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N-Substituted-1,2,3-triazoles: Synthesis, characterization and antimicrobial activity studies

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ABSTRACT

Triazoles display a broad range of biological activities and are found in many potent, biologically active compounds, such as trazodone (antidepressant drug), rizatriptan (antimigrane drug), hexaconazole (antifungal drug) and alprazolam (hyptonic, sedative and tranquilizer drug). So far, modifications of the triazole ring have proven highly effective with improved potency and lesser toxicity.1,2,3-triazole appended bromoquinoline carboxylic acid derivatives were synthesized from Isatin and keto-functionalized triazole derivative. Chemical structures of the synthesized compounds were confirmed by various spectral techniques. All the compounds were subjected to antimicrobial and anti fungal studies showed moderate to good properties.

Keywords: 1,2,3-triazole, quinoline carboxylic acid, antibacterial activity, antimicrobial activity, antifungal activity.

INTRODUCTION

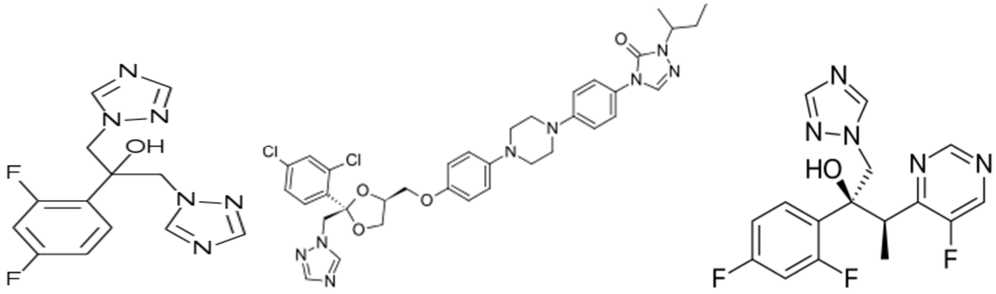
Quinoline is a heterocyclic scaffold of paramount importance to human race. Several quinoline derivatives isolated from natural resources or prepared synthetically are significant with respect to medicinal chemistry and biomedical use. Indeed quinoline derivatives are some of the oldest compounds which have been utilized for the treatment of a variety of diseases. The bark of Cinchona plant (also known as Jesuit's or Cardinal's bark) containing quinine was utilized to treat palpitations, [1] fevers and tertian's since more than 200 years ago. Quinidine, a diastereoisomer of quinine was in the early 20th century acknowledged as the most potent of the antiarrhythmic compounds isolated from the Cinchona plant. [2] Compounds containing quinoline moiety are most widely used as antimalarial, [3] antibacterial, [4] antifungal [5] and anticancer agents. [6] Additionally, quinoline derivatives find use in the synthesis of fungicides, virucides, biocides, alkaloids, rubber chemicals and flavouring agents. [7] They are also used aspolymers, catalysts, corrosion inhibitors, preservatives, and as solvent for resins and terpenes. Furthermore, these compounds find applications in chemistry of transition metal catalyst for uniform polymerization and luminescence chemistry. [8] Quinoline derivatives also act as antifoaming agent in refineries. [9] It has been shown [10] that quinoline exhibit antitumor activity due to the formation of stable complex with DNA. Recent demonstrations [11-14] showed that quinoline carboxylic acid can be used as potential anti-HIV agents that stimulated further interest on these molecules with yet another perspective. Owing to such significance, the synthesis of substituted quinolines has been a subject of great focus in organic chemistry.

It has been observed that incorporation of certain bioactive pharmacophores in the existing drug molecules sometimes exert a profound influence on the biological activity of the parent molecules, it was thought of interest to incorporate active pharmacophore like triazole framework with quinoline moiety could produce interesting series of

compounds with enhanced biological activities.In recent years hundreds of different heterocyclic compounds have been synthesized and screened for anticonvulsant activity. These include mostly five and six membered ring systems containing up to three or four heteroatom and seven membered ring systems related to the diazepines. Among the nitrogen containing heterocycles, a considerable amount of work has been done in the areas of five membered rings bearing one or two nitrogen atoms. Five membered rings with 3 or 4 nitrogen atoms that have been investigated include mostly, 1,2,4- triazoles and some tetrazoles. However very little research has been done on 1,2,3-triazoles.

