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Synthesis, Biological Evaluation of 4-aminoquinoline 1, 2, 4-triazole Conjugated Benzothiazole as Potent Analgesic, Anti-inflammatory

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ABSTRACT

In this study the synthesis of novel 4-aminoquinoline 1,2,4-triazole derivatives containing benzothiazole (4a-4j and 5a-5j) done by three major steps and characterization of synthesized derivatives assigned by Infra-Red (IR), Proton Nuclear Magnetic Resonance (^{1}H-NMR), Mass Spectra (MS), Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR). The biological evaluation for in vivo analgesic and anti-inflammatory activity was performed for primed derivatives, among the tested compounds 5a, 5c, 5e, 5g, 5h, 5i showed more potent anti-inflammatory and 4i, 5a, 5b, 5f, 5g, 5h showed analgesic activity compared standard drug meloxicam and diclofenac sodium.

Keywords: 4-Aminoquinoline, *In vivo* analgesic, Meloxicam, Anti-inflammatory

INTRODUCTION

Inflammation is an imperative element of the body's impervious response. This mechanism makes body endeavor to cure from damage, protect beside foreign invaders like viruses and bacteria and refurbish injured tissue [1]. Prostaglandins are the chemicals produced by the cells of the body and they promote inflammation obligatory for healing. Prostaglandins are formed in body cells having enzyme Cyclooxygenase (COX-1 and COX-2) [2]. Nonsteroidal Anti-inflammatory Drugs (NSAIDs) block the COX enzymes and reduce prostaglandins in body which decreases enduring inflammation, pain, and fever [3]. However NSAIDs blocks COX-1 that produces prostaglandins that facilitate platelets and shield the gastric layer and blood clotting that leads to ulcers in the stomach and endorse hemorrhage but reticence of COX-2 leads to the anti-inflammatory, analgesic and antipyretic effects [4,5]. The most commonly used NSAIDs are aspirin, diclofenac, ibuprofen (non-selective COX-1 and COX-2 inhibitors) while meloxicam is selective COX-2 inhibitor used as typical model for anti-inflammatory activity in present study [6-8]. In the past few years the new research were focused on heteroaromatic rings with numerous structural modifications. Literature survey suggested that among the heterocyclic compounds aminoquinoline and its analog act as anti-inflammatory [9], antibacterial [10], antimalarial [11], anticancer [12], antifungal [13], antiviral [14], antitubercular [15], anti-HIV [16]. Endeavor of the current research is to explore the analgesic and anti-inflammatory activity of 4-aminoquinoline so we had designed and synthesized the 4-(Substituted Benzothiazol-2-ylamino)-5-[2-(7-chloro-quinolin-4-yl amino)-ethyl amino] substituted methyl-2,4-dihydro-[1,2,4]triazole-3-thione (4a-4j and 5a-5j) where 4-aminoquinoline incorporated with two pharmacological active moieties-1,2,4 Triazole and Substituted benzothiazol-2-ylamine to hoping to produce synergistic effect in anti-nociceptive and anti-inflammatory activity the of aminoquinoline as core moiety in target compounds. Literature survey reveals that 1,2,4-triazole possess diversified pharmacological activities such as analgesic [17], anti-inflammatory [18], antihypertensive [19], antimicrobial [20], anticonvulsant [21], antiviral [22], while benzothiazole derivatives previously reported as anti-inflammatory [23], antibacterial [24], anticancer [25], antihelminthes [26], analgesic [27], diuretics [28], antidiabetic [29].

MATERIALS AND METHODS

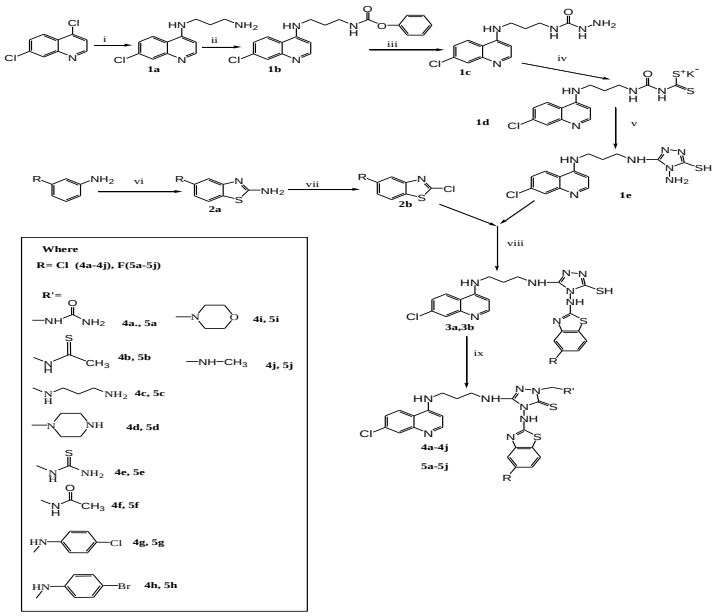
Materials and instrumentation

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 Fourier Transform Infra-Red (FTIR) (2.0 cm^{-1}, flat, smooth, abex) were recorded on Perkin Elmer RX-I Spectrophotometer. Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectra were recorded in Deuterated Dimethyl Sulfoxide (DMSO-d_6) using Bruker Avance II 400 NMR and ^{13}C-NMR spectra on Bruker Avance II 100 NMR spectrometer in DMSO-d_6 using Tetramethylsilane (TMS) as internal standard. Mass spectra were obtained on VG-Autospec spectrometer equipped with electrospray ionization sources.

Methods

A series of derivatives of 4-aminoquinoline 1,2,4-triazole clubbed benzothiazole were synthesized *via* three major step protocol. In the first major step formation of 4-Amino-5-[2-(7-chloro-quinolin-4-ylamino)-ethylamino]-4H-[1,2,4] triazole-3-thiol take place *via* five series of

reaction where at first step 4,7-dichloroquinoline react with 1,3-diamino propane however second step involve reaction between derivative (1a) with phenyl chloroformate in presence of triethylamine leads to the formation of 1-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-carbamic acid phenyl ester (1b). Formation of N-{2-[(7-chloroquinolin-4 yl)amino] ethyl} hydrazine carboxamide (1c) achieved in third step where hydrazine monohydrate react with compound 3 using methanol as solvent further forth step involve potassium dithiocarbazinate (1d) synthesis where derivative 1c 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)-ethyl amino]-4H-[1,2,4]triazole-3-thiol (1e) where derivative (1d) unite with hydrazine hydrate. The second major step formation of 2-Chloro-substituted Benzothiazole (2b) *via* two step reaction involving the substituted aniline with ammonium thiocyanate followed by bromination in the presence of glacial acetic acid leads to formation of substituted benzothiazol-2-yl amine (2a), further it is reacted with sodium nitrite in the solution of phosphoric acid followed by addition of brine solution and copper sulphate solution. The third major step involve the formation of 4-(Substituted Benzothiazol-2-ylamino)-5-[2-(7-chloro-quinolin-4-yl amino)-ethylamino]-2 substituted methyl-2,4-dihydro-[1,2,4] triazole-3-thione(4) *via* formation of 4 (substituted Benzothiazol-2-ylamino)-5-[2-(7-chloro-quinolin-4-ylamino)-ethylamino]-4H-[1,2,4]triazole-3-thiol (3) where 2b reacts with 1e in the presence of equimolar amount of the 4-chlorobenzoyl chloride in the presence of sodium hydroxide solution. The compound 4a-4j and 5a-5j was formed by addition of amines in the presence formaldehyde to derivative 3 mentioned in Scheme 1. Newly synthesized compounds were characterized by their melting points IR, NMR (^{1}H and ^{13}C), elemental analysis, and mass spectroscopy.



