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Design, Synthesis, SAR, Docking and antibacterial evaluation: Aliphatic amide bridged 4-aminoquinoline clubbed 1,2,4- triazole derivatives

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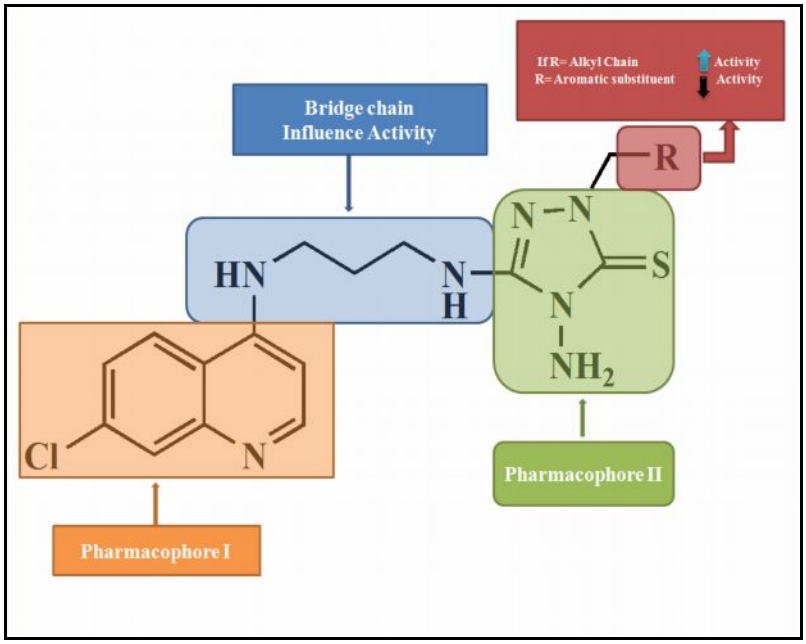
Abstract: A series (7a-7k) of aliphatic amide bridged 4-aminoquinoline clubbed 1,2,4- triazole derivatives were designed on the basis of field alignment and mapping and finally synthesized via six step synthesis protocol. The antibacterial activities of all the synthesized molecules were performed on different gram positive and gram negative bacterial strains. Compound 7a, 7d and 7h were found most active against all the strains in comparison with reference ciprofloxacin. Molecular docking studies of most active compounds were performed on DNA gyrase protein pdb: 1ZI0 and results revealed that functional groups and its positions seems to be critical for their antibacterial activities.
Key words : 4-aminoquinoline, 1, 2, 4- triazole, antibacterial activity, Molecular docking, DNA gyrase, Field analysis.

Introduction

In previous decades, the human population affected with lethal contagious diseases caused by multi-drug resistant gram-positive and gram-negative pathogenic bacteria. Drug resistance enlarged to an alarming level around the world [1] and one of the major cause of death [2-3]. Penicillin used in human therapeutics since 1940s and during the past 60 years, various other antibiotics have been used .The antibiotics developed not just to treat human infectious diseases, but also their property used in veterinary, plant agriculture and aquaculture. Extensive use has created a robust discerning burden, which unswervingly has resulted in the spreading of resistant bacteria, the advent of resistance had shown multifaceted mechanisms by which resistance genes spread across the bacterial demesne, with deceptive disregard for species barriers. But the bacterial evolutionary response has not been restricted to the acquisition of resistance of genetic factor [4]. The drug resistance emerged as serious global issue that appeal the attention of chemists towards new antibacterial agents design and development. In this direction heterocyclic compound shows their vital position due to their varied range of activities. Merging of two or more heterocyclic ring makes molecule more competent and pharmacological dynamic [5-8]. Quinoline, heterocyclic ring, is a very important moiety of various antibacterial agents like ciprofloxacin, ofloxacin, norfloxacin etc. The 1,2,4-triazole and its derivatives were reported to

exhibit various pharmacological activities such as antimicrobial, analgesic, anti-inflammatory, anticancer and antioxidant properties [9-11]. Some of the current day drugs such as Ribavirin (antiviral agent), Rizatriptan (antimigraine agent), Alprazolam (anxiolytic agent), Fluconazole and Itraconazole (antifungal agents) are the preeminent examples for potent molecules possessing triazole nucleus [12]. Here in present communication we clubbed 1,2,4 triazole with 4-aminoquinoline using aliphatic bridge for development towards novel and proficient antibacterial agent.

Graphical abstract



Materials and methods

All commercially available solvents and reagents were of analytical grade and used without further purification. Melting points were determined on a Veego, MPI melting point apparatus and FTIR (2.0 cm-1, flat, smooth, abex) were recorded on Perkin Elmer RX-I Spectrophotometer. ^{1}H NMR spectra were recorded in DMSO-d6 using Bruker Avance II 400 NMR and ^{13}C NMR spectra on Bruker Avance II 100 NMR spectrometer in DMSO-d6 using TMS as internal standard. Mass spectra were obtained on VG-AUTOSPEC spectrometer equipped with electrospray ionization (ESI) sources. Elemental analysis was carried out on Vario EL-III CHNOS elemental analyzer.

Chemistry

A series of eleven clubbed derivatives of 4-aminoquinoline were synthesized via six step protocol. In the first step formation of N-(7-chloro-quinolin-4-yl)-ethane-1,2-diamine (2) take place via the reaction of 4,7-dichloroquinoline with 1,3-diamino propane however second step involve reaction between derivative (2) with phenyl chloroformate in presence of triethylamine leads to the formation of 1-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-carbamic acid phenyl ester (3). Construction of N-{2-[(7-chloroquinolin-4 yl)amino] ethyl} hydrazinecarboxamide (4) achieved in third step where hydrazine monohydrate react with compound 3 using methanol as solvent further forth step involve potassium dithiocarbazinate (5) synthesis where derivative 4 reacted with KOH and carbon disulfide using ethanol as solvent. Fifth synthetic protocol involve synthesis of 4-amino-5-[2-(7-chloro-quinolin-4-ylamino)-ethylamino]-4H-[1,2,4]triazole-3-thiol (6) where derivative (5) unite with hydrazine hydrate using distilled water as solvent. Final synthetic protocol involve synthesis of 4-amino-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino Substituted methyl 2,4-dihydro-[1,2,4]triazole-3-thione (7a-7k) viz from derivative 6, formaldehyde and various amines.

Synthetic procedure for N-(7-chloro-quinolin-4-yl)-ethane-1,2-diamine (2)

A mixture of 4,7-dichloroquinoline (2.0 g, 10.1 mmol) (1) and 1,3 diaminopropane (50.0 mmol), was heated at 80°C for 1 h without stirring for 1 h and then at 110°C for 4–6 h with continued stirring to drive the reaction to completion. The reaction mixture was cooled to room temperature and diluted with dichloromethane. The organic layer was successively washed with 5% NaOH (30 mL) followed by water wash and then finally with brine. The organic layer was dried over anhydrous Na_2SO4 and solvent was removed under reduced pressure to afford the compounds **2** , at 80–90% yield [13].

Synthesis of 1-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-carbamic acid phenyl ester (3)

Phenyl chloroformate (1.41 g, 9.02 mmol) was added to a stirred and cooled (0^oC) solution of N-(7-chloro-quinolin-4-yl)-ethane-1,2-diamine (2.00 g, 9.02 mmol) (2) and triethylamine .(1.26 ml,9.02 mmol) in DMF (10 ml). The mixture was stirred at room temperature for 45 min, diluted with water (50 ml) and extracted with chloroform (3-50 ml). The combined organic layers were washed with water (3· 50 ml), brine (50 ml), dried ($MgSO_4$) and concentrated to give a yellow residue [14].