The chemistry of N-bridged heterocyclic compounds, such as triazole, has received considerable attention due to their interesting biological activities. Triazole is one of a pair of isomeric chemical compounds with the molecular formula $C_2H_3N_3$. It is a basic aromatic heterocyclic ring [15]. Triazole derivatives are known to exhibit various pharmacological properties such as antimicrobial [16-20], antitubercular[21], anticancer [22,23], anticonvulsant [24], anti-inflammatory, analgesic [25] and antiviral [26]. Triazoles have also been incorporated in a wide variety of therapeutically interesting drugs including H_1/H_2 histamine receptor blockers, CNS stimulants, anti-anxiety agents and sedatives [27]. The most important use, however, is as antimycotics in drugs such as fluconazole, itraconazole and voriconazole[28,29] shown in fig 1.

Figure:1



Fluconazole **Itraconazole** **voriconazole**

The triazole moiety is stable to metabolic degradation and capable of hydrogen bonding, which could be favourable in binding bimolecular targets as well as increasing solubility(30).Moreover, triazoles can function as attractive linker units which could connect two pharmacophores to give an innovative bifunctional drugs, thus have become increasingly useful and important in constructing bioactive and functional molecules (31-33).

It is decided to combine above phamacophoric fragments viz., quinoline and triazole in a single molecule through covalent bond. Chemotherapeutic activity of nitrogen heterocycles are almost always associated with compounds having the nitrogen containing group attached to C-5 of the five-membered heterocycle containing appropriate substituents at C-2 and C-5 of six membered heterocycle.

MATERIALS AND METHODS

The target molecule C(a–j) were synthesized according to the Scheme 1 using 5-Bromo isatin**1a** and substituted 1,2,3-triazole **2(a-j).**The yield of the desired derivatives was around 90 % prior to purification. IR spectra were recorded on FT-IR Shimadzu 8300 spectrophotometer in KBr pellet and ^{1}H-NMR spectra were recorded on a Bruker 400 MHz spectrometer in DMSO-d_6 using tetramethylsilane as the internal standard. Mass spectra were obtained on Shimadzu LCMS-2010EV. TLC was performed on aluminum-backed silica plate with visualization using UV-light and columnchromatography using silica gel. HPLC were obtained on Agilent-1200 series with DAD (Diode Array Detector), Zorbax C-18 Column. 1.0 mg of the compound was taken for spectral elucidation, whereas 0.1 mg of the compound was taken for the HPLC studies.

General procedure for the preparation and purification of compound **3(a–j)**

Preparation of 5-Bromoindolin-2,3-dione:
N-(4-Bromophenyl)-2-hydroxyiminoacetamide
4-Bromoaniline(0.50 mol) was added slowly to 10% aqueous HCl solution.This suspension was added to a mixture of chloral hydrate(0.55 mol) and sodium sulphate(50g) in 75 mL water with mechanical stirring. Hydroxylamine

hydrochloride(1.63 mol)dissolved in 25mL water was added, and the resulting slurry was heated at 100^0C.After this temperature was reached, the heating mantle was removed immediately, and the solution was cooled to room temperature. The formed precipitate was collected by filtration, washed with water, and dried in a vacuum oven at 60^0C .Concentrated sulphuric acid(20mL) was heated at 50^0C, and N-(4-bromophenyl)-2-hydroxyiminoacetamide was slowly added.The black solution was carefully heatedand maintained at 90^0C for half an hour.The dark-red solution was cooled to room temperature and poured into ice water. The precipitated solid was filtered and dried to get 5-Bromoindolin-2,3-dione**.**The product was fairly pure for further reactions.

Synthesis of substituted azidobenzene

Aniline (20 g, 0.144 mol) was dissolved in (91.4 ml) 1:1 ratio of HCl, water and taken in a round bottom flask equipped with stirrer. The reaction was agitated at 0-5°C sodium nitrite (10 g, 0.144 mol) was dissolved in 50 ml of icecold water and added drop wise, sodium azide (9.36 g 0.144 mol) dissolved in 100 ml of water and added dropwise, then reaction is allowed to continue for 30 min. The resultant oil was extracted with chloroform and washed successively with water. The organic layer was dried over anhydrous sodium sulphate, and the solvent stripped out in rotary evaporator to get azidobenzene, yield 93.5% (18.7 g).The similar procedure was followed for other substituted anilines.

