.67 (s, 2H, -NH_2), 7.76 (dd, 1H, ArH, J = 7.7 Hz, J = 1.4 Hz), 8.10 (s, 1H, ArH), 8.45 (d, 1H, ArH, J = 8.4 Hz), 8.90 (s, 1H, ArH), 10.57 (s, 1H, -NH), 12.88 (s, 1H, -OH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 110.68, 123.20, 126.53, 130.06, 131.25, 146.79, 160.96,

174.49; MS: m/z = 272 (M+1). Anal. calcd. For $C_{11}H_8F_3N_3O_2$; Calcd: C, 48.72; H, 2.97; N, 15.49; found: C, 48.75; H, 2.97; N, 15.59%.[28]

4.5. General method for the preparation of 4-hydroxy-7-(trifluoromethyl) quinoline-3-carbohydrazide derivatives (6a-e and 7a-g)

An equimolar mixture of 4-hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid hydrazide **5** (0.5 g, 0.0018 mol), pyrazole-4-carbaldehyde or aromatic aldehydes (0.002 mol) and catalytic amount of acetic acid in dry ethanol (5 mL) were stirred at 25 °C for 1 h. Completion of the reaction was monitored by TLC. The precipitated solid was filtered under suction, washed with ethanol and recrystallized from ethanol.

4.5.1. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (1,3-diphenyl-1H-pyrazol-4-ylmethylene)-hydrazide (6a)

IR (Neat, ν_{max} cm^{-1}): 3410 (O-H), 3138 (N-H), 3064, 2923 (C-H-str), 1668 (C=O), 1604 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.15-7.50 (m, 6H, ArH), 7.52 (d, 1H, ArH, J = 8.4 Hz), 7.75 (d, 2H, ArH, J = 8.0 Hz), 7.96 (d, 2H, ArH, J = 8.0 Hz), 8.07 (s, 1H, N=CH), 8.42 (d, 1H, ArH, J = 8.4 Hz), 8.43 (s, 1H, ArH), 8.96 (s, 2H, ArH), 12.90 (s, 1H, -OH), 13.10 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 10.51, 117.29, 117.77, 120.32, 128.00, 128.73, 128.91, 130.06, 138.11, 139.38, 139.23, 146.15, 155.07, 152.01, 160.55, 173.91; MS: m/z = 502 (M+1). Anal. calcd. For $C_{27}H_{18}F_3N_5O_2$; Calcd: C, 64.67; H, 3.62; N, 13.97; found: C, 64.65; H, 3.63; N, 13.90%.

4.5.2. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid [3-(4-metho xy-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-hydrazide (6b)

IR (Neat, ν_{max} cm^{-1}): 3403 (O-H), 3114 (N-H), 3067, 3010 (C-H-str), 1672 (C=O), 1607 (C=N), 1176 (O-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.86 (s, 3H, OCH_3), 7.10 (d, 2H, ArH, J = 8.6 Hz), 7.38 (t, 1H, ArH, J = 7.4 Hz), 7.55 (t, 2H, ArH, J = 8.1 Hz), 7.76 (d, 2H, ArH, J = 8.6 Hz), 7.82 (d, 1H, ArH, J = 9.0 Hz), 8.02 (d, 2H, ArH, J = 7.7 Hz), 8.13 (s, 1H, N=CH), 8.45 (s, 1H, ArH), 8.48 (d, 1H, ArH, J = 8.6 Hz), 8.98 (s, 1H, ArH), 9.03 (s, 1H, ArH), 12.99 (s, 1H, -OH), 13.15 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 55.62, 111.43, 118.72,

119.23, 121.28, 124.67, 124.90, 126.69, 127.32, 127.67, 127.88, 129.74, 130.07, 138.96, 146.26, 152.23, 159.54, 175.85. MS: m/z = 532 (M+1). Anal. calcd. For $C_{28}H_{20}F_3N_5O_3$; Calcd: C, 63.28; H, 3.79; N, 13.18; found: C, 63.34; H, 3.72; N, 13.20%.