Scheme 1: Synthesis of 4-(Substituted Benzothiazol-2-ylamino)-5-[2-(7-chloro-quinolin-4-yl amino)-ethyl amino] substituted methyl-2,4-dihydro-[1,2,4]triazole-3-thione (4a-4j and 5a-5j)

Materials: (i) 5.0 equivalent of $NH_2(CH_2)_2NH_2$, reflux, 6 h, (ii) 1.0 equivalent of chloro formate, 1.0 equivalent of Et_3N, DMF/DCM (1:1), 0°C, 1 h, (iii) 10 equivalent of $H_2NNH_2.H_2O$, MeOH, reflux, 12 h, (iv) CS_2/KOH, EtOH, (v) 10 equivalent of $H_2NNH_2.H_2O$ (vi) NH_4SCN, EtOH, HCl/Br_2, glacial acetic acid, (vii) 8 5% H_3PO_4, $NaNO_2$, $CuSO_4$, 75% NaCl, (viii) 10% NaOH, (ix) Formaldehyde (1.5 ml, 40% solution), amines

General procedure

Synthetic procedure for N-(7-chloroquinolin-4-yl) alkyl-diamine) (1a) [30]

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 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_2SO_4 and solvent was removed under reduced pressure to afford the compounds 2, at 80-90% yield.

Synthesis of 1-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-carbamic acid phenyl ester (1b) [31]

Phenyl chloroformate (1.41 g, 9.02 mmol) was added to a stirred and cooled (0°C) 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 Dimethylformamide (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.

Synthesis of N-{2-[(7-chloroquinolin-4-yl)amino]ethyl} hydrazinecarboxamide (1c) [31]

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°C for 12 h. The reaction mixture was concentrated to give a white residue.

Synthesis of potassium dithiocarbazinate derivative (1d) [32]

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 dithiocarbazinate 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.

Synthesis of 4-amino-5-[2-(7-chloro-quinolin-4-ylamino)-ethylamino]-4H[1,2,4] triazole-3-thiol (1e) [32]

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 dilute 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: Ethyl acetate: Formic acid (5:4:1, v/v/v) as solvent system.

Synthesis of substituted benzothiazol-2-ylamine (2a) [33]

Equimolar quantities of substituted aniline (0.02 mol), and ammonium thiocyanate (1.5 g, 0.02 mol) were dissolved in ethanol containing 2 ml of Conc. H_2SO_4. To this bromine in glacial acetic acid (2.7 ml, 0.05 mol) was added and the reaction mixture was refluxed for 1 h. Then, it was cooled in ice-water mixture. The precipitate obtained, strained well, filtered washed with cold water and dried. The crude product was recrystallized from rectified spirit.

Synthesis of 2-chloro-substituted benzothiazole (2b) [34]

Solution of 85% H_3PO_4, substituted benzothiazol-2-yl amine (1 mmol), and $NaNO_2$ (1 mmol) was refluxed for 7 h followed by the addition of aqueous $CuSO_4$ and NaCl with continuous stirring, precipitate was filtered.

Synthesis of 4-(substituted benzothiazol-2-ylamino)-5-[2-(7-chloro-quinolin-4-yl amino) -ethyl amino]-4H-[1,2,4]triazole-3-thiol (3a,3b)

The compound 1e (1 mmol) in 20 ml of 10% NaOH was treated drop wise with an equimolar amount of the 4-chlorobenzoyl chloride at 0°C, which was stirred for 30-45 min. At the end of stirring, precipitate was observed. It was then filtered, washed thoroughly with water, and crystallized.

4-(5-Chloro-benzothiazol-2-ylamino)-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-4H-[1,2,4]triazole-3-thiol (3a)

Pale yellow solid; Yield: 62%; m.p. 167-168°C, R*f*, 0.59 (Toulene: Ethanol: Dimethyamine, 4:2:1); Mol. Wt, 517.46, Elemental analysis calculated for $C_{21}H_{18}C_{l2}N_8S_2$: C, 48.74; H, 3.51; N, 21.65. Found: C, 48.70; H, 3.48; N, 21.62. FTIR (ν_{max}; cm^{-1} KBr): 3289, 3147, 2831, 2436, 1754, 1653, 1105, 782. ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.56 (*d*, 1H, J=4.7, H-2'), 8.05 (*d*, 1H, J=8.2, H-5'), 7.76 (*d*, 1H, J=1.9, H-8'), 7.72 (*d*, 1H, J=2.5, H-7'), 7.67 (*d*, 1H, J=1.8, H-5'), 6.72 (*d*, 1H, J=4.7, H-2'), 3.42 (*d*, 1H, J=6.8, H-2), 3.12 (*m*, C_2H_5); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm= 174.5, 151.4, 149.5, 149.4, 148.2, 134.6, 130.4, 128.1, 125.8, 121.8, 126.2, 124.1, 122.5, 120.7, 115.9, 104.5, 78.3, 76.5, 49.4, 48.6, 43.6, 33.3. MS (m/z (relative abundance, %)): 520.04 (M^+, 20), 519.05 (17), 516.05 (100), 518.04 (73), 517.05 (28).

5-[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-4H-[1,2,4]triazole-3-thiol(3b)

Light cream solid; Yield: 62%; m.p. 167-168°C, R*f*, 0.63 (Toluene: Ethanol: Dimethyamine: 4:2:1); Mol. Wt, 501.00, Elemental analysis calculated for $C_{21}H_{18}ClFN_8S_2$: C, 50.33; H, 3.60; N, 22.35. Found: C, 50.31; H, 3.56; N, 22.30. FTIR (ν_{max}; cm^{-1} KBr): 3265, 3143, 3019, 2789, 2521, 1768, 1641, 1322, 1107, 1045, 773, 782. ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.59 (*d*, 1H, J=4.6, H-2'), 7.87 (*d*, 1H, J=5.2, H-5'), 7.74 (*d*, 1H, J=2.6, H-8'), 7.29 (*d*, 1H, J=1.6, H-7'), 6.98 (*d*, 1H, J=2.6, H-5'), 6.65 (*d*, 1H, J=5.6, H-2'), 3.46 (*d*, 1H, J=6.1, H-2), 3.18 (*m*, C_2H_5), 1.48 (*m*, C_2H_5'). ^{13}C-NMR (100 MHz, DMSO-d_6), δppm= 174.5, 158.7, 151.4, 149.7, 149.5, 149.4, 148.2, 148, 134.6, 130.7, 128.1, 125.8, 124.6, 121.8, 118.4, 109.4, 104.5, 77.5, 49.4, 48.7, 33.3. MS (m/z (relative abundance, %)): 502.01 (M^+, 45), 501.3 (27), 502.5 (100), 503.4 (35), 504.5 (23).

Synthesis of 4-(substituted benzothiazol-2-ylamino)-5-[2-(7-chloro-quinolin-4-yl amino) -ethyl amino] Substituted methyl-2,4-dihydro-[1,2,4]triazole-3-thione (4a-4j and 5a-5j)

Formaldehyde (1.5 ml, 40% solution) was added to a solution of compound 3 (0.5 g, 1.8 mmol) 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.