^{1}H-NMR(400 MHz, DMSO-d6):6.89-6.92 (q,, 1H,Ar-H),7.39-7.46 (m, 1H, Ar–H),11.40 (broad, NH). ESI–MS m/z: [M+1]: 165.90

Synthesis of benzyl azide:

Benzyl chloride(6.72ml;0.0584 mol) was taken in a round bottom flask with 80 ml of dimethyl sulphoxide equipped with stirrer,sodium azide(7.6g;0.1169 mol) added and stirred overnight. The reaction mixture was quenched in ice-water and extracted with diethyl ether,washed successively with 5% bicarbonate solution and dried over anhydrous sodium sulphate. The solvent was removed by rotary evaporator to give benzyl azide,yield 71.4%(4.8ml) .

Synthesis of 1-[phenyl]-4-acetyl-5-methyl-1,2,3-triazole

The azidobenzene (3 g, 0.018 moles), acetyl acetone(1.8 g, 0.018 moles), potassium carbonate (8 g, 0.057 moles), absolute ethanol (95%, 45 ml) were taken in a round bottom flask equipped with stirrer. The reaction mixture was agitated at 75°C for 30 min. The progress of the reaction was monitored by TLC. After the completion of the reaction, the solvent was removed under vacuum. The residual mass was quenched in the ice-water mixture and neutralized with 10% HCl solution. The product was extracted with diethyl ether dried over anhydrous sodium sulfate.Evaporation of the solvent yield crude product, which was purified by column chromatography using chloroform as eluent and recrystallized from absolute ethanol, yield 79.5% (3.9 g).The remaining substituted triazoles were prepared in the similar manner and their characterized .

^{1}H-NMR(400 MHz, DMSO-d6):2.63(d,3H,CH_3),2.75(d,3H,-$COCH_3$),7.46 (m, 2H, Ar–H),7.60(m, 3H, Ar–H);ESI–MS m/z: [M+1]: 201.90.

Synthesis of 6-Bromo-2-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid)

1-(1-phenyl-1H-1,2,3-triazol-4-yl)ethanone (0.0099 mol) was dissolved in ethanol(20ml). 4-Bromoisatin(0.0099mol) was added in lot wise. The reaction mixture was stirred at 25- 28^0C. Sodium hydroxide (0.0792mol) dissolved in water(20ml) and added drop wise to the reaction mixture. Heated to reflux for 24 hrs(till completion of reaction) .Cooled to room temperature and the solvent was stripped out in rotary evaporator .The residue was adjusted to pH-3.5 using cooled dil. HCl(10ml). The precipitated solid was dried at 50^0C. The compound was further purified by column chromatography using cyclohexane and THF mixtures. The remaining compounds were prepared in the similar manner and their characterization data are as follows:

6-Bromo-2-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid

IR (KBr, cm-1): 2926.1(OH), 1232.7 (C–N), 1719.82 (C=O), 1500.48 (C-C); 1H NMR(400 MHz, DMSO-d6): 2.82 (s, 3H–CH3), 7.62-7.70 (m,, 5H,Ar-H),7.84-7.86 (d, 1H, Ar–H), 7.94-7.96 (d, 1H, Ar–H), 8.60 (s, 1H, Ar–H),9.06 (s, 1H, Ar–H), 7.34(broad,OH);13C NMR (DMSO-d6) d (ppm): 167.20 (C=O), 152.27 (C=C), 147.24(Ar–C), 142.01(Ar–C), 130.39(Ar–C), 125.88 (Ar–C), 125.02(Ar–C), 121.50(Ar–C), 121.40(Ar–C), 10.92(C–C, CH3). ESI–MS m/z: [M+1]: 411.15.

2-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-6-Bromoquinoline-4-carboxylic acid
IR (KBr, cm-1): 2990.42(OH), 1236.1 (C–N), 1706.3 (C=O), 1448.55 (C-C); 1H NMR(400 MHz, DMSO-d6): 2.75 (s, 3H–CH3),5.69(s,2H,) 7.24-7.26 (d, 2H,Ar-H),7.30-7.40 (m, 3H, Ar–H), 7.74-7.76 (d, 1H, Ar–H),7.83-7.86(s,1H,Ar-H),8.37 (s, 1H, Ar–H),9.10-9.107(s,1H,Ar-H);13C NMR (DMSO-d6) d (ppm): 168.48 (C=O), 152.64(C=C), 148.90(Ar–C), 146.99(Ar–C), 132.18(Ar–C), 127.76 (Ar–C), 126.62(Ar–C), 118.85(Ar–C), 118.45(Ar–C), 51.10(Ar-CH_2)9.87(C–C, CH3). ESI–MS m/z: [M+1]: 423.05.