4.5.3. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid [3-(4-chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-hydrazide (6c)

IR ((Neat, ν_{max} cm^{-1}): 3411 (O-H), 3129 (N-H), 3015, 2913 (C-H-str), 1667 (C=O), 1605 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.34-7.55 (m, 4H, ArH), 7.75 (d, 2H, ArH, J =8.0 Hz), 7.83 (d, 2H, ArH, J = 8.0 Hz), 7.96-7.98 (m, 3H, ArH), 8.06 (s, 1H, N=CH), 8.42 (s, 1H, ArH), 8.95 (s, 1H, ArH), 8.97 (s, 1H, ArH), 12.96 (s, 1H, -OH), 13.10 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 111.02, 117.49, 117.82, 120.45, 129.02, 129.14, 130.23, 131.15, 138.33, 139.54, 139.77, 146.48, 156.00, 153.15, 160.78, 175.02; MS: m/z = 536 (M+1). Anal. calcd. For $C_{27}H_{17}ClF_3N_5O_2$; Calcd: C, 60.51; H, 3.20; N, 13.07; found: C, 60.58; H, 3.21; N, 13.05%.

4.5.4. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid [3-(4-nitro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-hydrazide (6d)

IR (Neat, ν_{max} cm^{-1}): 3464 (O-H), 3233 (N-H), 3073, 2923 (C-H-str), 1650 (C=O), 1596 (C=N), 1534 (N-O), 1495 (N-O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.41-7.45 (m, 1H, ArH), 7.56-7.60 (m, 2H, ArH), 7.83 (dd, 1H, ArH, J = 8.76 Hz, J = 1.56 Hz), 8.04-8.07 (m, 2H, ArH), 8.14 (s, 1H, N=CH), 8.23-8.26 (m, 2H, ArH), 8.36-8.39 (m, 2H, ArH), 8.49 (d, 1H, ArH, J = 8.4 Hz), 8.56 (s, 1H, ArH), 9.0 (s, 2H, ArH), 13.03 (s, 1H, -OH), 13.14 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 111.81, 117.72, 117.81, 120.63, 129.84, 129.97, 131.04, 131.26, 138.59, 139.87, 139.94, 147.00, 156.12, 153.25, 161.02, 175.49; MS: m/z = 547 (M+1). Anal. calcd. For $C_{27}H_{17}F_3N_6O_4$; Calcd: C, 59.34; H, 3.14; N, 15.38; found: C, 59.38; H, 3.11; N, 15.40%.

4.5.5. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (1-phenyl-3-p-tolyl-1H-pyrazol-4-ylmethylene)-hydrazide (6e)

IR (Neat, ν_{max} cm^{-1}): 3385 (O-H), 3130 (N-H), 3014, 2919 (C-H-str), 1663 (C=O), 1605

(C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.41(s, 3H, CH_3), 7.36-7.40 (m, 3H, ArH), 7.55 (t, 2H, ArH, J = 8.0 Hz), 7.70 (d, 2H, ArH, J = 7.8 Hz), 7.83 (d, 1H, ArH, J = 8.7 Hz), 8.01 (d, 2H, ArH, J = 8.0 Hz), 8.13 (s, 1H, N=CH), 8.46 (s, 1H, ArH), 8.47 (d, 1H, ArH, J = 8.7 Hz), 8.98 (s, 1H, ArH), 9.02 (s, 1H, ArH), 12.96 (s, 1H, -OH), 13.13 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 21.18, 111.68, 117.25, 117.42, 121.32, 127.88, 128.83, 129.80, 130.06, 138.51, 139.12, 139.38, 139.56, 141.27, 146.11, 152.07, 160.96, 175.49. MS: m/z = 516 (M+1). Anal. calcd. For $C_{27}H_{17}F_3N_6O_4$; Calcd: C, 65.24; H, 3.91; N, 13.59; found: C, 65.30; H, 3.89; N, 13.55%.

4.5.6. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (4-methoxy-benzylidene)-hydrazide (7a)

IR (Neat, ν_{max} cm^{-1}): 3407 (O-H), 3163 (N-H), 3063, 2978 (C-H-str), 1647 (C=O), 1605 (C=N), 1165 (O-CH_3); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 2.95 (s, 3H, OCH_3), 6.17 (d, 2H, ArH, J = 8.2 Hz), 6.86 (d, 2H, ArH, J = 8.2 Hz), 6.96 (d, 2H, ArH, J = 8.0 Hz), 7.28 (s, 1H, N=CH), 7.53 (s, 1H, ArH), 7.63 (d, 1H, ArH, J = 8.0 Hz), 8.15 (s, 1H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 55.13, 108.83, 112.10, 117.32, 121.34, 122.01, 127.33, 127.85, 128.61, 139.41, 146.05, 148.55, 149.52, 151.38, 160.98, 160.99, 175.87; MS: m/z = 390 (M+1). Anal. calcd. For $C_{19}H_{14}F_3N_3O_3$; Calcd: C, 58.61; H, 3.62; N, 10.79; found: C, 58.72; H, 3.62; N, 10.78%.