{4-(5-Chloro-benzothiazol-2-ylamino)-3-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-5-thioxo-4,5-dihydro-[1,2,4]triazol-1-ylmethyl}-urea (4a)

Light white solid; Yield: 85.7%; m.p. 252-254°C, R_f, 0.78 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol wt, 589.52; Elemental Analysis Calculated for $C_{23}H_{22}Cl_2N_{10}OS_2$: C, 45.91; H, 3.50; N, 24.34% Found: C, 45.93; H, 3.56; N, 24.23 %.; FTIR (ν_{max}; cm^{-1} KBr): 3381, 3052, 2124, 1648, 1681, 765; ^{1}H-NMR (400MHz, DMSO-d_6, TMS) δppm=8.24 (*d*, 1H, J=1.6, H-2'), 7.88 (*d*, 1H, J=4.2, H-5'), 7.77 (*s*, 1H, H-6), 7.54 (*s*, 1H, H-8), 7.33 (*d*, 1H, J=1.9, H-8'), 7.01- 7.13 (*t*, 2H, -NH_2), 6.26- 6.23 (*d*, 1H, J=1.2, H-2'), 6.17 (*s*, 2H, H-1), 3.50 (*d*, 2H, J=6.1, H-2'), 3.45-3.57 (*t*, 2H, H-6'), 3.38-3.35 (*t*, 2H, H-9), 1.87 (*s*, 1H, NH). ^{13}C- NMR (100 MHz, DMSO-d_6), δppm=186.54, 174.23, 154.23, 151.86, 150.10, 149.14, 130.04, 127.49, 124.10, 122.50, 120.72, 115.93, 104.32, 98.82, 67.68, 38.53, 33.56; MS (m/z (relative abundance, %)): 591.08 (M^+, 20.1), 590.08 (74), 589.08 (30), 588.08 (100).

N-{4-(5-Chloro-benzothiazol-2-ylamino)-3-[3-(7-chloro-quinolin-4-ylamino)-propyl amino]-5-thioxo-4,5 -dihydro-[1,2,4]triazol-1-ylmethyl}-acetamide(4b)

Light yellow solid; Yield: 89%; m.p. 243-245°C, R_f, 0.72 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 588.54; Elemental analysis calculated for $C_{24}H_{23}Cl_2N_9OS_2$: C, 48.98; H, 3.94; N, 21.42% Found: C, 47.98; H, 3.56 N, 20.78%, FTIR (ν_{max}; cm^{-1} KBr): 3775.14, 3739.15, 3573.19, 3394.09, 3032.55, 23473.52, 2311.13, 1836.87, 1725.74, 1660.60, 1515.52, 1351.19, 1280.52, 1156.97, 922.84; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.25(*d*, 1H, J=4.2, H-2'), 7.91 (*d*, 1H, J=5.6, H-5'), 7.89-7.85 (*d*, 1H, J=1.5, H-5'), 7.32 (*d*, 1H, J=4.2, H-6), 6.23 (*d*, 1H, J=5.6, H-9'), 5.78 (*d*, 1H, J=6.5, H-3'), 5.28 (*s*, 1H, -CH_2), 3.40 (*d*, 2H, J=6.5, H-4'), 2.82 (*d*, 2H, J=7.2, H-3'), 2.45 (*s*, 1H, -NH), 1.89-1.84 (*m*, 2H, H-4), 1.65-1.58 (*m*, 2H, H-6). ^{13}C-NMR (100 MHz, DMSO-d_6), δ ppm: 186.22, 174.56, 171.07, 154.26, 151.42, 149.08, 134.09, 126.49, 125.09, 122.05, 127.78, 65.12, 49.98, 38.45, 33.25, 27.62; MS (m/z (relative abundance, %)): 590.08 (23), 589.08 (M^+, 73), 588.09 (27), 587.08 (100).

2-[(3-Amino-propylamino)-methyl]-4-(5-chloro-benzothiazol-2-ylamino)-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-2,4-dihydro-[1,2,4]triazole-3-thione(4c)

Yellow solid; Yield: 78%; m.p. 238-240 ºC, R_f, 0.62 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol wt, 687.46; Elemental Analysis Calculated for $C_{27}H_{22}BrCl_2N_9S_2$: C, 49.75; H, 4.68; N, 23.21% Found: C, 49.35; H, 4.53;N, 22.01%. FTIR (ν_{max}; cm^{-1} KBr): 3193.86, 3135.22, 3109.92, 3007.89, 2904.27, 2347.85, 2332.16, 2155.92, 1687.69, 1579.65, 1545.25, 1492.08, 1177.66, 849.49, 726.57; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm: 8.24 (*d*, 1H, J=4.2, H-2'), 7.92 (*d*, 1H, J=5.6, H-5'), 7.91 (*s*, 1H, NH), 7.85 (*s*, 1H, -NH), 7.82 (*s*, 1H, H-8'), 7.54 (*s*, 1H, -NH), 7.29 (*d*, 1H, J=4.2, H-6), 6.92(*d*, 1H, J=5.6, H-9'), 6.54-6.53 (*t*, 1H, H-6'), 6.27 (*d*, 1H, J=1.5, H-5'), 5.72 (*s*, 2H, H-4), 5.66 (*s*, 2H, H-6), 3.51- 3.44 (*t*, 2H, H-7'), 3.38-3.365 (*t*, 2H, H-3'), 1.84-1.78 (*m*, 2H, H-6'). ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=186.01, 174.51, 154.65, 149.09, 130.08, 126.45, 122.06, 120.09, 104.05, 71.19.23, 117.65, 49.12, 49.76, 38.54, 33.52; MS (m/z (relative abundance, %)): 690.00 (15), 689.00 (M^+, 42), 688.00 (31), 687.10 (100), 686.00 (20), 685.00 (58).

4-(5-Chloro-benzothiazol-2-ylamino)-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-2-piperazin-1-ylmethyl-2,4-dihydro-[1,2,4]triazole-3-thione (4d)

Colourless solid; Yield: 64%; m.p. 188-190°C, R_f, 0.92; Mol. Wt, 615.60 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Elemental analysis calculated for $C_{26}H_{28}Cl_2N_{10}S_2$: C, 50.73; H, 4.58; N, 22.75%; Found: C, 49.95; H, 4.25; N, 23.04%. FTIR (ν_{max}; cm^{-1} KBr): 3775.58, 3394.16, 3037.07, 2861.22, 2861.22, 1925.81, 1662.61, 1225.62, 1042.91, 975.90; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.27 (*d*, 1H, J=5.6, H-5'), 7.92-7.81 (*d*, 1H, J=4.2, H-2'), 7.80 (*s*, 1H, N-H), 7.54 (*s*, 1H, -NH), 7.32 (*d*, 1H, J=4.2, H-6), 7.08-6.97 (*t*, 1H, -NH), 6.25 (*d*, 1H, J=5.6, H-9'), 5.64 (*s*, 2H, -NH_2), 5.3178 (*d*, 1H, J=6.5, H-3'), 3.51- 3.46 (*t*, 2H, H-9'), 3.38-3.35 (*t*, 2H, -CH_2), 1.83-1.82 (*m*, 2H, -C_2H_5),); ^{13}C-NMR (100 MHz, DMSO-d_6), δ ppm: 186.51, 174.15, 148.09, 130.68, 128.05, 125.06, 121.09, 127.45, 105.12, 75.25, 49.76, 38.32, 33.31; MS (m/z (relative abundance, %)): 617.13 (24), 616.13 (M^+, 74), 615.14 (29), 614.13 (100).