2-(1-(2,6-dimethylphenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6-Bromoquinoline-4-carboxylic acid
IR (KBr, cm-1): 2922.93(OH), 1226.06 (C–N), 1708.1 (C=O), 1485.5 (C-C); 1H NMR(400 MHz, DMSO-d6):1.94 (s, 6H–CH3),2.58 (s, 3H–CH3), 7.35-7.37 (d,, 2H,Ar-H),7.45-7.49 (m, 1H, Ar–H), 7.94-7.96 (m, 1H, Ar–H),8.01-8.04 (d, 1H, Ar–H), 8.83 (s, 1H, Ar–H),9.01-9.01 (s, d,1H, Ar–H);13C NMR (DMSO-d6) d (ppm): 167.28 (C=O), 152.40 (C=C), 147.38(Ar–C), 141.80(Ar–C), 135.75(Ar–C), 125.12 (Ar–C), 121.62(Ar–C), 121.14(Ar–C), 9.85(C–C, CH3)), 17.25(C–C, CH3). ESI–MS m/z: [M+1]: 439.15.

6-Bromo-2-(1-(4-fluorophenyl)-5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid
IR (KBr, cm-1): 2926.47(OH), 1233.8 (C–N), 1719.4 (C=O), 1513.8 (C-C); 1H NMR(400 MHz, DMSO-d6): 2.80 (s, 3H–CH3), 7.51-7.55 (m,, 2H,Ar-H),7.77-7.80 (m, 2H, Ar–H), 7.95-7.97 (q, 1H, Ar–H), 7.98-8.05 (d, 1H, Ar–H), 8.79 (s, 1H,Ar-H) ,9.002-9.007 (d, 1H, Ar–H);13C NMR (DMSO-d6) d (ppm): 167.24 (C=O), 161.72 (C=C), 152.32(Ar–C), 142.00(Ar–C), 131.99(Ar–C), 128.49 (Ar–C), 128.40(Ar–C), 125.09(Ar–C), 117.28(Ar–C), 117.05(Ar–C), 10.84(C–C, CH3). ESI–MS m/z: [M+1]: 427.10

2-(1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6- Bromoquinoline-4-carboxylic acid
IR (KBr, cm-1): 2925.35(OH), 1234.2 (C–N), 1719.16 (C=O), 1498.6 (C-C); 1H NMR(400 MHz, DMSO-d6): 2.83 (s, 3H–CH3), 7.73-7.75 (d, 5H,Ar-H),7.91-7.93 (t,1H, Ar–H), 8.00-8.02 (d, 1H, Ar–H),8.71 (s, 1H, Ar–H), 9.02 (s, 1H, Ar–H);13C NMR (DMSO-d6) d (ppm): 167.66 (C=O), 152.2 (C=C), 147.24(Ar–C), 142.36(Ar–C), 135.00(Ar–C), 135.26 (Ar–C), 127.72(Ar–C), 125.43(Ar–C), 121.06(Ar–C), 120.70(Ar–C), 10.88(C–C, CH3). ESI–MS m/z: [M+1]:444.20

2-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6- Bromoquinoline-4-carboxylic acid
IR (KBr, cm-1): 2925.7(OH), 1238.7 (C–N), 1716.77 (C=O), 1494.65 (C-C); 1H NMR(400 MHz, DMSO-d6): 2.78 (s, 3H–CH3), 7.66-7.68 (d, 2H,Ar-H),7.86-7.92 (m,4H, Ar–H), 8.71 (s, 1H, Ar–H),8.94 (s, 1H, Ar–H), 13C NMR (DMSO-d6) d (ppm): 167.18 (C=O), 152.12 (C=C), 147.22(Ar–C), 142.10(Ar–C), 135.49(Ar–C), 131.88 (Ar–C), 123.60(Ar–C), 125.02(Ar–C), 123.60(Ar–C), 121.64(Ar–C), 10.88(C–C, CH3). ESI–MS m/z: [M+1]: 489.15