4.5.7. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (3,4-dimethoxy-benzylidene)-hydrazide (7b)

IR (Neat, ν_{max} cm^{-1}): 3410 (O-H), 3170 (N-H), 3015, 2969 (C-H-str), 1661 (C=O), 1582 (C=N), 1173 (O-CH_3), 1156 (O-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 7.06 (d, 1H, ArH, J = 4.4 Hz), 7.28 (dd, 1H, ArH, J = 8.3 Hz, J = 1.8 Hz), 7.39 (d, 1H, ArH, J = 1.8 Hz), 7.82 (dd, 1H, ArH, J = 8.6 Hz, J = 1.3 Hz), 8.15 (s, 1H, N=CH), 8.38 (s, 1H, ArH), 8.50 (d, 1H, ArH, J = 8.4 Hz), 9.02 (s, 1H, ArH), 13.02 (s, 1H, -OH), 13.11 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 55.97, 108.80, 112.09, 117.39, 121.35, 122.06, 127.33, 127.93, 128.53, 139.33, 146.04, 148.60, 149.51, 151.28, 160.97, 160.97, 175.74. MS: m/z = 420 (M+1). Anal. calcd. For $C_{20}H_{16}F_3N_3O_4$; Calcd: C, 57.28; H, 3.85; N,

10.02; found: C, 57.35; H, 3.85; N, 10.00%.

4.5.8. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid thiophen-2-ylmethylene-hydrazide (7c)

IR (Neat, ν_{max} cm^{-1}): 3407 (O-H), 3061 (N-H), 2983, 2922 (C-H-str), 1649 (C=O), 1607 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.15-7.18 (m, 1H, ArH), 7.47 (d, 1H, ArH, J = 7.6 Hz), 7.69-7.70 (m, 1H, ArH), 7.83 (d, 1H, ArH, J = 8.5 Hz), 8.15 (s, 1H, N=CH), 8.49 (d, 1H, ArH, J = 8.4 Hz), 8.71 (s, 1H, ArH), 9.01 (s, 1H, ArH), 13.03 (s, 1H, -OH), 13.12 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 120.31, 122.54, 122.81, 123.10, 127.13, 127.74, 146.29, 149.15, 151.06, 160.27, 161.92, 174.35; MS: m/z = 366 (M+1). Anal. calcd. For $C_{16}H_{10}F_3N_3O_2S$; Calcd: C, 52.60; H, 2.76; N, 11.50; found: C, 52.66; H, 2.72; N, 11.53%.

4.5.9. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (4-dimethyl amino-benzylidene)-hydrazide (7d)

IR (Neat, ν_{max} cm^{-1}): 3417 (O-H), 3100 (N-H), 3075, 3010 (C-H-str), 1645 (C=O), 1602 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.97 (s, 6H, NCH_3), 6.77 (d, 2H, ArH, J = 8.9 Hz), 7.58 (d, 1H, ArH, J = 8.9 Hz), 7.76 (d, 1H, ArH, J = 8.4 Hz), 8.12 (s, 1H, N=CH), 8.26 (s, 1H, ArH), 8.48 (d, 1H, ArH, J = 8.4 Hz), 9.00 (s, 1H, ArH), 13.11 (s, 1H, -OH), 13.14 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 47.05, 53.04, 111.73, 112.04, 117.72, 121.36, 122.38, 127.02, 129.09, 138.96, 145.97, 148.92, 151.27, 160.14, 175.51. MS: m/z = 403 (M+1). Anal. calcd. For $C_{20}H_{17}F_3N_4O_2$; Calcd: C, 59.70; H, 4.26; N, 13.92; found: C, 59.68; H, 4.25; N, 13.82%.