Synthesis of N-{2-[(7-chloroquinolin-4-yl)amino]ethyl}hydrazinecarboxamide (4)

To a solution of [2-(7-chloro-quinolin-4-ylamino)-ethyl]-carbamic acid phenyl ester (3) (1.26 g, 3.70 mmol) in dry methanol (10 ml) was added hydrazine monohydrate(1.85, 37 mmol) and the resulting mixture was stirred at 90^oC for 12 h. The reaction mixture was concentrated to give a white residue [15].

Synthesis of Potassium dithiocarbazinate derivative (5)

Potassium hydroxide (0.03 mol) was dissolved in absolute ethanol (50 mL). The solution was cooled in an ice bath and acid hydrazide (0.02 mol) **(4)** was added with stirring. To this, carbon disulfide (0.025 mol) was added in small portions with constant stirring. The reaction mixture was stirred continuously for 12 h at room temperature. The precipitated potassium dithiocarb- azinate was collected by filtration, washed with anhydrous ether and dried in vacuum. The potassium salt thus obtained was used in the next step without further purification [16].

Synthesis of 4-amino-5-[2-(7-chloro-quinolin-4-ylamino)-ethylamino]-4H[1,2,4]triazole-3-thiol (6)

A suspension of potassium dithiocarbazinate derivatives (5) (0.02 mol) and hydrazine hydrate (99%, 0.04 mol) in water (50 mL) was refluxed for 10-15 h with occasional shaking. The colour of the reaction mixture changed to light green with evolution of hydrogen sulfide gas. A homogenous mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with cold water (20 mL). On acidification with dil.HCl the required triazole was precipitated as white precipitate. It was filtered, washed with cold water, dried and recrystallized from ethanol. The compound was found pure in TLC analysis using toluene : ethylacetate : formic acid (5:4:1, v/v/v) as solvent system [16].

General synthetic procedure 4-amino-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino Substituted methyl 2,4-dihydro-[1,2,4]triazole-3-thione (7a-7k)

Formaldehyde (1.5 mL, 40% solution) was added to a solution of derivative (6) (1.8 m mol) in ethanol (15 mL) and the reaction mixture was refluxed for 1 h. The appropriate amine (0.001 mol) was added and the reaction mixture was refluxed for 4 h. After cooling, the formed precipitate was filtered and recrystallized with ethanol.

1-((4-amino-3-(3-(7-chloroquinolin-4-ylamino)propylamino)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)methyl)urea (7a)

Light white solid; Yield: 85.7%; m.p. 252-254 ºC, R_f, 0.78; Mol wt, 421.91; ; FTIR (ν_{max} ; cm^{-1} KBr): 3380 –(N-H stretch), 3050 (C-H stretch, Aromatic),), 2125 (N=C=S stretch), 1645 (C=C, stretch), 1680 (C=O stretch), 760 (C-Cl stretch); ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 1.88-1.81 (p, 2H, -CH_2), 3.39-3.35 (t, 2H, -CH_2), 3.51-3.45 (t, 2H, -CH_2), 4.98-4.93 (d, 2H, -CH_2), 6.18 (s, 2H, -NH_2), 6.27- 6.23 (d, 1H, quinoline), 7.01- 7.14 (t, 2H, -NH_2), 7 .32- 7.26 (d, 1H, quinoline), 7.55 (s, 1H, -NH), 7.78 (s, 1H, quinoline), 7.88 – 7.80 (d, 1H, quinoline), 8.25- 8.20 (d, 1H, quinoline); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 171.56,

158.23, 157.0, 151.86, 150.10, 149.15, 135.04, 127.49, 125.01, 124.10, 117.32, 98.82, 54.68, 43.78, 40.56, 28.37; Mass: 422.16 ($C_{16}H_{20}ClN_9OS$, [M+H]+). Elemental Analysis Calculated: C, 45.55; H, 4.78; N, 29.88; O, 3.79; S, 7.60% Found: C, 45.95; H, 4.56; O, 3.62; N, 29.84%.

4-amino-1-((3-aminopropylamino)methyl)-3-(3-(7-chloroquinolin-4-ylamino) propylamino) -1H -1,2,4-triazole-5(4H)-thione (7b)

Light yellow solid; Yield: 89 %; m.p. 243-245 ºC, R_f, 0.72; Mol wt, 435.17; FTIR (ν_{max} ; cm^{-1} KBr): 3380(N-H stretch), 3050 (C-H stretch, Aromatic), 2120 (N=C=S stretch), 1630 (C=C, stretch), 760 (C-Cl stretch); ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 1.65-1.58 (m, 2H, -CH_2), 1.89-1.84 (m, 2H, -CH_2), 2.45 (s, 1H, -NH), 2.82-2.75 (d, 2H, -NH_2), 3.40-3.35 (d, 2H, -CH_2), 5.28 (s, 2H, -CH_2), 5.78-5.72 (d, 1H, -NH), 6.23-6.18 (d, 1H, quinoline), 7.32- 7.28 (d, 1H, quinoline), 7.89-7.85 (d, 1H, quinoline), 7.91-7.90 (d, 1H, quinoline), 8.25-8.23 (d, 1H, quinoline); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 171.49, 158.22, 151.62, 150.08, 149.08, 135.05, 127.49, 124.09, 124.05, 117.78, 98.78, 65.32, 46.98, 43.72, 40.62, 40.41, 33.20, 28.32; Mass: 436.12 ($C_{18}H_{26}ClN_9S$, [M+H]+). Elemental Analysis Calculated: C, 49.59; H, 6.01; N, 28.91; S, 7.35 % Found: C, 49.35; H, 6.28; N, 29.02%.

4-amino-3-(3-(7-chloroquinolin-4-ylamino)propylamino)-1-((phenylamino)methyl)-1H-1,2,4-triazole-5(4H)-thione (7c)

Yellow solid; Yield: 78%; m.p. 238-240 ºC, R_f, 0.62; Mol wt, 454.98; FTIR (ν_{max} ; cm^{-1} KBr): 3380 (N-H stretch), 3050 (C-H stretch, Aromatic), 2122 (N=C=S stretch), 1630 (C=C, stretch), 760 (C-Cl stretch), 1465 (CH_2 stretch), 1254 (C=N stretch); ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 1.87-1.80 (m, 2H, -CH_2), 3.39-3.36 (t, 2H, -CH_2), 3.52- 3.45 (t, 2H, -CH_2), 5.64 (s, 2H, -NH_2), 5.74 (s, 2H, -CH_2), 6.28-6.24 (d, 1H,quinoline), 6.56-6.52 (t, 1H, Ar-CH), 6.93-6.89 (d, 2H, Ar CH), 7.30-7.35 (d, 1H, quinoline), 7.55 (s, 1H, -NH), 7.80 (s, 1H, quinoline),7.86 (s, 1H, -NH), 7.90 (s, 1H, quinoline), 7.92-7.95 (d, 1H, quinoline), 8.25-8.27 (d, 1H, Quinoline); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 171.51, 151.65, 150.09, 149.08, 147.45, 135.06, 127.09, 124.05, 119.23,117.65, 113.25, 98.64, 64.92, 43.76, 28.31; Mass: 455.18 ($C_{21}H_{23}ClN_8S$, [M+H]+). Elemental Analysis Calculated: C, 55.44; H, 5.10; N, 24.63; S, 7.25 % Found: C, 55.35; H, 5.24; N, 25.01%.