{4-(5-Chloro-benzothiazol-2-ylamino)-3-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-5-thioxo-4,5-dihydro-[1,2,4]triazol-1-ylmethyl}-thiourea (4e)

White solid; Yield: 58%; m.p. 188-190°C, R*f*, 0.87 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 605.59; Elemental Analysis Calculated for $C_{24}H_{23}Cl_2N_9S_3$: C, 45.62; H, 3.66; N, 23.13% Found: C, 44.35; H, 3.71; N, 22.98%. FTIR (ν max; cm^{-1} KBr); 3698.39, 3679.11, 3655.12, 3632.64, 2956.45, 2373.52, 2311.13, 1933.08, 1855.89, 1783.93, 1660.60, 1530.22, 1156.97, 1107.79, 824.16; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δ ppm: 8.47 (*d*, 1H, J=1.6, H-2'), 7.86 (*d*, 1H, J=4.2, H-5'), 7.64 (*s*, 1H, -NH), 7.36 (*d*, 1H, J=1.9, H-8'), 7.09 (*d*, 1H, J=1.6, Ar -H), 6.85 (*d*, 1H, J=1.2, H-2'), 6.41-6.39 (*d*, 1H, J=5.8, H-9'), 6.16 (*s*, 1H, H-2), 5.75 (*s*, 2H, H-4'), 5.71 (*s*, 2H, H-5), 3.49-3.45 (*m*, 2H, H-7'), 3.42-3.35 (*m*, 2H, H-6'); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=186.51, 174.25, 154.53, 151.35, 149.09, 135.21, 13.045, 129.25, 128.56, 127.29, 125.65, 124.15, 122.93, 117.65, 72.34, 49.56, 38.31, 33.56; MS (m/z (relative abundance, %)): 608.05 (20), 607.06 (22), 606.05 (M^+, 77), 605.06 (28), 604.06 (100).

N-{4-(5-Chloro-benzothiazol-2-ylamino)-3-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-5-thioxo-4,5-dihydro-[1,2,4]triazol-1-ylmethyl}-acetamide (4f)

Light white solid; Yield: 64%; m.p. 248-250°C, R_f, 0.82 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 588.54; Elemental analysis calculated for $C_{24}H_{23}Cl_2N_9OS_2$: C, 48.98; H, 3.94; N, 21.42% Found: C, 47.88; H, 3.67; N, 20.87%; FTIR (ν_{max}; cm^{-1} KBr): 3775.14, 3739.15, 3573.19, 3394.02, 3032.55, 23473.52, 2311.13, 1836, 1725.74, 1660.60, 1515.52, 1351.19, 1280.52, 1156.97, 922.84; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δ ppm: 9.12 (*s*, 1H, H-9); 9.10 (*s*, 1H, H-8), 8.46 (*d*, 1H, J=1.6, H-2'), 7.90 (*d*, 1H, J=4.2, H-5'), 7.37 (*d*, 1H, J=1.9, H-8'), 6.41-6.39 (*s*, 1H, H-4'), 6.29 (*s*, 2H, H-9'), 6.17 (*s*, 1H, H-6'), 5.68 (*s*, 2H, H-4), 5.48 (*s*, 1H, H-2'), 4.42 (*s*, 2H, H-3'), 3.58-3.54 (*m*, 2H, H-2') 3.49-3.45 (*m*, 2H, H-8'); ^{13}C- NMR (100 MHz, DMSO-d_6), δppm=186.52, 174.51, 171.23, 154.63, 149.09, 128.45, 121.18, 120.23, 107.65, 65.67, 38.38, 33.35; MS (m/z (relative abundance, %)): 590.08 (23), 589.08 (M^+, 73), 588.09 (27), 587.08 (100).

4-(5-Chloro-benzothiazol-2-ylamino)-2-[(4-chloro-phenylamino)-methyl]-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-2,4-dihydro-[1,2,4]triazole-3-thione(4g)

Dark yellow solid; Yield: 68%; m.p. 248-250°C, R_f, 0.65 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 657.04; Elemental analysis calculated for $C_{28}H_{24}Cl_3N_9S_2$: C, 51.18; H, 3.68; N, 19.19% Found: C, 50.88; H, 3.34; N, 18.76%; FTIR (ν_{max}; cm^{-1} KBr): 3389, 3030, 2865, 2304, 2128, 1468, 754: ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.81 (*s*, 1H, -NH), 8.47 (*d*, 1H, J=4.2, H-2'), 7.87 (*d*, 1H, J=1.5, H-5'), 7.47 (*s*, 2H, -NH_2), 7.33 (*d*, 1H, J=4.2, H-6), 6.39 (*d*, 1H, J=5.6, H-9'), 5.66 (*s*, 2H, -NH_2), 6.18 (*s*, 1H, -NH), 5.38 (*s*, 1H, -NH), 4.48 (*s*, 2H, -CH_2), 3.55 (*d*, 2H, J=6.5, H-4'), 3.47-3.44 (*m*, 2H, H-8'); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=186.71, 174.55, 154.29, 151.64, 149.25, 149.14, 134.19, 13.087, 129.85, 128.83, 127.65, 125.34, 115.89, 104.53, 73.25, 63.64, 49.92, 38.76, 33.56; MS (m/z (relative abundance, %)): 658.04 (M^+, 37), 657.05 (31), 656.05(100), 655.05 (32), 654.05 (94)

2-[(4-Bromo-phenylamino)-methyl]-4-(5-chloro-benzothiazol-2-ylamino)-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-2,4-dihydro-[1,2,4]triazole-3-thione(4h)

Colourless solid; Yield: 55%; m.p. 221-223°C, R_f, 0.52 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 687.46; Elemental analysis calculated for $C_{27}H_{22}BrCl_2N_9S_2$: C, 47.94; H, 3.45; N, 17.97% Found: C, 46.98; H, 3.27; N, 18.77%. FTIR (ν_{max}; cm^{-1} KBr): 3193.86, 3135.22, 3109.92, 3007.89, 2904.27, 2347.85, 2332.16, 2155.92, 1687.69, 1579.65, 1545.25, 1492.08, 1177.66, 849.49, 726.57; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.50 (*d*, 1H, J=4.2, H-2'), 7.86 (*d*, 1H, J=5.6, H-5'), 7.66 (*s*, 1H, H-5'), 7.37 29 (*d*, 1H, J=4.2, H-6), 6.38-6.40 (*d*, 1H, J=5.6, H-9'), 6.18 (*s*, 1H, H-4'), 5.77 (*s*, 2H, -NH_2), 5.14 (*s*, 2H, H-9), 3.57-3.59 (*d*, 1H, J=4.2, H-6'), 3.47-3.44 (*m*, 4H, H-4); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=186.51, 178.32, 151.65, 149.10, 142.67, 134.56, 128.23, 126.87, 125.43, 124. 43, 120.65, 121.88, 120.76, 115.87, 114.89, 111.54, 73.49, 49.76, 38.65, 33.55. MS (m/z (relative abundance, %)): 690.00 (15), 689.00 (42), 688.00 (M^+, 31), 687.10 (100), 686.00 (20), 685.00 (58).