4.5.10. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (3-ethoxy-2-hydroxy -benzylidene)-hydrazide (7e*)*

IR (Neat, ν_{max} cm^{-1}): 3400 (O-H), 3222 (O-H), 3189 (N-H), 3049, 2985 (C-H-str), 1660 (C=O), 1615 (C=N), 1206 (O-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.37 (t, 3H, CH_3, J = 6.8 Hz), 4.07 (q, 2H, OCH_2, J = 4.4 Hz), 6.87 (t, 1H, ArH, J = 8.4 Hz), 7.03 (d, 1H, ArH, J = 7.7 Hz), 7.12 (d, 1H, ArH, J = 7.0 Hz), 7.84 (d, 1H, ArH, J = 7.1 Hz), 8.15 (s, 1H, N=CH), 8.50 (d, 1H, ArH, J = 7.0 Hz), 8.69 (s, 1H, ArH), 9.04 (s, 1H, ArH), 11.14 (s, 1H, -OH), 13.13 (s, 1H,

-OH), 13.18 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 21.40, 64.52, 111.84, 117.13, 121.98, 122.45, 123.08, 126.33, 127.81, 128.05, 140.00, 145.99, 150.21, 159.27, 160.87, 160.97, 176.02; MS: m/z = 420 (M+1). Anal. calcd. For $C_{20}H_{16}F_3N_3O_4$; Calcd: C, 57.28; H, 3.85; N, 10.02; found: C, 57.30; H, 3.86; N, 10.00%.

4.5.11. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (4-diethylamino-2-hydroxy-benzylidene)-hydrazide (7f)

IR (Neat, ν_{max} cm^{-1}): 3466 (O-H), 3237 (O-H), 3110 (N-H), 2975, 2927 (C-H-str), 1621 (C=O), 1586 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.12 (t, 6H, CH_3, J = 6.9 Hz), 3.37 (q, 4H, NCH_2, J = 7.0 Hz), 6.13 (s, 1H, ArH), 6.29 (dd, 1H, ArH, J = 8.8 Hz, J = 2.4 Hz), 7.22 (d, 1H, ArH, J = 8.8 Hz), 7 .83 (dd, 1H, ArH, J = 8.6 Hz, J = 1.4 Hz), 8.14 (s, 1H, N=CH), 8.46 (s, 1H, ArH), 8.49 (d, 1H, ArH, J = 8.4 Hz), 9.00 (s, 1H, ArH), 11.37 (s, 1H, -OH), 12.91 (s, 1H, -OH), 13.10 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 17.54, 54.01, 111.66, 117.62, 121.87, 122.50, 123.16, 126.28, 127.15, 128.10, 145.83, 150.12, 159.30, 160.88, 161.00, 175.95; MS: m/z = 447 (M+1). Anal. calcd. For $C_{22}H_{21}F_3N_4O_3$; Calcd: C, 59.19; H, 4.74; N, 12.55; found: C, 59.20; H, 4.72; N, 12.15%.

4.5.12. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (6-bromo-pyridin-3-ylmethylene)-hydrazide (7g)

IR (Neat, ν_{max} cm^{-1}): 3464 (O-H), 3048 (N-H), 2974, 2926 (C-H-str), 1541 (C=O), 1580 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.84 (dd, 1H, ArH, J = 8.6 Hz, J = 1.5 Hz), 8.18 (s, 1H, N=CH), 8.34-8.35 (m, 1H, ArH), 8.52 (s, 1H, ArH), 8.76-8.78 (m, 2H, ArH), 8.89 (d, 1H, ArH, J = 8.2 Hz), 9.06 (s, 1H, ArH), 13.16 (s, 1H, -OH), 13.28 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 117.98, 119.26, 120.99, 121.16, 122.72, 123.15, 126.32, 127.31, 145.89, 150.15, 159.39, 160.87, 161.03, 175.72; MS: m/z = 440 (M+1). Anal. calcd. For $C_{17}H_{10}BrF_3N_4O_2$; Calcd: C, 46.49; H, 2.30; N, 12.76; found: C, 46.45; H, 2.32; N, 12.71%.

4.6. Synthesis of 4-chloro-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinoline (8)

A mixture of 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide **5** (5.0 g, 0.018 mol), benzoic acid (2.44 g, 0.020 mol) and phosphorous oxychloride (50 mL) were heated at 100 °C for 10 h. The reaction mixture was then cooled to room temperature, the excess of $POCl_3$ was

removed by distillation under vacuum. The residue obtained was quenched to crushed ice and the solid separated was filtered off and dried through pump. The crude product was purified by column chromatography using pet ether and ethyl acetate (9:1) as the eluent.