N-((4-amino-3-(3-(7-chloroquinolin-4-ylamino)propylamino)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)acetamide (7d)

Colourless solid; Yield: 64%; m.p. 188-190 ºC, R_f, 0.92; Mol wt, 420.92; FTIR (ν_{max} ; cm^{-1} KBr): 3380 (N-H stretch), 3050 (C-H stretch, Aromatic), 2125(N=C=S stretch), 1680 (C=O stretch); ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 1.84-1.80 (m, 2H, -CH_2), 3.39-3.36 (t, 2H, -CH_2), 3.52- 3.47 (t , 2H, -CH_2), 5.30 - 5.24 (d, 2H, -CH_2), 5.63 (s, 2H, -NH_2), 6.26-6.24 (d, 1H, quinoline), 7.09-6.98 (t, 1H, -NH), 7.32-7.28 (d, 1H, quinoline), 7.55 (s, 1H, -NH), 7.81 (s, 1H, quinoline), 7.92-7.83 (d, 1H, quinoline), 8.27- 8.24 (d, 1H, uinoline); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 171.51, 117.15, 158.09, 151.68, 150.05, 149.06, 137.09, 127.45, 135.12,127.65, 124.25, 124.07, 117.08, 98.64, 54.92, 43.76, 40.71, 28.32, 22.31; Mass: 420.92 ($C_{15}H_{17}ClN_7S$, [M+H]+). Elemental Analysis Calculated: C, 48.51; H, 5.03; N, 26.62; S, 7.62 % Found: C, 48.35; H, 5.25; N, 27.04%.

2-((4-amino-3-(2-(7-chloroquinolin-4-ylamino)ethylamino)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)hydrazinecarboxamide (7e)

Light white solid; Yield: 64%; m.p. 248-250 ºC, R_f, 0.82; Mol wt, 422.90; FTIR (ν_{max} ; cm^{-1} KBr): 3372(N-H stretch), 3025 (C-H stretch), 2125 (N=C=S stretch) , 1680 (C=O stretch), 1612 (C=C stretch, Aromatic), 760 (C-Cl stretch); ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 3.49-3.45 (m, 2H, -CH_2), 3.58-3.54 (m, 2H, -CH_2), 4.42 (s, 2H, -CH_2), 5.48 (s, 1H, -NH), 5.68 (s, 2H, NH_2), 6.17 (s, 1H, aliphatic -NH), 6.29 (s, 2H, -NH_2), 6.41-6.39 (s, 1H, quinoline), 7.37-7.33 (d, 1H, quinoline), 7.90-7.84 (d, 1H, quinoline), 8.46-8.44 (d, 1H, quinoline), 9.10 (s, 1H, -NH), 8.46-8.44 (d, 1H, quinoline), 9.12 (s, 1H, -NH); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 171.51, 158.63, 158.09, 151.65, 150.12, 149.06, 137.09, 127.45, 124.18, 119.23,117.65, 113.25, 99.24, 64.92, 43.26, 38.38; Mass: 424.28 ($C_{21}H_{23}ClN_8S$, [M+H]+). Elemental Analysis Calculated: C, 42.60; H, 4.53; N, 33.12; S, 7.58 % Found: C, 42.37; H, 4.42; N, 32.97%.

2-((4-amino-3-(2-(7-chloroquinolin-4-ylamino)ethylamino)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)hydrazinecarbothioamide (7f)

Dark yellow solid; Yield: 68%; m.p. 248-250 ºC, R_f, 0.65; Mol wt, 438.98; FTIR (ν_{max} ; cm^{-1} KBr): 3380 (N-H stretch), 3035 (C-H stretch), 2125 (N=C=S stretch), 1465(CH_2 stretch), 752 (C-Cl stretch) ^{1}H

NMR (400MHz, DMSO-d_6, TMS) δ ppm: 3.48-3.45 (m, 2H, -CH_2), 3.55-3.50 (d, 2H, -CH_2), 4.47 (s, 2H, -CH_2), 5.39 (s, 1H, -NH), 5.65 (s, 2H, -NH_2), 6.16 (s, 1H, -NH), 6.39-6.38 (d, 1H, quinoline), 7.33-7.25 (d, 1H, quinoline), 7.48 (s, 2H, -NH_2), 7.87-7.84 (d, 1H, quinoline), 8.47- 8.45 (d, 1H, quioline), 8.82 (s, 1H, -NH); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 182.11, 171.55, 158.29, 151.64, 150.25, 149.14 135.19, 127.85, 124.83,117.65, 99.25, 63.64, 44.92, 39.76; Mass: 438.96 ($C_{15}H_{19}ClN_{10}S_2$, [M+H]+). Elemental Analysis Calculated: C, 41.04; H, 4.36; N, 31.91; S, 14.61 % Found: C, 41.55; H, 5.28; N, 15.03%.

4-amino-3-(2-(7-chloroquinolin-4-ylamino)ethylamino)-1-(morpholinomethyl)-1H-1,2,4-triazole-5(4H)-thione (7g)

Colourless solid; Yield: 55%; m.p. 221-223 ºC, R_f, 0.52; Mol wt, 434.95; FTIR (ν_{max} ; cm^{-1} KBr): 3380 (N-H stretch), 3032 (C-H stretch, Aromatic), 2125 (N=C=S stretch), 1465 (CH_2 stretch), 1125 (C=O stretch), 760 (C-Cl stretch) ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 2.69-2.66 (m, 4H, marpholine), 3.48-3.45 (m, 4H, -marpholine), 3.58-3.55 (d, 2H, -CH_2), 5.16 (s, 2H, -CH_2), 5.76 (s, 2H, -NH_2), 6.16 (s, 1H, -NH), 6.39-6.41 (d, 1H, quinoline), 7.38-7.32 (d, 1H, quinoline), 7.65 (s, 1H, -NH), 7.85-7.82 (d, 1H, quinoline), 8.48-8.42 (d,1H, quinoline); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 171.51, 158.32, 151.65, 150.10, 149.08, 135.21, 127.41, 124.14, 124.05,117.65, 99.00, 70.42, 66.25, 51.31, 43.24,39.20; Mass: 435.18 ($C_{18}H_{23}ClN_8$ OS, [M+H]+). Elemental Analysis Calculated: C, 49.71; H, 5.33; N, 25.76; S, 7.33 % Found: C, 55.35; H, 5.24; N, 25.01%.

4-amino-3-(2-(7-chloroquinolin-4-ylamino)ethylamino)-1-(piperazin-1-ylmethyl)-1H-1,2,4-triazole-5(4H)-thione (7h)

Light yellow solid; Yield: 62%; m.p. 245-247 ºC, R_f, 0.93; Mol wt, 433.96; FTIR (ν_{max} ; cm^{-1} KBr): 3378 (N-H stretch), 3036 (C-H stretch, Aromatic), 2125 (N=C=S stretch), 1465 (CH_2 stretch), 755 (C-Cl stretch), ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 2.52 -2.49 (m, 4H, piperazine), 2.80-2.70 (m, 4H, piperazine), 3.53- 3.53 (m, 2H, -CH_2), 5.04 (s, 2H, CH_2) 6.16 (s, 1H, -NH), 7.34- 7.28 (d, 1H,quinoline), 7.90-7.88 (d, 1H, quinoline), 8.28-8.23 (d, 1H, quinoline); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 172.51, 158.38, 151.69, 150.08, 149.04, 135.26, 127.09, 124.05, 119.23,117.65, 98.64, 69.92, 53.21, 46.21, 43.76, 38.31; Mass: 434.18 ($C_{18}H_{24}ClN_9S$, [M+H]+). Elemental Analysis Calculated: C, 49.82; H, 5.57; N, 29.05; S, 7.39 % Found: C, 49.35; H, 6.24; N, 29.25%.