4-(5-Chloro-benzothiazol-2-ylamino)-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-2-morpholin-4-ylmethyl-2,4-dihydro-[1,2,4]triazole-3-thione(4i)

Light yellow solid; Yield: 62%; m.p. 245-247°C, R_f, 0.93 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 546.50; Elemental analysis calculated for $C_{22}H_{21}Cl_2N_9S_2$: C, 50.65; H, 4.41; N, 20.44% Found: C, 49.78; H, 3.89; N, 20.12%. FTIR (ν_{max}; cm^{-1} KBr): 3378, 3276, 3036, 2864, 2125, 1465, 1109, 755, ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.28 (*d*, 1H, J=4.2, H-2'), 7.90 (*d*, 1H, J=5.6, H-5'), 7.34 (*d*, 1H, J=4.2, H-6), 6.16 (*s*, 1H, -NH), 5.04 (*s*, 2H, CH_2), 3.53 (*m*, 2H, -CH_2), 2.80 (*m*, 4H, C_2H_4); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=186.67, 174.55, 154.34, 151.42, 149.65, 134.25, 130.45, 128.65, 126.22, 125.82, 124.12, 122.53, 121.85, 120.73, 104.53, 71.52, 54.34, 38.56, 33.54; MS (m/z (relative abundance, %)): 550.07 (4), 548.07 (21), 547.07 (M^+, 73), 546.08 (24), 545.07 (100).

4-(5-Chloro-benzothiazol-2-ylamino)-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-2-methyl amino methyl-2,4-dihydro-[1,2,4]triazole-3-thione (4j)

Light yellow solid; Yield: 62%; m.p. 245-247°C, R*f*, 0.64 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 638.59; Elemental analysis calculated for $C_{28}H_{25}Cl_2N_9OS_2$: C, 49.28; H, 4.14; N, 22.49% Found: C, 48.88; H, 4.09; N, 21.89%; FTIR (ν_{max}; cm^{-1} KBr): 3372, 3036, 2760, 2451, 2126, 1464, 1284, 1109, 758; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm: 8.28 (*d*, 1H, J=4.2, H-2'), 7.91 (*d*, 1H, J=5.6, H-5'), 7.33 (*d*, 1H, J=6.1, H-9'), 6.15 (*s*, 1H, H-4'), 5.03 (*s*, 1H, H-7'), 3.54- 3.52 (*m*, 2H, -C_2H_4); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=172.51, 156.38, 154.69, 151.08, 149.04, 132.26, 128.09, 124.05, 118.23, 117.65, 98.64, 69.92, 53.21, 46.21, 43.76, 37.31; MS (m/z (relative abundance, %)): 641.09 (16), 640.10 (24), 639.10 (74), 638.10 (36), 637.10 (100).

[3-[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-5-thioxo-4,5-dihydro-[1,2,4]triazol-1-ylmethyl]-urea (5a)

Light brown solid; Yield: 85.7%; m.p. 289-286°C, R_f, 0.71 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 559.04; Elemental analysis calculated: C, 48.20; H, 3.87; N, 24.44% Found: C, 47.87; H, 3.56; N, 23.80%; FTIR (ν_{max}; cm^{-1} KBr): 3368, 3189, 3034, 2851, 2490, 2124, 1876, 1598, 1666, 1201, 1103, 784; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.26-8.28 (*d*, 1H, J=4.2, H-2'), 7.66 (*d*, 1H, J=5.6, H-5'), 7.57 (*s*, 1H, H-6), 7.44 (*s*, 1H, H-2), 7.23 (*d*, 1H, J=3.1, H-3'), 6.99-7.14 (*t*, 2H, H-8), 6.27 (*d*, 1H, J=1.8, H-5), 6.13 (*s*, 2H, H-8), 4.98-4.97 (*d*, 2H, J=5.6, H-7), 3.51-3.48 (*t*, 2H, H-5), 3.36-3.38 (*t*, 2H, H-2), ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=186.54, 178.23, 174.4, 162.3, 158.7, 155.23, 152.86, 151.10, 149.14, 132.04, 127.49, 123.10, 122.50, 121.72, 116.93, 103.32, 98.82, 67.68, 38.53, 33.56; MS (m/z (relative abundance, %)): 558.09 (100), 560.09 (41), 559.10 (24), 561.09 (12), 559.09 (5), 562.09 (3).

N-[3-[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-5-thioxo-4,5-dihydro-[1,2,4]triazol-1-ylmethyl]-thioacetamide(5b)

Light orange solid; Yield: 89%; m.p. 275-278°C, R_f, 0.81(Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 588.15; Elemental analysis calculated for $C_{23}H_{21}ClFN_9OS_2$: C, 49.01; H, 3.94; N, 21.43% Found: C, 48.89; H, 3.89; N, 21.45% for FTIR (ν_{max}; cm^{-1} KBr): 3377, 3107, 3038, 2891, 2659, 2376, 2044, 1488, 1107, 786; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.26 (*d*, 1H, J=4.2, H-2'), 7.76 (*d*, 1H, J=3.1, H-3'), 7.44 (*d*, 1H, J=3.1, H-3'), 7.40 (*d*, 1H, J=4.3, H-5'), 6.22 (*d*, 1H, J=3.6, H-3), 5.48 (*d*, 1H, J=6.5, H-9'), 5.11 (*s*, 2H, H-6'), 3.36 (*d*, 2H, J=5.7, H-4), 2.44 (*d*, 2H, J=4.6, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=199.32, 185.22, 173.56, 158.7, 157.67, 153.26, 148.08, 133.09, 127.49, 125.09, 122.05, 118.78, 115.56, 112.34, 108.48, 70.98, 49.98, 38.45, 33.28; MS (m/z (relative abundance, %)): 591.09 (20), 590.09 (14), 589.09 (M^+, 47), 588.09 (32), 587.09 (100).

2-[(3-Amino-propylamino)-methyl]-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-2,4-dihydro-[1,2,4]triazole-3-thione (5c)

Pale yellow solid; Yield: 84%; m.p. 229-232°C, R_f, 0.62 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 587.14; Elemental analysis calculated for $C_{25}H_{28}ClFN_{10}S_2$: C, 51.14; H, 4.81; N, 23.86% Found C, 51.11; H, 4.76; N, 23.65%; FTIR (ν_{max}; cm^{-1} KBr): 3369, 3035, 2765, 2489, 2128, 1640, 1429, 1255, 1103, 749; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.54 (*d*, 1H, J=4.2, NH-2'), 7.90 (*s*, 1H, H-5'), 7.65 (*d*, 1H, J=3.6, H-3), 7.29-7.35 (*d*, 1H, J=4.2, H-6), 6.47 (*d*, 1H, J=5.6, H-9'), 6.98 (*d*, 1H, J=5.6, H-5'), 6.24 (*d*, 1H, J=5.6, H-8'), 5.66 (*s*, 2H, H-4'), 5.43 (*s*, 2H, H-2), 3.52-3.56 (*t*, 2H, H-3'), 3.32-3.37 (*t*, 2H, H-6'), 2.53 (*d*, 1H, J=4.2, H-6'), 1.77-1.81 (*m*, 2H, H-8'); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=187.9, 186.01, 178.4, 177.6, 174.51, 155.1, 154.65, 153.9, 151.26, 147.3, 134.09, 131.08, 133.0, 128.45, 127.2, 123.45, 121.06, 120.09, 103.05, 70.87, 48.12, 38.54, 36.65, 33.52; MS (m/z (relative abundance, %)): 590.15 (31), 589.16 (13), 588.16 (M^+, 42), 587.16 (33), 586.16 (100).