Yield: 3.1 g, 45 %; m.p: 131-133 °C; IR (Neat, ν_{max} cm^{-1}): 3049, 2921 (C-H-str), 1596 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.58-7.64 (m, 2H, ArH), 8.10 (t, 2H, ArH, J = 8.0 Hz), 8.15 (d, 2H, ArH, J = 8.0 Hz), 8.53 (s, 1H, ArH), 8.62 (d, 1H, ArH, J = 8.0 Hz), 9.62 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 123.35, 125.03, 127.17, 127.17, 127.76, 127.92, 129.03, 129.73, 129.91, 130.06, 131.22, 132.54, 133.32, 151.48, 165.30, 167.78; MS: m/z = 376 (M+1). Anal. calcd. For $C_{18}H_9ClF_3N_3O$; Calcd: C, 57.54; H, 2.41; N, 11.18; found: C, 57.55; H, 2.38; N, 11.20%.

4.6.1. General method for the preparation of N-alkyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolin-4-amine derivatives (9a-f)

A suspension of compound **8** (0.5 g, 0.0013 mol) in dry dimethylformamide 5 mL was taken in a 25 mL round bottomed flask (RBF), dry potassium carbonate (0.26 g, 0.0019 mol) and substituted amine (0.0019 mol) were then added to the round bottomed flask (RBF). The reaction mixture was heated at 110 °C for 8 h. After the reaction completion, the reaction mixture was poured into ice-cold water. The product was extracted in ethyl acetate and concentrated. The crude product was purified by column chromatography using pet ether and ethyl acetate as the eluent.

4.6.2. 4-(4-Methyl-piperazin-1-yl)-3-(5-phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinoline (9a)

IR (Neat, ν_{max} cm^{-1}): 3060, 2929 (C-H-str), 1589 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.26 (s, 3H, NCH_3), 2.57 (t, 4H, NCH_2, J = 4.2 Hz), 3.21 (t, 4H, NCH_2, J = 4.6 Hz), 7.67-7.70 (m, 3H, ArH), 7.97 (dd, 1H, ArH, J = 8.8 Hz, J = 1.84 Hz), 8.12-8.15 (m, 2H, ArH), 8.39 (s, 1H, ArH), 8.43 (d, 1H, ArH, J = 8.8 Hz), 9.08 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 46.29, 51.91, 55.22, 55.59, 111.59, 115.86, 122.72, 123.68, 127.14, 127.23, 127.33, 127.50, 127.66, 130.04, 149.36, 151.50, 153.56, 155.80, 162.37, 165.28. MS: m/z = 440 (M+1). Anal. calcd. For $C_{23}H_{20}F_3N_5O$; Calcd: C, 62.86; H, 4.59; N, 15.94; found: C, 62.96; H,

4.55; N, 15.98%.

4.6.3. 4-Morpholin-4-yl-3-(5-phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinoline (9b)

IR (Neat, ν_{max} cm^{-1}): 3062, 2959 (C-H-str), 1576 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.15 (t, 4H, NCH_2, J = 4.2 Hz), 4.92 (t, 4H, OCH_2, J = 4.4 Hz), 7.65-7.78 (m, 3H, ArH), 7.97-8.14 (m, 3H, ArH), 8.35 (s, 1H, ArH), 8.42 (d, 1H, ArH, J = 8.8 Hz), 9.06 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 50.68, 55.98, 115.82, 123.31, 127.10, 127.35, 127.48, 127.54, 127.69, 129.11, 129.17, 130.72, 133.82, 152.00, 165.16; MS: m/z = 427 (M+1). Anal. calcd. For $C_{22}H_{17}F_3N_4O_2$; Calcd: C, 61.97; H, 4.02; N, 13.14; found: C, 62.00; H, 4.03; N, 13.10%.