4-amino-3-(2-(7-chloroquinolin-4-ylamino)ethylamino)-1-(piperidin-1-ylmethyl)-1H-1,2,4-triazole-5(4H)-thione (7i)

White solid; Yield: 86%; m.p. 258-260 ºC, R_f, 0.72; Mol wt, 432.97; FTIR (ν_{max} ; cm^{-1} KBr): 3382 (N-H stretch), 3042 (C-H stretch, Aromatic), 2132 (N=C=S stretch), 1450 (CH_2 stretch), 755 (C-Cl stretch), ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 1.43-1.38 (m, 2H, -CH_2), 1.51-1.46 (m, 2H, piperidine), 2.67- 2.58 (d, 4H, piperidine), 3.63 -3.59 (m, 4H, piperidine), 3.88-3.56 (d, 2H, -CH_2), 5.62(s, 2H, -NH_2), 6.16 (s, 1H, -NH), 6.42-6.38 (d, 1H, quinoline), 7.34-7.28 (d, 1H, quinoline), 7.85- 7.83 (d, 1H, quinoline); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 171.51, 158.23, 151.09, 150.08, 149.45, 135.45, 127.42, 124.21, 117.65, 99.64, 70.92, 51.76, 43.21, 39.20, 25.90, 24.31; Mass: 433.86 ($C_{19}H_{25}ClN_8S$, [M+H]+). Elemental Analysis Calculated: C, 52.71; H, 5.82; N, 25.88; S, 7.41 % Found: C, 53.35; H, 5.64; N, 25.94%.

4-amino-1-((4-chlorophenylamino)methyl)-3-(2-(7-chloroquinolin-4ylamino)ethylamino)-1H-1,2,4-triazole-5(4H)-thione (7j)

White solid; Yield: 58%; m.p. 188-190 ºC, R_f, 0.87; Mol wt, 475.41; FTIR (ν_{max} ; cm^{-1} KBr) 3378 (N-H stretch), 3036 (C-H stretch, Aromatic), 2125 (N=C=S stretch), 1465 (CH_2 stretch), 755 (C-Cl stretch); ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 3.49-3.45 (m, 2H, -CH_2), 3.42-3.35 (m, 2H, -CH_2), 5.71 (s, 2H, -NH_2), 5.75 (s, 2H, -CH_2), 6.16 (s, 1H, -NH), 6.41-6.39 (d, 1H, quinoline), 6.85-6.83 (d, 2H, Ar -CH), 7.09-7.07 (d, 1H, Ar -CH), 7.36-7.34 (d, 1H, quinoline), 7.64 (s, 1H, -NH), 7.86-7.84 (d, 1H, Quinoline), 8.47-8.45 (d, 1H, Quinoline); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 171.51, 158.35, 150.09, 150.18, 149.45, 147.16, 135.21, 129.25, 127.29, 124.15, 121.93,117.65, 114.25, 99.44, 64.92, 43.76, 38.31; Mass: 473.42 ($C_{20}H_{20}Cl_2N_8S$, [M+H]+). Elemental Analysis Calculated: C, 50.53; H, 4.24; N, 23.57; S, 6.24 % Found: C, 51.35; H, 4.71; N, 24.12%.

4-amino-1-((4-bromophenylamino)methyl)-3-(2-(7-chloroquinolin-4-ylamino)ethylamino)-1H-1,2,4-triazole-5(4H)-thione (7k)

Yellow solid; Yield: 78%; m.p. 238-240 ºC, R_f, 0.62; Mol wt, 454.98; FTIR (ν_{max} ; cm^{-1} KBr): 3380 (N-H stretch), 3036 (C-H stretch, Aromatic), 2115 (N=C=S stretch), 1468 (CH_2 stretch), , 640(C- Br stretch); ^{1}H NMR (400MHz, DMSO-d_6, TMS) δ ppm: 3.59-3.55 (m, 2H, -CH_2), 3.50-3.45 (m, 2H, -CH_2), 5.69 (s, 2H, -NH_2), 5.75 (s, 2H, -CH_2), 6.17 (s, 1H, -NH), 6.41-6.39 (d, 1H, quinoline), 6.80-6.78 (d, 2H, Ar –CH) 7.24-7.21 (d, 2H, Ar -CH), 7.37-7.34 (d, 1H, quinoline), 7.64 (s, 1H, -NH), 7.87-7.84 (d, 1H, Quinoline), 7.88 (s,1H, Quinoline), 8.47-8.45 (d, 1H, Quinoline); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 171.51, 158.56, 151.49, 150.15, 149.08, 147.46, 135.91, 132.05, 127.23,124.15, 117.25,114.26, 109.64, 99.92, 65.25, 43.31, 32.21; Mass: 519.08 ,517.03 ($C_{20}H_{20}BrClN_8S$, [M+H]+). Elemental Analysis Calculated: C, 46.21; H, 3.88; N, 21.56; S, 6.17 % Found: C, 48.35; H, 4.14; N, 22.08%.

Minimum Inhibitory Concentration [17-18]

All synthesized compounds were screened for their antibacterial potential in the form of minimum inhibitory concentration (MIC, µg/mL) against selected gram positive organisms viz. *Bacillus subtilis* (NCIM-2063), *Bacillus cereus* (NCIM-2156), *Staphylococcus aureus* (NCIM-2079) and gram-negative organism viz. *Pseudomonas aeruginosa* (NCIM-2036), *Escherichia coli* (NCIM-2065), *Proteus mirabilis* (NCIM-2241), *Proteus vulgaris* (NCIM-2027) by the broth dilution method as recommended by the European Committee for Antimicrobial Susceptibility Testing with minor modifications. Ciprofloxacin was used as standard antibacterial agent. The test compounds and reference drug and control were prepared for analysis. The test compounds and reference drug were prepared in dimethyl sulfoxide (DMSO) at concentrations of 100, 50, 25, 12.5, 6.25, 3.125 µg/mL. The inoculum can be prepared by making a direct broth or saline suspension of isolated colonies of the same strain from 18 to 24 h Müeller-Hinton agar plate and test bacteria were adjusted to a volume of 0.5–2.5 x 10^5 colony-forming units/ml by matching with the turbidity of 0.5 McFarland reagent. A volume of 1 ml of the standardized broth culture was added to 1 ml of each serially diluted test tube. The capped tubes with cotton plugs were incubated at 37±2°C for 24 h and compared with standardized 0.5 McFarland reagent and the MIC was characterized as the lowest concentration able to inhibit any visible microorganism growth.

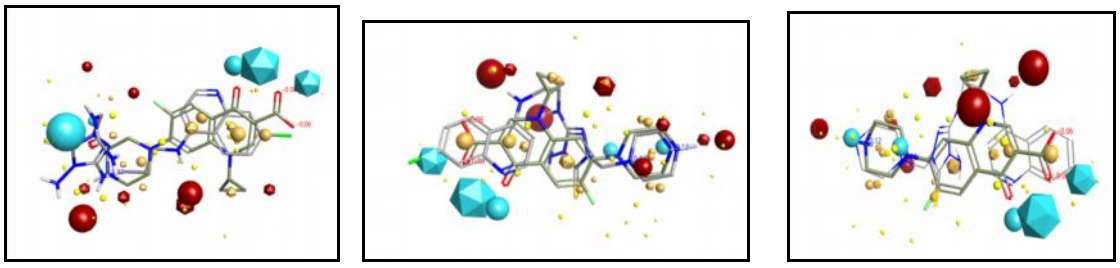
Molecular docking study

Molecular docking provides a powerful tool in understanding different protein functions. Molecular field mapping and alignment studies were performed using FORZE V10 software. The 2D oriented structures of all the synthesized ligands drawn using Chem Draw and analyze for the correct bond order. Docking calculations were carried out on using bacterial DNA gyryase with newely synthesize quinoline derivatives. Desired protein downloaded from RSCB protein Data bank (PDB ID: 1ZI0) and prepare for docking studies. Docking calculations were carried out using AutoDock Tools 1.4.6 and MGL Tools 1.5.4 packages [19]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on ligand protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools Affinity (grid) maps of 0.275 A^0 grid points and 0.375 A^0 spacing were generated using the Autogrid program [20]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [21]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Results and Discussion