5-[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-2-piperazin-1-yl methyl-2,4-dihydro-[1,2,4]triazole-3-thione (5d)

Light yellow solid; Yield: 69%; m.p. 187-190°C, R_f, 0.7562 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 599.15; Elemental analysis calculated for $C_{25}H_{26}BrClN_{10}S_2$: C, 52.12; H, 4.71; N, 23.38% Found: C, 52.01, H, 4.65, N, 23.29%; FTIR (ν_{max}; cm^{-1} KBr): 3365, 3172, 3048, 2807, 2457, 2237, 2127, 1864, 1677, 1204, 1108, 752; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.37 (*d*, 1H, J=4.2, H-2'), 7.89 (*d*, 1H, J=2.2, H-3'), 7.78 (*s*, 1H, H-1), 7.44 (*s*, 1H, H-4'), 7.33 (*d*, 1H, J=4.2, H-6), 7.01 (*t*, 1H, H-5), 6.89 (*d*, 1H, J=5.6, H-5'), 5.38 (*s*, 2H, H-6'), 5.29 (*d*, 1H, J=5.6, H-8'), 3.73- 3.68 (*t*, 2H, H-7'), 3.35-3.34 (*t*, 2H, H-8'), 1.88-1.86 (*m*, 2H, H-3); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=186.51, 187.2, 174.6, 173.15, 155.9, 154.2, 153.09, 149.6, 148.68, 133.26, 127.05, 123.06, 121.09, 118.67, 116.34, 113.45, 108.67, 104.12, 75.25, 49.76, 38.52, 36.0, 33.51; MS (m/z (relative abundance, %)): 602.15 (3), 601.16 (13), 600.16 (42), 599.16 (34), 598.16 (100).

[3-[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-5-thioxo-4,5-dihydro-[1,2,4]triazol-1-ylmethyl]-thiourea (5e)

Cream solid; Yield: 82%; m.p. 183-187°C, R*f*, 0.65 (Ethanol: Ethyl acetate: Dichloro methane, 7:2:1); Mol. Wt: 589.13; Elemental analysis calculated for $C_{23}H_{22}ClFN_{10}S_3$: C, 46.89; H, 3.76; N, 23.78% Found: C, 46.91; H, 3.58; N, 23.77%; FTIR (ν_{max}; cm^{-1} KBr), 3379, 3264, 3065, 2851, 2637, 2404, 2168, 1868, 1469, 1113, 755; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.36-8.36 (*d*, 1H, J=4.2, H-2'), 7.98 (*d*, 1H, J=1.8, H-2'), 7.57 (*d*, 1H, quinoline), 7.39 (*s*, 1H, H-1), 7.06 (*d*, 1H, J=2.2, H-3'), 6.67 (*d*, 1H, J=5.6, H-5'), 6.45-6.40 (*d*, 2H, J=3.4, H-8'), 6.12 (*s*, 1H, H-4), 5.78 (*s*, 2H, H-5), 5.65 (*s*, 2H, H-6), 3.38-3.36 (*m*, 2H, H-9), 3.46-3.44 (*m*, 2H, H-8), ^{13}C-NMR (100 MHz, DMSO-d_6),

δppm=186.71, 175.87, 172.53, 158.7, 155.38, 154.2, 152.46, 149.49, 141.5, 134.6 133.21, 128.67, 125.85, 124.36, 122.93, 121.54, 118.65, 112.56, 109.21, 104.67, 65.78, 33.59; MS (m/z (relative abundance, %)): 592.08 (5), 591.09 (12), 590.08 (M^+, 45), 589.09 (28), 588.09 (100).

2-[(4-Chloro-phenylamino)-methyl]-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-2,4-dihydro-[1,2,4]triazole-3-thione (5f)

Light pink solid; Yield: 70%; m.p. 251-253°C, R_f, 0.86 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 640.58; Elemental analysis

calculated for $C_{28}H_{24}Cl_2FN_9S_2$: C, 52.50; H, 3.78; N, 19.68% Found: C, 52.48; H, 3.73; N, 19.65%; FTIR (ν_{max}; cm^{-1} KBr): 3255, 3186, 3174, 2872, 2451, 2317, 2144, 2017, 1644, 1638, 1401, 1119, 848, 742; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=9.86 (*s*, 1H, H-7'), 8.66 (s, 1H, H-6'), 8.53 (*d*, 1H, J=4.2, H-2'), 8.28 (*d*, 1H, J=4.2, H-3'), 7.62 (*d*, 1H, J=1.8, H-2'), 7.29 (*d*, 1H, J=5.6, H-8'), 6.55 (*s*, 2H, H-4'), 6.38 (*s*, 1H, H-3'), 5.85 (*s*, 1H, H-2'), 5.44 (*s*, 1H, H-8'), 4.88 (*s*, 2H, H-2), 4.66 (*s*, 2H, H-6'), 3.65 (*m*, 2H, H-4'), 3.48 (*m*, 2H, H-5'); ^{13}C-NMR (100 MHz,

DMSO-d_6), δppm=187.5, 186.45, 184.7, 177.49, 174.23, 159.63, 158.39, 151.47, 150.5, 148.72, 149.01, 145.9, 129.78, 125.40, 124.23, 121.68, 118.65, 115.65, 112.23, 109.15, 104.55, 73.63, 49.77, 38.38, 33.35; MS (m/z (relative abundance, %)): 642.10 (20), 641.09 (M^+,72), 640.10 (33), 639.10 (100).

2-[(4-Bromo-phenylamino)-methyl]-5-[3-(7-chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-2,4-dihydro-[1,2,4]triazole-3-thione (5g)

Dark brown solid; Yield: 65%; m.p. 301-298°C, R_f, 0.86 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol wt, 685.04; Elemental analysis calculated for $C_{28}H_{24}BrClFN_9S_2$: C, 49.09; H, 3.53;N, 18.40% Found: C, 49.07; H, 3.48; N, 18.36%; FTIR (ν_{max}; cm^{-1} KBr): 3278, 3058, 2876, 2645, 2498, 2124, 1815, 1518, 1258, 1103, 765; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.56 (*d*, 1H, J=4.2, H-2'), 8.42 (*s*, 1H, H-6'), 7.58 (*d*, 1H, J=1.8, H-2'), 7.36 (*d*, 1H, J=4.5), 6.78 (*d*, 1H, J=3.6, H-3'), 6.55 (*s*, 2H, H-4'), 5.44 (*s*, 1H, H-8'), 5.50 (*s*, 2H, H-2'), 5.76 (*s*, 1H, H-3'), 3.64 (*s*, 2H, H-4'), 3.71 (*d*, 2H, J=7.2, H-3'), 3.58-3.51 (*m*, 2H, H-4'); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=185.00, 176.42, 158.75, 153.44, 150.70, 154.47, 147.95, 133.78, 134.46, 127.65, 124.55, 121.32, 120.88, 117.99, 114.68, 113.78, 112.96, 104.55, 73.94, 38.85, 33.86; MS (m/z (relative abundance, %)): 685.04 (100), 683.05 (72), 687.04 (32), 686.05 (29), 684.05 (22), 688.04 (11).