6- Bromo -2-(1-(2-fluorophenyl)-5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid
IR (KBr, cm-1): 2928.7(OH), 1241.92 (C–N), 1722.85 (C=O), 1509.12 (C-C); 1H NMR(400 MHz, DMSO-d6): 2.72 (s, 3H–CH3), 7.51-7.62 (m, 1H,Ar-H),7.67-7.75 (m,1H, Ar–H),7.77-7.82 (m, 2H, Ar–H),7.94-7.97 (d, 1H, Ar–H), 8.01-8.04 (m, 1H, Ar–H), 8.04 (s, 1H, Ar–H) ,9.01 (s, 1H, Ar–H);13C NMR (DMSO-d6) d (ppm): 167.23 (C=O), 157.61 (C=C), 155.11(Ar–C), 152.08(Ar–C), 133.748(Ar–C), 123.45 (Ar–C), 123.33(Ar–C), 121.73(Ar–C), 117.67(Ar–C), 117.48(Ar–C), 10.23(C–C, CH3). ESI–MS m/z: [M-1]:427.00

2-(1-(3-chlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6- Bromoquinoline-4-carboxylic acid
IR (KBr, cm-1): 3082.13(OH), 1233.96 (C–N), 1721.34 (C=O), 1591.29 (C-C); 1H NMR(400 MHz, DMSO-d6): 2.80 (s, 3H–CH3), 7.71 (s, 3H,Ar-H),7.87-7.96 (m,3H, Ar–H),8.72 (s,1H, Ar–H),8.95 (s, 1H, Ar–H) ;13C NMR (DMSO-d6) d (ppm): 167.17 (C=O), 152.09 (C=C), 147.22 (Ar–C), 142.04(Ar–C), 135.53(Ar–C), 135.42 (Ar–C), 134.38(Ar–C), 125.04(Ar–C), 124.73(Ar–C), 124.73(Ar–C), 10.86(C–C, CH3). ESI–MS m/z: [M-1]:444.55

2-(1-(2,5dichlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6- Bromoquinoline-4-carboxylic acid
IR (KBr, cm-1): 2925.48(OH), 1234.19 (C–N), 1719.20 (C=O), 1498.63 (C-C); 1H NMR(400 MHz, DMSO-d6): 2.68 (s, 3H–CH3), 7.82-7.92 (m, 3H,Ar-H),7.99-8.08 (d,2H, Ar–H),8.68(s,1H, Ar–H), 9.06 (s, 1H, Ar–H) ;13C NMR (DMSO-d6) d (ppm): 167.81 (C=O), 152.05 (C=C), 147.27 (Ar–C), 141.96(Ar–C), 136.48(Ar–C), 132.36 (Ar–C), 125.73(Ar–C), 120 .82(Ar–C), 120.18(Ar–C), 10.23(C–C, CH3). ESI–MS m/z: [M+1]:477.35

2-(1-(2,6-difluorobenzyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6- Bromoquinoline-4-carboxylic acid
IR (KBr, cm-1): 2926.22(OH), 1235.23 (C–N), 1713.84 (C=O), 1471.60 (C-C); 1H NMR(400 MHz, DMSO-d6): 2.88 (s, 3H–CH3), 5.69 (s, 2H–CH_2) 7.18-7.22 (t,2H,Ar-H),7.49-7.54 (m,1H, Ar–H),7.93-8.02 (m,2H, Ar–H),8.68