Primarily, we performed field analysis of the designed series and compared with known antibacterial agent ciprofloxacin (Figure 1). The field point pattern is a sophisticated ‘pharmacophore’ which can be used to define a template for binding. Molecules can be overlaid using their fields, rather than structure, and the field similarity between two molecules can be quantified and converted to a similarity value. This is anticipated that compounds having similar arrangement of field points bind to the receptor in similar fashion and affinity. Four

molecular fields to represent the binding properties of a ligand are positive electrostatic (colored red), negative electrostatic (colored blue), Van der Waals attractive i.e. 'steric' (colored yellow), hydrophobic (colored orange). Compounds owning field similarity more than 50% with field of reference ciprofloxacin were selected for synthesis. The field similarity data of these compounds are presented in Table 1. The syntheses of aliphatic amide bridged 4-aminoquinoline clubbed 1,2,4- triazole derivatives are outlined in scheme 1 (Figure 2). Reaction progress and completion was monitored by thin layer chromatography. The ^{1}H NMR, ^{13}C NMR, FTIR and mass spectral data of synthesized derivatives were recorded and found in full agreement with the proposed structures. Elemental analysis data confirmed the purity of the compounds.



7a **7d** **7h**

Fig. 1: Field point alignment of the 4-aminoquinoline derivatives (7a, 7d and 7h) on ciprofloxacin

The size of the point indicates the potential strength of the interaction. Round-shaped field points are of test compounds 7a and 7h. Diamond-shaped field points are of reference compound (ciprofloxacin). Sky blue color: negative ionic fields; magenta color: positive ionic fields; light yellow color: vanderwaal interactions; dark yellow color: hydrophobic fields. Field similarity score of derivative 7a: 0.580; 7d: 0.57; 7h: 0.58 respectively.

Reaction scheme

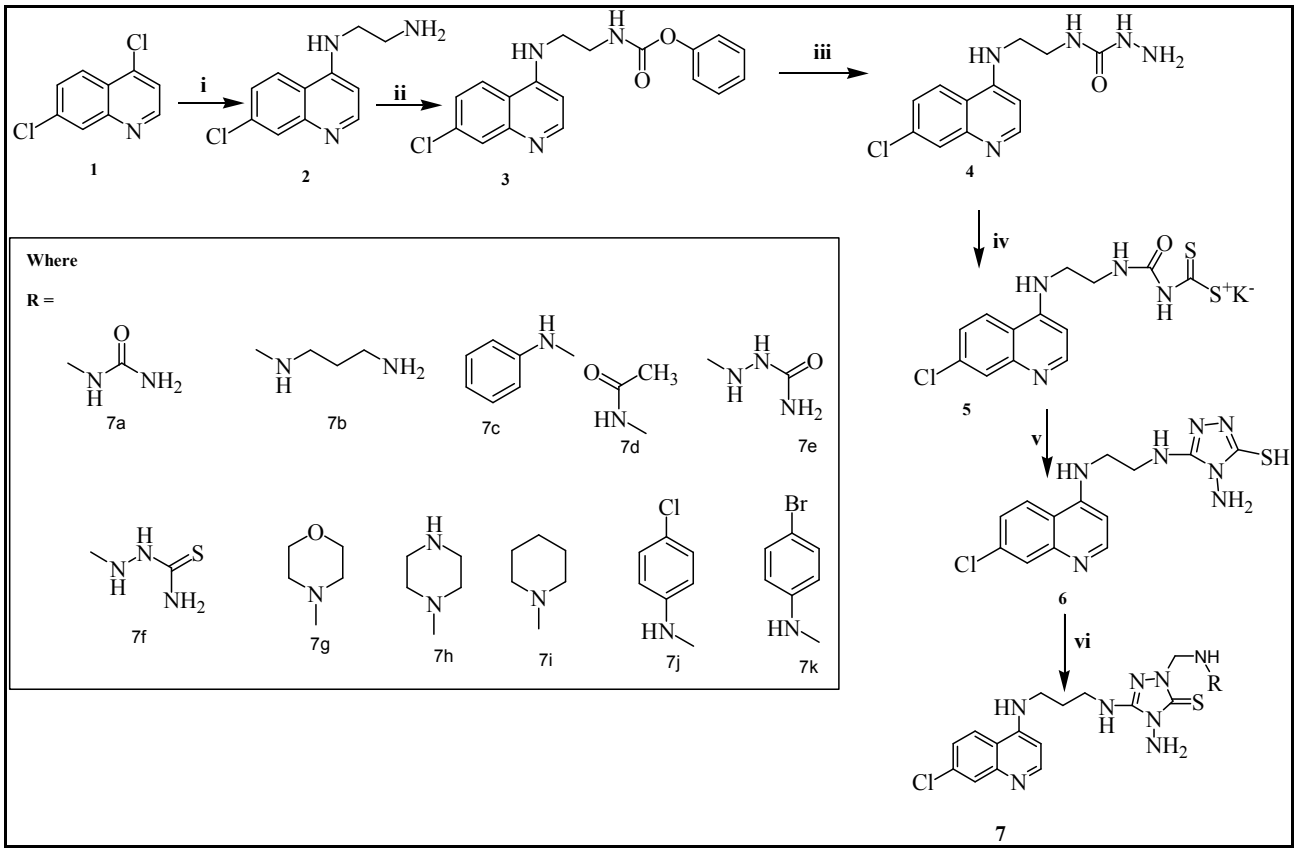


Fig. 2: Synthesis of Aliphatic amide bridged 4-aminoquinoline clubbed 1,2,4- triazole derivatives