5-[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-2-morpholin-4-ylmethyl-2,4-dihydro-[1,2,4]triazole-3-thione (5h)

Light violet colour solid; Yield: 43%; m.p. 242-239°C, R_f, 0.62; Mol. Wt, 600.13 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Elemental analysis calculated for $C_{26}H_{27}ClFN_9OS_2$: C, 47.94; H, 3.45; N, 17.97% Found: C, 46.98; H, 3.27; N, 18.77%. FTIR (ν_{max}; cm^{-1} KBr): 3266, 3138, 3098, 2743, 2513, 2307, 2044, 1455, 1113, 786; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.48(*d*, 1H, J=4.2, H-2'), 7.84 (*d*, 1H, J=1.8, H-2'), 7.66 (*s*, 1H, -NH), 7.39 (*d*, 1H, J=4.5), 6.38 (*d*, 1H, J=3.6, H-3'), 6.15 (*s*, 1H, H-4'), 5.77 (*s*, 2H, H-3'), 5.17 (*s*, 2H, H-4'), 3.58 (*m*, 2H, H-4'), 3.48-3.44 (*m*, 4H, H-3'), 2.69-2.65 (*m*, 4H, H-4), ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=185.01, 184.1, 173.56, 159.75,158.7, 155.98, 154.2, 151.3, 150.32, 148.21, 135.56, 129.22, 125.7, 124.86, 123. 33, 121.6, 120.89, 118.34, 115.89, 114.97, 112.21, 73.49, 53.65, 42.76, 42.66; MS (m/z (relative abundance, %)): 602.15 (9), 601.14 (40), 600.15 (29), 599.15 (100).

5-[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-2-morpholin-4-ylmethyl-2,4-dihydro-[1,2,4]triazole-3-thione (5i)

Light orange solid; Yield: 75%; m.p. 294-292°C, R_f, 0.85 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Mol. Wt, 600.13; Elemental analysis calculated for $C_{26}H_{27}ClFN_9OS_2$: C, 52.03; H, 4.53; N, 21.01% Found: C, 51.03; H, 3.55; N, 20.88%.; FTIR (ν_{max}; cm^{-1} KBr): 3284, 3125, 2813, 2591, 2342, 2149, 1871, 1500, 1407, 796, 669; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.64 (*d*, 1H, J=4.2, H-2'), 7.80 (*d*, 1H, J=5.8, H-6'), 7.23 (*d*, 1H, J=1.8, H-2'), 5.24 (*s*, 1H, H-3'), 4.24 (*s*, 2H, H-4'), 3.64- 3.66 (*m*, 2H, H-3'), 2.78-2.82 (*m*, 4H, H-4'); ^{13}C-NMR (100 MHz, DMSO-d_6), δppm=185.66, 174.2, 173.55, 158.0, 157.44, 153.43, 151.42, 149.75, 134.6, 133.65, 131.45, 127.69, 126.32, 124.6, 119.82, 116.12, 114.53, 109.85, 104.73, 49.1, 38.56, 33.54; MS (m/z (relative abundance, %)): 602.15 (9), 601.14 (M^+, 40), 600.15 (29), 599.15 (100).

5-[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-4-(5-fluoro-benzothiazol-2-ylamino)-2-methyl aminomethyl-2,4-dihydro-[1,2,4]triazole-3-thione (5j)

Light cream solid; Yield: 76%; m.p. 221-218°C, R*f*, 0.80 (Ethanol: Ethyl acetate: Dichloromethane, 7:2:1); Elemental analysis calculated for $C_{23}H_{25}ClFN_9S_2$: C, 50.77; H, 4.26; N, 23.17, %Found: C, 50.68; H, 4.19; N, 22.87%; Mol. Wt, 545.13; FTIR (ν_{max}; cm^{-1} KBr): 3344, 3123, 3076, 2841, 2698, 2571, 2471, 2138, 2031, 1892, 1521, 1295, 771, 668; ^{1}H-NMR (400 MHz, DMSO-d_6, TMS), δppm=8.42 (*d*, 1H, J=4.2, H-2'), 7.88 (*d*, 1H, *J=1.8*, H-2'), 7.85 (*d*, 1H, J=4.5), 6.35 (*s*, 1H, H-4'), 6.29 (*s*, 1H, H-5'), 4.97 (*s*, 2H, H-6'), 3.44 - 3.37 (*m*, 2H, H-4'), 2.89-2.86 (*m*, 4H, H-3'), 2.68 -2.67 (*m*, 4H, H-4); ^{13}C-NMR (100 MHz, DMSO-d_6), δ ppm=186.76, 182.6, 174.59, 158.6, 154.41, 151.08, 149.05, 134.26, 128.56, 125.09, 121.34, 118.23, 115.9, 112.83, 109.12, 104.65, 74.34, 38.31, 33.35; MS (m/z (relative abundance, %)): 548.14 (8), 547.13 (40), 546.14 (26), 545.13 (100).

Biological activity

Acute toxicity

According to Organisation for Economic Co-operation and Development (OECD) guidelines for acute toxicity study. Adult and healthy Swiss albino mice weighing between 20-25 g were used in this experiment. Animals were divided into group of five each and they were starved for at least 24 h before the experiment. The Control group receives 1% solution of sodium carboxy methyl cellulose. Test group receive the test compound solution in sodium carboxy methyl cellulose (1%) in doses of 100-1000 mg/kg body weight and administered intraperitoneally. The animals were observed for 48 h from the time of administration of test compounds to record the mortality. The compounds used for toxicity studies are found safe as no mortality is reported at the doses of 100-1000 mg/kg body weight.

In vivo anti-inflammatory activity

In vivo anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema assay model for the compounds (4a-4j and 5a-5j). The male albino rats (170-220 g) of either sex were used in a group of six animals in each. They were starved overnight with water ad libitum prior to the day of experiment. One hour prior to the 1% w/v solution injection of 0.1 ml carrageenan into the plantar region of left hind paw. The marking was just made beyond the tibia-tarsal junction of (knee joint) left hind paw in each animal of groups. The Control group received 1 ml of 0.5% sodium carboxymethyl cellulose (Na-CMC), standard group received 13.5 mg/kg meloxicam and test groups received 200 mg/kg of synthesized compounds orally. One hour later; a sub plantar injection of 0.05 ml of 1% solution of carrageenan (Sigma) in sterile distilled water was administered to the left hind footpad of each animal. The paw edema volume was measured with a digital plethysmometer at 0, 1, 2, 3, 4, 5 and 6 h after carrageenan injection. Paw edema volume was compared with vehicle control group and percent reduction was calculated as

$$\% \text{ edema inhibition} = 1 - Vt/Vc \times 100$$

Where, Vt and Vc were edema volume in treated and control groups, respectively. The results are analyzed statistical significance (expressed as mean ± SEM) between the vehicle control and the treated groups using one-way ANOVA followed by Dunnett's test using Graph Pad Instant software.

Analgesic activity by tail-flick method in mice

The analgesic activity was performed on Swiss albino mice. Overnight fasted healthy and adult male Swiss albino mice weighing between 20-25

g, in a group of six each were taken for the investigation. The animals were kept into a small cage with an opening for the tail at the rear wall. The tail was held gently and a light beam exerting radiant heat was directed to the proximal third of the tail. The tip of the tail of the mice was individually placed on the radiant heat source at constant temperature 55°C. The cut-off reaction time was fixed at 15 sec to avoid tissue damage. The tail flick response was measured at 0, 1, 2, 3 and 4 h after treatment of test compounds by digital analgesiometer. The control group received normal saline solution at 10 mg/ml intraperitoneal and second group received diclofenac sodium (3.9 mg/kg, intraperitoneal) was used as standard drug for comparison and test groups received synthesized derivatives at 100 mg/kg oral administration.