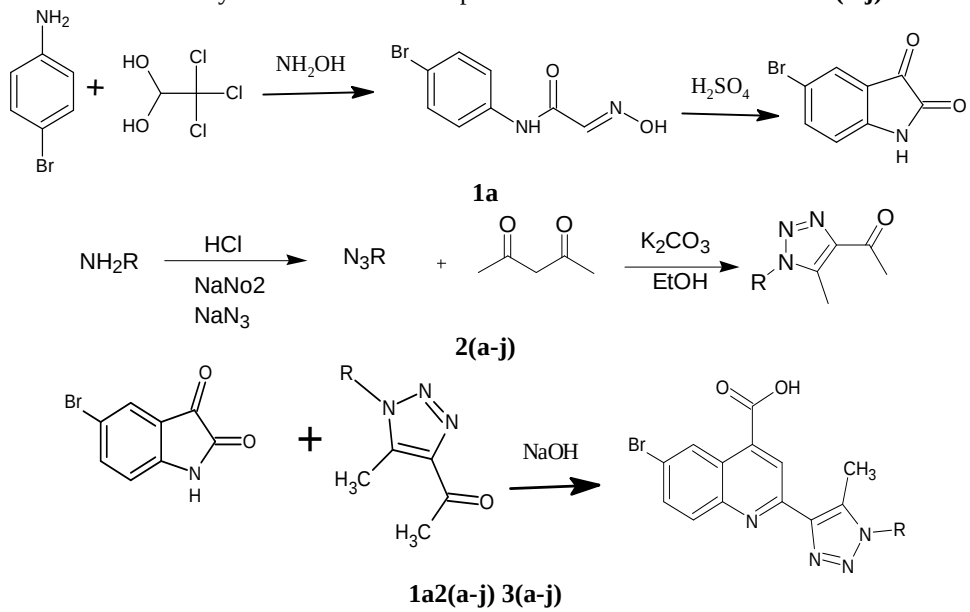
(s, 1H, Ar–H), 8.97 (s, 1H, Ar–H) ;13C NMR (DMSO-d6) d (ppm): 167.27 (C=O), 162.61 (C=C), 147.29 (Ar–C), 141.57(Ar–C), 134.86(Ar–C), 125.03 (Ar–C), 121.50(Ar–C), 121.35(Ar–C), 111.21(Ar–C), 111.40(Ar–C), 9.64(C–C, CH3). ESI–MS m/z: [M+1]:459.00

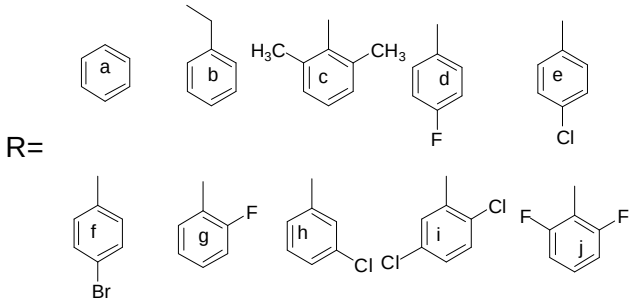
RESULTS AND DISCUSSION

4.1 Chemistry:

1,2,3-triazole appended quinoline carboxylic acid derivatives were successfully synthesized from Isatin and ketofunctionalizedtriazole derivative. The triazole compounds were synthesized through 1,3-dipolar cycloaddition of aromatic azides and 1,4-diketone under basic condition. All the aromatic azides were prepared using conventional techniques except benzyl azide which was prepared using DMSO/sodium azide. The structure of the triazole derivatives were confirmed using ^{1}H-NMR,mass spectra analysis.The ^{1}H-NMR of compound **2a**showed doublet in the region of δ2.63ppm which is due to triazole methyl proton and a doublet in the region of δ2.75ppm which is due to acetyl methyl proton. similarly multiplet in the region of δ7.46-7.60 which is due to aromatic ring proton .The mass spectrum of compound **2a** showed molecular ion peak at m/z 200.90, which is in good agreement with the molecular formula $C_{11}H_{11}N_3O$.The compound thus obtained was treated with isatin prepared by the literature method (21) pitfzinger reaction conditions.We tried different inorganic base such as Lithium hydroxide,Sodium hydroxide,Potassium hydroxide etc. The rate of reaction was very slow in Lithium hydroxide,Sodium hydroxide, for halogen substituted molecule(~48hrs).In Potassium hydroxide reaction goes to completion in ~24 hrs.The better purity of compound achieved using adjusting pH to 3-3.5.For monohalogenated compounds unreacted starting material were removed by leaching with cyclohexane/2-methyl THF and for dihalogenated compounds unreacted starting material was removed by leaching with methanol/dichloromethane.The targeted compound structures were confirmed by IR,^{1}H-NMR,^{13}C-NMR,mass spectral analysis.For compound **3a** absorption band at 2926cm^{-1} due to acid OH functional group and weak absorption band at 1232.7, 1719.82, 1500.48 cm^{-1} due to C–N group, C=O stretching, and C-C bond stretching respectively. The ^{1}H -NMR of compound **3a**showed singlet in the region of δ2.82ppm which is due to triazole methyl proton and a multiplet in the region of δ7.62-7.70 ppm is due to quinoline ring aromatic proton. Similarly broad peak in the region of δ 7.34 is due to acid functional group proton. The mass spectrum of compound **3a** showed molecular ion peak at m/z 411.15 which is agreed with the molecular formula $C_{19}H_{14}N_4BrO_2$.The entire scheme of the synthesis is given in the Scheme 1. Similarly a series of compounds with varying substitutions with aromatic ring attached to triazole ring were synthesized and subjected to antimicrobioal studies.