Antibacterial activity

The *in vitro* antibacterial activity of newly synthesized compounds **7a - 7k** was determined by broth dilution method on three gram positive and four gram negative bacterial strain. The result were summarized in Table 2 .The antibacterial screening revealed that tested compounds **7a, 7d, 7h** showed good inhibition against almost tested microbial strains. The result indicated that tested derivative **7a** showed excellent activity against *E. coli* at concentration 3.125 µg/mL, equipotent activity against *S. aureus, B. subtilis, B. cereus* at concentrations of 6.25, 12.5, 3.125 µg/mL, while mild to moderate activity shown against *P. aerugenosa*, , *P. mirabilis* and *P. vulgaris* at 50, 12.5, 12.5 µg/mL respectively. Derivative **7b** consist 1,3 diaminopropane substitution showed moderate activity against *S. aureus, P. aerugenosa, P. vulgaris* and mild activity on *B. subtilis, B. cereus, E. coli and P. mirabilis* at 6.25, 50, 12.5, 12.5, 3.125, 3.125, 12.5 µg/mL respectively. Substitution of aliphatic diaminopropane side chain with aromatic aniline (**7c**) showed higher activity against *P. aerugenosa* at 12.5µg/mL, equipotent activity against *P. vulgaris* at 12.5 µg/mL and mild to moderate activity against *S. aureus, B. subtilis, E. coli, P. mirabilis* and *B. cereus* at 50, 25, 25, 6.25 µg/mL respectively. Derivative **7d** posses acetamide substitution exhibited equipotent activity against *S. aureus, B. subtilis, P.aerugenosa, P. mirabilis, P. vulgaris* at 6.25, 12.5, 25, 12.5, 12.5 µg/mL respective concentrations, moderate activity showed on *B. cereus, E. coli* at 6.25 and 12.5µg/mL. Semicarbazide substitution at triazole sidechain **(7e)** exhibited equipotent activity against *B. subtilis* at 12.5 µg/mL while mild to moderate MIC showed on *E. coli, S. aureus, B. cereus, P. aerugenosa, P. mirabilis* and *P. vulgaris* at 50, 12.5, 6.25, 50, 25, 25µg/mL respectively. Derivative **7f** having thiosemicabazide substitution inplace of semicarbazide exhibited equipotent activity against *P. aerugenosa at 25* µg/mL*, P. vulgaris* at 2.5 µg/mL and mild to moderate activity exhibited against *B.cereus* at 12.5 µg/mL*, E. coli* at 50 µg/mL*, P. mirabilis* at 50 µg/mL, *S. aureus* at 12.5 µg/mL and *B. subtilis* at 25 µg/mL. Introduction of marpholine (**7g)** showed equipotent activity against *B. subtilis, P. aerugenosa, P. mirabilis* at 12.5, 25, 12.5 µg/mL respectively while mild to moderate activity observed against *S. aureus* at 25 µg/mL, *B. cereus* at 12.5 µg/mL, *E. coli* at 25 µg/mL, and *P. vulgaris* at 25 µg/mL. Derivative **7h** having piperazine substitution exhibited highly activity against *S. aureus* at 3.125 µg/mL, while euipotent activity of ciprofloxacin against rest of the bacterial strains. In derivative **7i** piprazine replaced with piperidine and found suppression in antibacterial activity due to decrease in lipophilicity in term of strain it showed equipotent activity against *B. subtilis* at 12.5 µg/mL, *P. aerugenosa* at 25 µg/mL while mild to moderate activity *S. aureus, B. cereus, E. coli, P. mirabilis, P. vulgaris* at 25, 25, 12.5, 25, 25 µg/mL respective concentration. Further addition of 4- chloroaniline **7j** showed decreased mild antibacterial activity against *S. aureus, B. subtilis, B. cereus, P. aerugenosa, E. coli, P. mirabilis* at 25, 50, 12.5, 100, 25, 50µg/mL respectively and moderate activity exhibited on *P. vulgaris* at 25µg/mL. Derivative **7k** obtained through replacenet of chlorine atom with other bromine which enhanced activity like higher activity against *P. vulgaris* at 3.125 µg/mL, equipotent activity against *P. mirabilis* at 12.5 µg/mL and mild to moderate efficacy exhibited against *S. aureus, B. cereus, E. coli, B. subtilis, P. aerugenosa* at 50, 12.5, 25, 25, 50 µg/mL respective concentration.

SAR analysis of the synthesize series revealed that aliphatic side chain containing derivatives showed more antibacterial activity as compared to aromatic substituted side chain derivatives. Increasing no of electronegative atom makes molecules lipophilic and plays a vital role enhancing antibacterial activity. It was depicted from the results of *in vitro* activity that minor variation in structural fragment would be able to confer drastic alteration in activity. It is worthwhile to mention here that presence of piperazine and amine bridge (connecting side chain at 4^{th} position of quinoline) was termed as the important predictor for escalation and generation of activity. In context with result we can conclude that our designed derivatives may provide novel insight about the development of next generation of 4-aminoquinoline clubbed 1,2,4- triazole derivatives as antibacterial agent. Our studies are in progress towards the direction of designing their higher derivatives and elucidate its putative mechanism of action as antibacterial and will be reported subsequently in future.

Molecular Modeling Study

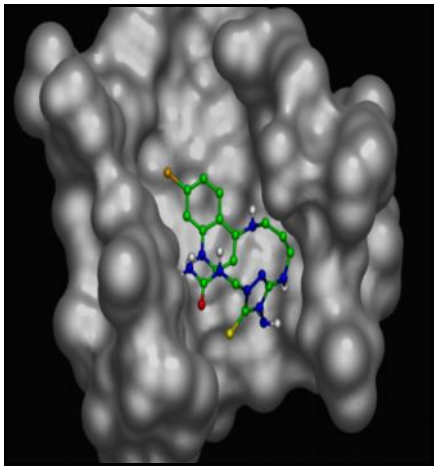
Molecular modeling studies are the key for the creation of models which justify experimental evidences. These models give consistent and precise result of biologically active molecules at molecular level and help to generate new thought towards newer novel biological active agents. The molecular docking was performed with the aim to predict anti bacterial activity of synthesized derivatives. Derivatives 7a, 7d, 7h show good interaction energy in agreement with experimental evidence which indicate that derivatives 7a, 7d, 7h are potent inhibitor of DNA gryase as compared to other synthesize compounds and reference standard. Derivatives 7a shows five hydrogen bond interactions, THR-632 (2.56 A), ILE-683 (3.12 A, 3.40 A), VAL-733 (2.91 A, 3.47 A), SER-734 (2.75 A, 2.96 A), ALA-786 (3.00 A), one polar interaction, SER-734 (3.86 A, 3.23 A, 3.10

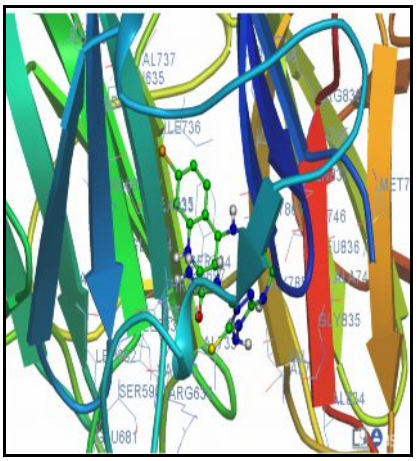
A), one hydrophobic interaction, LEU-836 (3.35 A, 3.84 A) and two halogen interaction VAL-685 (3.79 A), LEU-735 (3.51 A) while compound 7d shows four hydrogen bond interaction with ASP-579 (2.81 A), VAL-733 (3.35 A, 3.43 A), VAL-784 (2.90 A), LEU-836 (3.16 A, 2.92 A), four polar interaction i.e. ASP-579 (3.76 A), ARG-580 (3.15 A, 3.74 A), SER-734 (3.64 A), GLN-837 (3.45 A, 3.66 A), one hydrophobic interaction VAL-733 (3.44 A, 3.53 A, 3.76 A) and one halogen interaction ILE-631 (3.76 A). Compound 7h exhibits hydrogen bond interaction ASP-579 (2.51 A), ILE-683 (2.87 A), ASP-686 (2.57 A), LEU-735 (2.6 A), four polar interaction ASP-579 (2.13 A, 2.16 A), ARG-580 (3.01 A, 3.90 A), ASP-686 (2.43 A), GLN-837 (2.54 A, 3.31 A, 3.32 A), one hydrophobic interaction ILE-736 (3.42 A, 3.78 A) and one halogen interaction VAL-737 (3.34 A). All compounds were compared with the standard ciprofloxacin which showed two hydrogen interaction VAL-685 (3.33 A, 3.23 A), ASP-686 (2.76 A), two polar ASP-686 (2.46 A, 2.20 A), SER-734 (3.56 A, 3.76 A), two halogen THR-632 (2.77 A), ILE-683 (2.77 A) and three hydrophobic interaction, LEU-735 (3.21 A, 3.68 A), ILE-736 (3.63 A, 3.86 A), VAL-787 (3.50 A). Docking study give conclusion that compound 7a, 7d and 7h have significant affinity to bind with catalytic site of DNA gyrase. All energy related results to binding of ligand and 1ZI0 are given in Table 3 (Figure 3).