4.6.4. 2-[3-(5-Phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinolin-4-ylamino]-ethanol (9c)

IR (Neat, ν_{max} cm^{-1}): 3272 (N-H), 3072, 2921 (C-H-str), 1587 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.38 (s, 1H, OH), 3.73 (q, 2H, NCH_2, J = 5.0 Hz), 3.93 (q, 2H, OCH_2, J = 5.0 Hz), 5.08 (s, 1H, NH), 7.65-7.78 (m, 3H, ArH), 7.75 (dd, 1H, ArH, J = 8.9, Hz, J = 1.5 Hz), 8.20-8.22 (m, 2H, ArH), 8.70 (d, 1H, ArH, J = 8.9 Hz), 8.96 (t, 1H, ArH, J = 8.7 Hz), 9.21 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 50.98, 60.32, 118.99, 119.70, 121.44, 123.60, 125.68, 126.99, 127.35, 128.06, 129.07, 130.87, 132.36, 148.92, 150.28, 151.24, 162.92, 163.23. MS: m/z = 401 (M+1). Anal. calcd. For $C_{20}H_{15}F_3N_4O_2$; Calcd: C, 60.00; H, 3.78; N, 13.99; found: C, 60.03; H, 3.88; N, 13.94%.

4.6.5. Acetic acid 2-[3-(5-phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinolin-4-ylamino]-ethyl ester (9d)

IR (Neat, ν_{max} cm^{-1}): 3260 (N-H), 3052, 2981 (C-H-str), 1741 (C=O), 1590 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.45 (s, 3H, CH_3), 4.15 (q, 2H, NCH_2, J = 5.1 Hz), 4.67 (q, 2H, OCH_2, J = 5.0 Hz), 5.21 (s, 1H, NH), 7.64-7.70 (m, 3H, ArH), 8.08-8.13 (m, 3H, ArH), 8.51 (d, 1H, ArH, J = 8.9 Hz), 8.61 (t, 1H, ArH, J = 8.6 Hz), 9.89 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 15.98, 51.00, 60.59, 119.13, 121.45, 123.65, 125.60, 127.00, 127.87, 127.91, 128.10, 130.64, 132.36, 149.08, 162.97, 164.02; MS: m/z = 443 (M+1). Anal. calcd. For $C_{22}H_{17}F_3N_4O_3$; Calcd: C, 59.73; H, 3.87; N, 12.66; found: C, 59.70; H, 3.81; N, 12.76%.

4.6.6. [3-(5-Phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinolin-4-yl]-(3,4,5-trimethoxy-phenyl)-amine (9e)

IR (Neat, ν_{max} cm^{-1}): 3260 (N-H), 3025, 2981 (C-H-str), 1590 (C=N), 1116 (O-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.10 (s, 3H, OCH_3), 3.55 (s, 6H, OCH_3), 6.24 (s, 1H, NH), 7.44-7.52 (m, 4H, ArH), 7.72-7.78 (m, 3H, ArH), 8.13 (s, 1H, ArH), 8.66 (d, 1H, ArH, J = 8.0 Hz), 8.82 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 55.83, 55.89, 119.23, 121.46, 123.32, 124.17, 125.82, 127.33, 127.39, 127.71, 129.11, 129.43, 130.07, 133.81, 152.45, 164.96; MS: m/z = 508 (M+1). Anal. calcd. For $C_{27}H_{21}F_3N_4O_4$; Calcd: C, 63.90; H, 3.97; N, 8.28; found: C, 63.90; H, 3.97; N, 8.30%.

4.6.7. (2-Methyl-quinolin-4-yl)-[3-(5-phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinolin-4-yl]-amine (9f)

IR (Neat, ν_{max} cm^{-1}): 3245 (N-H), 3061, 2921 (C-H-str), 1606 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.86 (s, 3H, CH_3), 6.30 (s, 1H, NH), 7.30-7.68 (m, 3H, ArH), 7.78 (t, 2H, ArH, J = 8.2 Hz), 7.96-8.06 (m, 3H, ArH), 8.24 (d, 2H, ArH, J = 8.3 Hz), 8.26 (s,1H, ArH), 8.42 (s, 1H, ArH), 8.61 (d, 1H, ArH, J = 8.0 Hz), 9.35 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 17.06, 118.29, 123.15, 123.92, 125.24, 127.00, 127.23, 127.91, 129.06, 129.37, 130.11, 132.07, 132.10, 132.27, 135.11, 137.46, 151.89, 163.17, 163.82; MS: m/z = 498 (M+1). Anal. calcd. For $C_{28}H_{18}F_3N_5O$; Calcd: C, 67.60; H, 3.65; N, 14.08; found: C, 67.65; H, 3.64; N, 14.10%.