Scheme 1: synthesis oftriazole compounds from isatin**1a** andtriazole**2(a-j)**





4.2Antimicrobial activity:

A series of 6-Bromo-2-(substituted)-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid 3(a-j) were synthesized in good yields and screened for their antibacterial activity by disc diffusion method. Antibacterial study was assessed by Minimum inhibitory concentration (MIC) plate method. Antibacterial studies against various bacterial strains show mixed results but comparing with standard compound viz., Nitrofurazone, the synthesized compounds were shown good MIC values. Compound **3a** shows good inhibition against Pseudomonas aeroinosa. Compound **3f** shows very good inhibition against Pseudomonas aeroinosa bacterial strains which is having Bromine at 4th position, compound**3i** where the 2,6-dichloro shows good inhibition against Pseudomonas aeroinosa and Klebsiella Pneumoniae.Most of halogen substituted compound shows good activity against bacteria. Compounds **3(d,e,j)** shows no inhibition or very mild activity against bacteria.

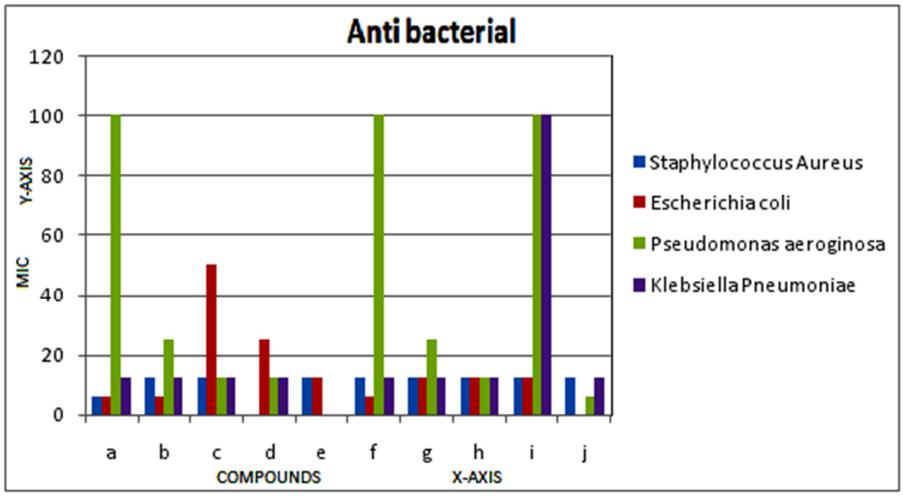
2.3Antibacterial activity

The newly synthesized compounds**3(a-j**) were screened for their antibacterial activity against *Escherichia coli* (ATTC- 25922), *Staphylococcus aureus*(ATTC-25923), *Psuedomonusaeruginosa*(ATTC-27853), *Bacillus subtilis*and *Klebsiellapneumoniae*(recultured) bacterial stains by disc diffusion method. The discs measuring 6.25mm in diameter were punched from Whatman No. 1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using dimethyl sulfoxide 1% (DMSO). One millilitre containing 100 times the amount of chemical required in each disc was added to each bottle which contains 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37.8°C for 24 h. Nitrofurazone was used as a standard drug. Solvent and growth controls were kept. The zone of inhibition and minimum inhibitory concentrations [MIC] was noted. The MIC values of tested compounds are given in Table 1

Table 1 :Minimum Inhibitory Concentration(MIC) of compound(a-j) against *Staphylococcus Aureus, Escherichia coli, Pseudomonas aeroginosa, Klebsiella Pneumoniae*