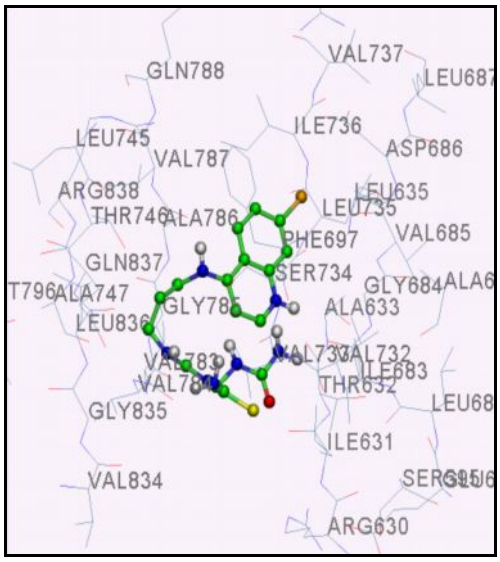
Synthesis protocol for the reaction:

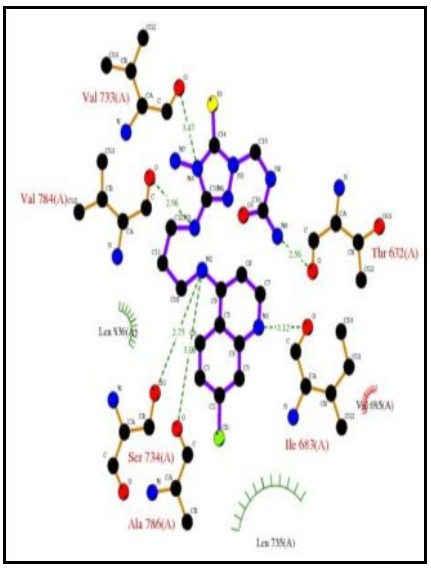
(i). 1,3 diaminopropane, NaOH, dichloromethane, 80-110^{0}C with stir. (ii). Phenyl chloroformate, triethylamine, DMF, stir at room temp. (iii). Hydrazine monohydrate, dry methanol, reflux at 90^{0}C with stir. (iv). Absolute ethanol, KOH, CS_2, stir for 12 hr with stir. (v) Hydrazine hydrate, H_2O, reflux for 10 hr. (vi) Formaldehyde, absolute ethanol, desired amine, reflux for 4 hr.

(7a)

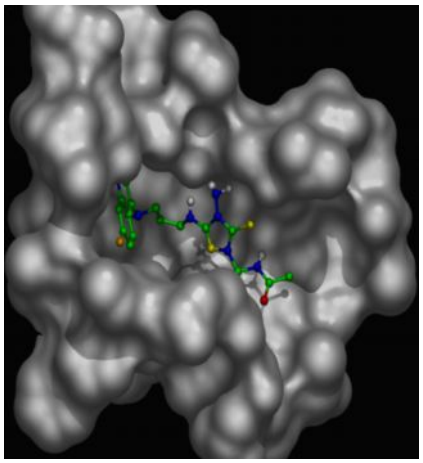


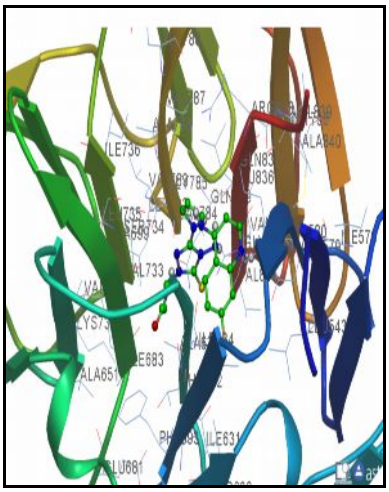


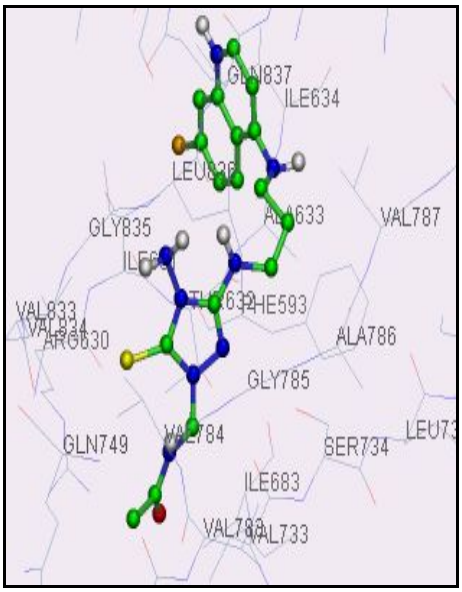




(7d)






GLN837
ILE634
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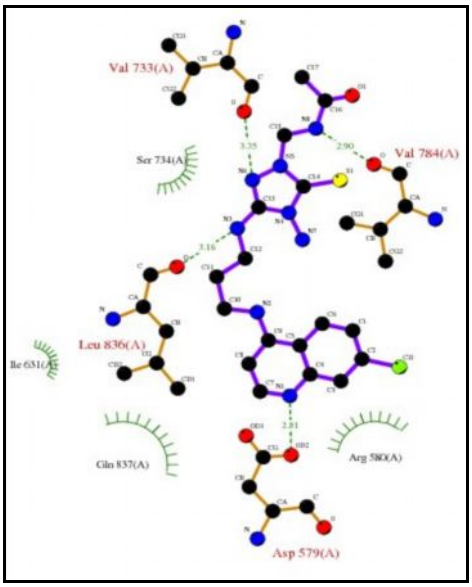
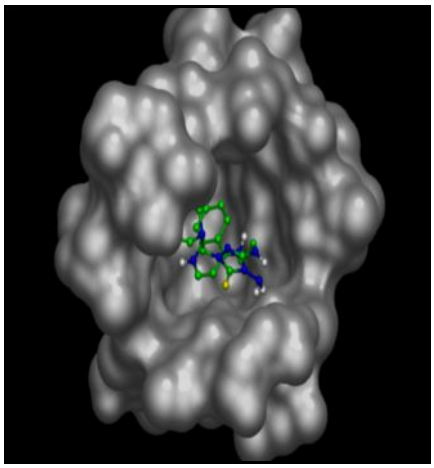
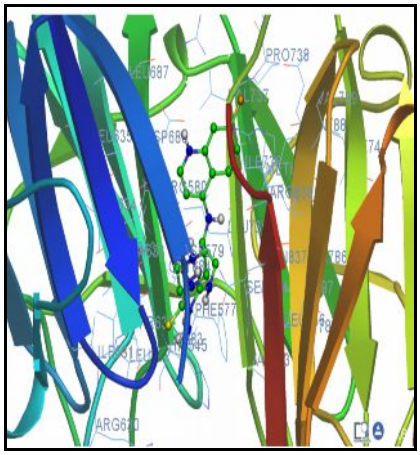
VAL787
VAL833
ARG630
PHE593
ALA786
GLY785
GLN749
SER734
ILE683
VAL733


Val 733(A)
Ser 734(A)
Val 784(A)
Leu 836(A)
Gln 837(A)
Asp 579(A)

(7h)