5.X-ray crystallographic study of compound 9a

The X-ray crystallographic analysis of the compound **9a** was carried out by fine-focus sealed tube graphite, with approximate dimensions 0.44 mm x 0.20 mm x 0.13 mm, grown from the slow evaporation of a dilute ethanol solution at room temperature. The crystal structure solution was worked out by Bruker SMART APEXII DUO CCD diffractometer. All the atoms were located in different Fourier maps and refined isotropically, using a riding model and all the projections were generated using ORTEP. The details of the crystal data and refinement are shown in Table 5. Also the single crystal image for compound **9a** is given in Fig. 2.[36]

Figure 2

Table 5

6.Antibacterial studies

The antibacterial activity of newly synthesized compounds (**6a-e**, **7a-g** and **9a-f**) were determined by well diffusion method in nutrient agar media.[30,31] *In-vitro* antibacterial activity of compounds against 24 hrs old bacterial culture of a *Mycobacterium smegmatis (*MTCC 943) and *Pseudomonas aeruginosa* (MTCC4676) was performed in well diffusion method. Nutrient agar media (about 15-20 millilitres) was poured into each petri plate and allowed to solidify by placing inside the laminar air flow for 15 min. 100 µL of 0.5 McFarland standard of bacterial suspension was inoculated on the agar media and spread on the whole surface with a sterile cotton bud. Using a sterile cork borer, five mm wells were made on the seeded agar plates and 50 µL of test compound at different concentrations (25 and 50 µg/mL) was transferred in to the wells. The plates were prepared in triplicate for each compound and incubated. *Pseudomonas aeruginosa* at 30 °C and *Mycobacterium smegmatis* at 37 °C for 12 hrs and observed for the zone of inhibition in millimeter. All the compounds are dissolved in DMSO and dilutions of the working solution were made using the same solvent. Ciprofloxacin was used as antibacterial standard.

7. Antifungal studies

Antifungal studies of synthesized compounds (**6a-e**, **7a-g** and **9a-f**) were carried out against *Candida albicans* (MTCC 183) and *Penicillium chrysogenum* (MTCC 6795) using the well diffusion method. Czapek Yeast extract agar media was used for *Penicillium chrysogenum* and yeast extract peptone dextrose (YEPD) agar media was used for *Candida albicans*.[32,33] Normal saline was used to make a suspension of spore of *Penicillium chrysogenum* for lawning. A loop full of *Penicillium chrysogenum spores* was transferred to 3 mL saline to get a spore suspension. Whereas, few colonies of *Candida albicans* were dispersed in 5 mL of YEPD broth and allowed to grow for few hours before used for spreading the agar plate. Twenty millilitres of agar media was poured into each petri dish and allowed for 15 minutes to solidify and followed the same protocol used for antibacterial activity explained above. Except for the petri plates incubated at 25 °C for 48 hours for *Penicillium chrysogenum* and 30 °C for *Candida albicans* for 12-24 hours. Antifungal activity was determined by measuring the diameter of inhibition zone in

mm. All the experiments were conducted in triplicates and Flucanazole was used as standard antifungal compound.

8. MIC against microorganisms

Minimum concentration of compounds required for the inhibition of bacteria *M. smegmatis, P. aeruginosa* and *Staphylococcus aureus* and a fungi *C. albicans* was determined using compound concentration ranging from 1.6-50 µg/mL . Test compounds are dissolved in DMSO and serially diluted to 50, 25, 12.5, 6.25, 3.125 and 1.6 µg/mL of concentration. In a 96 well plate 50 µL of bacterial or fungal suspension was taken and 50 µL of serially diluted above compounds were added to each well and mixed. Incubated the mixture for 12 hr and observed each well for the bacterial growth. The lowest concentration at which no microbial growth found was taken as MIC. For the confirmation, 10 µL of the mixture from each well was spread on a nutrient agar plate and incubated to check for any bacterial growth. Each test was repeated in triplicates. Ciprofloxacin and Flucanozole were used as standard for bacteria and fungi respectively.

Acknowledgements

AMI thanks Department of Atomic Energy, Board for Research in Nuclear Sciences, Government of India for the 'Young Scientist' award. Authors thank Department of Information & technology, Government of India, New Delhi for the financial support. Authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through research group project no. RGP-VPP-207.

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