	Anti bacterial			
Compounds	*Staphylococcus Aureus (Recultured)*	*Escherichia coli (Recultured)*	*Pseudomonas aeroginosa (Recultured)*	*Klebsiella Pneumoniae (Recultured)*
a	6.25	6.25	100	12.5
b	12.5	6.25	25	12.5
c	12.5	50	12.5	12.5
d	NI	25	12.5	12.5
e	12.5	12.5	NI	NI
f	12.5	6.25	100	12.5
g	12.5	12.5	25	12.5
h	12.5	12.5	12.5	12.5
i	12.5	12.5	100	100
j	12.5	NI	6.25	12.5
Nitrofurazone	<6.25	<6.25	<6.25	<6.25
DMSO (1%)	00	00	00	00

Table 2 :Grapical representation of Minimum Inhibitory Concentration(MIC) of compound3(a-j) against *Staphylococcus Aureus* , *Escherichia coli, Pseudomonas aeroginosa, Klebsiella Pneumoniae*



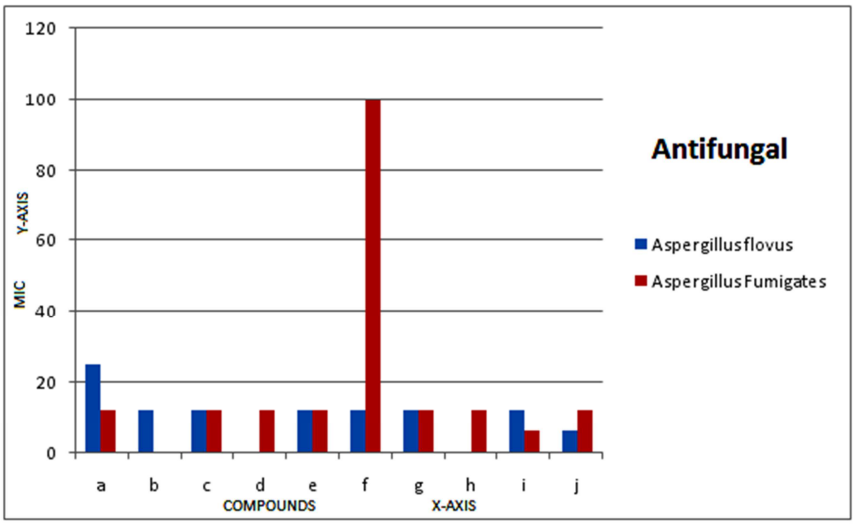
2.4Antifungal activity

Antifungal activity for newly prepared compounds was screened by serial plate dilution method. 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 a suspension of spores of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. A 20ml of agar media was poured in to each of the petridishes. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1 h. Using an agar punch wells were made on these seeded agar plates and 10 mg/ml of the test compounds in DMSO were added into each well labeled. A control was also prepared for the plates in the same way using solvent DMSO. The petridishes 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. Activity of each compound was compared with Amphotericin B as standard. The minimum inhibitory concentration (MIC) for the Amphotericin B in DMSO was more than 1 mg/ml against the tested species. The MIC values of tested compounds are given in Table 3.Antifungal activity of the synthesized compounds were analysed by disc diffusion method. Antifungal studies against two fungal stains show moderate to good activity. The activity of the synthesized compounds was compared with standard Amphotericin B. As like in antibacterial, compound **3f**shows good inhibition values. Compound **3b** and**3d** shows no inhibition against Asperillusflovus whereas the other compounds show medium to poor inhibition.

Table 3 :Minimum Inhibitory Concentration(MIC) of a-j against aspergillusflovus and asperillus fumigates

Compounds	*Aspergillus flovus*	*Aspergillus Fumigates*
a	25	12.5
b	12.5	NI
c	12.5	12.5
d	NI	12.5
e	12.5	12.5
f	12.5	100
g	12.5	12.5
h	NI	12.5
i	12.5	6.25
j	6.25	12.5
Amphotericin B	<6.25	<6.25

Table 4 :Grapical representation of Minimum Inhibitory Concentration(MIC) of a-j against *aspergillus flovus and asperillus fumigates*



CONCLUSION

A series of quinoline carboxylic acid derivative using pfitzinger reaction synthesized from isatin and various 1,2,3-triazole containing keto functional group. Structures of the synthesized compounds were investigated by usual spectral techniques to ascertain the chemical structure. The purity of the compounds was analyzed by HPLC and purity was found to above 99.5%. Microbial screenings of the compounds reveal that these compounds are moderate to good activity compared to market available standards.

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