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PHE577

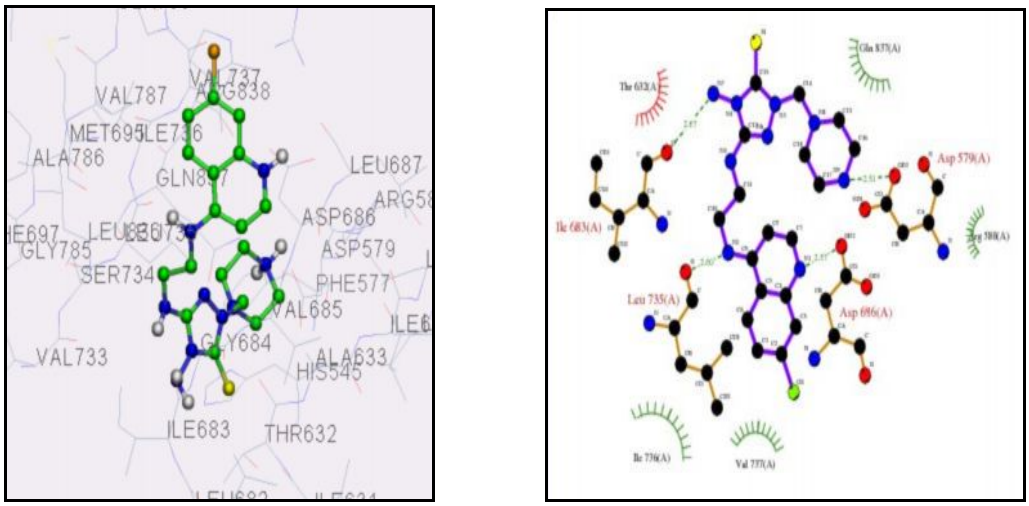
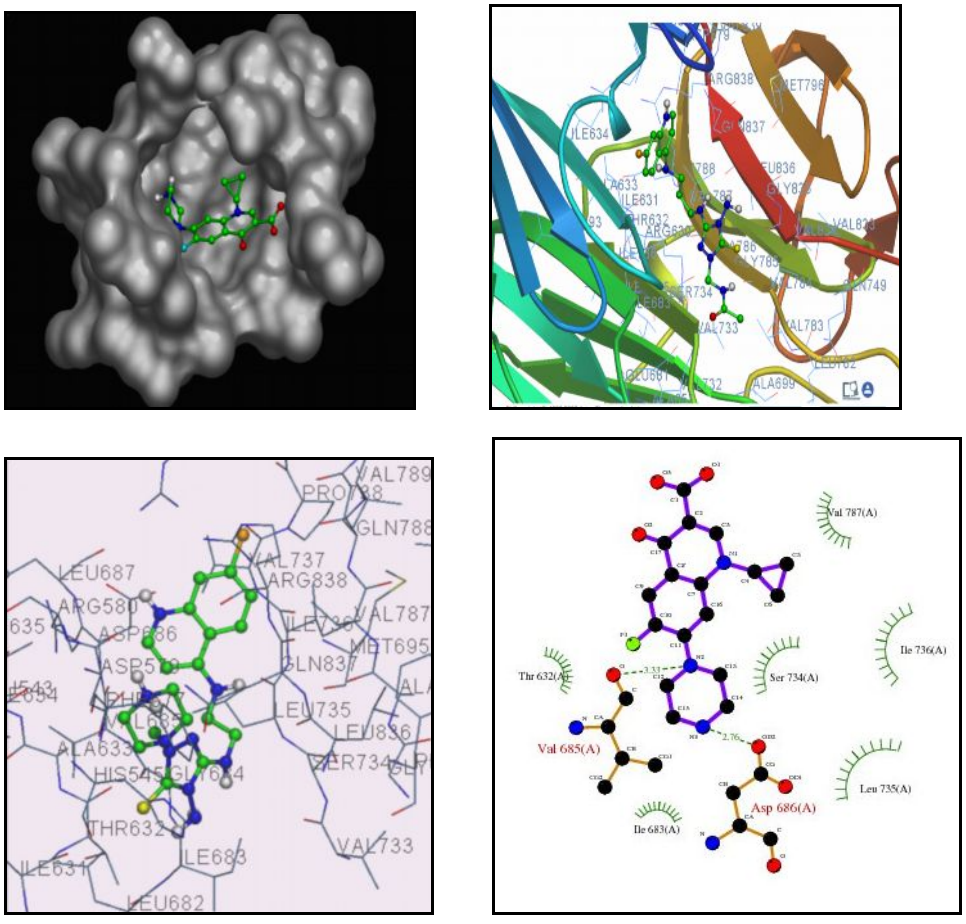


Fig.3: Docking of compounds (7a, 7d,7h) into the active site of DNA gyrase. Lower panel showed the ciprofloxacin –DNA gyrase active site complex (In pocket form, with protein structure, with amino acid trap and 2D interaction respectively)

Standard Ciprofloxacin



Executive summary

- A series of Aliphatic amide bridged 4-aminoquinoline clubbed 1,2,4- triazole derivatives (7a-7k) were designed and synthesized.
- Docking studies of 4-aminoquinoline clubbed 1,2,4- triazole derivatives 7a, 7d, 7h with DNA gyrase catalytic site (Pdb: 1ZI0) were performed and result found that better binding affinities were achieved with novel inhibitors than reference.
- All the synthesized derivatives were evaluated for their anti-bacterial activity for minimum inhibitory concentration using broth dilution method.
- Derivatives having aliphatic substitution (7a, 7d, 7h) exhibited most potent antibacterial activity against all gram positive and gram negative bacterial strains.

Table1: Field mapping data of designed derivatives

Compound	Similarity	SlogP	TPSA
7a	0.580	1.00	136.9
7b	0.562	1.2	126.1
7c	0.534	3.4	93.8
7d	0.577	1.4	110.9
7e	0.552	0.1	149
7f	0.571	0.3	131.9
7g	0.571	1.3	95.5
7h	0.580	0.8	98.3
7i	0.547	2.4	86.3
7k	0.538	3.7	93.8
7k	0.543	3.9	93.8

Table2: Antibacterial activity (Minimum Inhibitory Concentration of Title Compounds in µg/mL)

Compounds	*Gram +ve strains*			*Gram –ve strains*			
	S. aureus	*B. subtilis*	*B. cereus*	*P. aeruginosa*	*E. coli*	*P. mirabilis*	*P. vulgaris*
7a	6.25	12.5	3.125	50	3.125	12.5	12.5
7b	12.5	50	25	50	25	50	25
7c	100	50	12.5	12.5	25	.25	12.5
7d	6.25	12.5	6.25	25	12.5	12.5	12.5
7e	12.5	12.5	6.25	50	50	25	25
7f	12.5	25	12.5	25	50	50	12.5
7g	25	12.5	12.5	25	25	12.5	25
7h	3.1.25	12.5	3.125	25	6.25	12.5	12.5
7i	25	12.5	25	25	12.5	25	25
7j	25	50	12.5	100	25	50	25
7k	50	25	12.5	50	25	12.5	3.125
Ciprofloxacin (Standard)	6.25	12.5	3.125	25	6.25	12.5	12.5

Table 3: Energy table of active derivatives and ciprofloxacin

Compound	Est. free energy of binding (kcal/mol)	Est. inhibition constant, Ki (µM)	vdW +Hbond+ desolv energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total intermolec. energy (kcal/mol)	Interact. Surface
7a	-6.81	10.21	-8.76	-0.38	-9.14	725.75
7d	-6.52	16.62	-7.96	-1.06	-9.02	857.05
7h	-6.85	9.54	-7.12	-1.83	-8.96	844.69
Ciprofloxacin	-6.66	13.21	-7.09	-0.80	-7.89	630.25

Conclusion

In the present study, we reported synthesis of aliphatic amide bridged 4-aminoquinoline clubbed 1,2,4-triazole derivatives and evaluated their antibacterial activity against seven different bacterial strains. A systemic SAR of above designed molecules was discussed in the light of its antibacterial activity. It was depicted from the results of *in vitro* activity that minor deviation in structural fragment would be able to confer strong variation in activity. It is precious to mention here that presence of piperazine and amine Bridge (connecting side chain at 4^{th} position of quinoline) was termed as the important interpreter for escalation and generation of activity. In context with result we can conclude that our designed derivatives may provide novel insight about the development of next generation of 4-aminoquinoline clubbed 1,2,4- triazole derivatives as antibacterial agent. Molecular docking studies of these three compounds give certainty that synthesize derivatives have capability to make interaction with DNA gyrase active site. Our results revealed that 4-amionquinoline clubbed triazole derivatives are significantly active against gram positive and gram negative bacteria in comparison with standard ciprofloxacin and provide information towards development for lead antibacterial agents in future.

Conflict Of Interest

The authors have declared that there is no conflict of interest.

Acknowledgments

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