RESULTS AND DISCUSSION

In vivo anti-inflammatory activity

The anti-inflammatory activity of synthesized compounds were mentioned in Table 1 which shows that all the compounds show anti-inflammatory activity, the compounds 5a, 5c, 5e, 5g, 5h, 5i shows preeminent activity when compared with standard drug celecoxib.

Table 1: Anti-inflammatory activity of synthesized compounds on carrageenan-induced acute paws edema

Compound code	Mean difference in Paw volume in ml (Mean ± SEM)					Inhibition after 4 h (%)
	0 h	1 h	2 h	3 h	4 h	
C.D#	0.13 ± 0.01	0.23 ± 0.01	0.24 ± 0.02	0.24 ± 0.02	0.25 ± 0.02	-
S.D^	0.14 ± 0.02	0.13 ± 0.01	0.12 ± 0.01*	0.10 ± 0.01	0.08 ± 0.01*	68
4a	0.13 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	28
4b	0.13 ± 0.01	0.15 ± 0.01	0.17 ± 0.02	0.21 ± 0.01	0.23 ± 0.01	8
4c	0.13 ± 0.01	0.15 ± 0.01	0.16 ± 0.02	0.18 ± 0.02	0.19 ± 0.01	24
4d	0.13 ± 0.01	0.15 ± 0.01	0.17 ± 0.01**	0.21 ± 0.01	0.22 ± 0.01	12
4e	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	24
4f	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	32
4g	0.12 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	44
4h	0.13 ± 0.01	0.15 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.01*	48
4i	0.12 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.20 ± 0.02	0.16 ± 0.01*	36
4j	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	56
5a	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.15 ± 0.01	0.10 ± 0.01**	60
5b	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.02	0.13 ± 0.02	0.15 ± 0.01*	40
5c	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.09 ± 0.01	0.09 ± 0.02**	64
5d	0.12 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.18 ± 0.02	0.20 ± 0.01	20
5e	0.14 ± 0.02	0.13 ± 0.01*	0.13 ± 0.01	0.10 ± 0.01	0.08 ± 0.01*	68
5f	0.16 ± 0.02	0.13 ± 0.01*	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	32
5g	0.15 ± 0.02	0.13 ± 0.02*	0.11 ± 0.01	0.09 ± 0.01	0.10 ± 0.02**	60
5h	0.14 ± 0.02	0.13 ± 0.01	0.12 ± 0.01**	0.10 ± 0.01**	0.09 ± 0.02**	64
5i	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.09 ± 0.01**	64
5j	0.18 ± 0.02	0.16 ± 0.02*	0.17 ± 0.01	0.15 ± 0.01**	0.18 ± 0.01	28

*P<0.05, **P<0.01; #S.D: Standard Drug-Meloxicam (3.9 mg/kg i.p); ^C.D: Control Drug (0.5% CMC)

Analgesic activity

The analgesic activity of synthesized derivatives are mentioned in Table 2 it shows that the compounds 4i, 5a, 5b, 5f, 5g, 5h shows effective analgesic activity while 4g, 4h, 4j, 5b shows moderate activity when compared with standard drug Diclofenac sodium.

Table 2: Analgesic activity of synthesized compounds (4a-j and 5a-j)

Compound code	Tail withdrawing time in seconds (Mean ± SEM)				
	0 h	1 h	2 h	3 h	4 h
Control #	1.13 ± 0.33	1.66 ± 0.33	2.85 ± 0.25	3.35 ± 0.21	4.32 ± 0.75
Standard ^	2.33 ± 0.33	5.00 ± 0.40	8.06 ± 0.40	8.34 ± 0.35*	10.25 ± 0.33**
4a	3.25 ± 0.47	3.50 ± 0.28	4.00 ± 0.42*	5.32 ± 0.50	6.55 ± 0.47**
4b	2.00 ± 0.81	2.25 ± 0.25	3.75 ± 0.85	4.21 ± 0.32	5.21 ± 0.36
4c	1.75 ± 0.47	2.75 ± 0.62	3.25 ± 0.84*	3.79 ± 0.45*	4.23 ± 0.22
4d	2.00 ± 0.95	2.25 ± 0.25	3.27 ± 0.85	4.35 ± 0.33	5.75 ± 0.41*
4e	2.25 ± 0.95	2.75 ± 0.62	3.70 ± 0.25	4.52 ± 0.46*	6.34 ± 0.23*
4f	2.00 ± 0.81	3.63 ± 0.81	3.25 ± 0.43	4.15 ± 0.31	5.23 ± 0.31**
4g	1.25 ± 0.25	3.00 ± 0.43	2.75 ± 0.31	3.25 ± 0.22	4.25 ± 0.21
4h	1.75 ± 0.47	2.00 ± 0.40	3.25 ± 0.31	4.16 ± 0.33*	5.27 ± 0.35**
4i	1.75 ± 0.25	2.75 ± 0.25	4.00 ± 0.40*	5.23 ± 0.38	7.45 ± 0.22**
4j	2.50 ± 0.28	2.25 ± 0.47	3.50 ± 0.28	4.25 ± 0.29	6.21 ± 0.33*
5a	1.50 ± 0.28	2.33 ± 0.48	5.00 ± 0.80*	6.32 ± 0.21*	8.43 ± 0.21**
5b	2.00 ± 0.40	2.70 ± 0.25	4.29 ± 0.75*	6.58 ± 0.20	7.29 ± 0.33*
5c	1.25 ± 0.25	2.26 ± 0.47	3.75 ± 0.25	4.43 ± 0.31	6.57 ± 0.22*
5d	1.75 ± 0.47	2.25 ± 0.45	3.25 ± 0.31	4.16 ± 0.23	6.32 ± 0.27**
5e	1.50 ± 0.28	2.00 ± 0.40	4.25 ± 0.20	5.45 ± 0.54*	6.78 ± 0.67*
5f	1.75 ± 0.47	2.50 ± 0.64	4.50 ± 0.75*	6.75 ± 0.29**	9.56 ± 0.78**
5g	2.00 ± 0.57	2.50 ± 0.47	4.35 ± 0.35*	5.34 ± 0.31	7.23 ± 0.33*
5h	2.25 ± 0.47	2.25 ± 0.49	4.55 ± 0.19*	6.21 ± 0.22*	8.34 ± 0.23*
5i	1.75 ± 0.47	2.50 ± 0.75	3.00 ± 0.70	4.23 ± 0.33*	6.70 ± 0.45*
5j	1.50 ± 0.28	2.00 ± 0.40	3.50 ± 0.64**	4.78 ± 0.21*	6.75 ± 0.21*

*P<0.05, **P<0.01; #Control-vehicle (0.5% CMC); ^Std: Diclofenac sodium (3.9 mg/kg)

CONCLUSIONS

In the current study focused on the synthetic route of 4-(Substituted Benzothiazol-2-yl amino)-5-[2-(7-chloro-quinolin-4-yl amino)-ethyl amino] substituted methyl-2,4-dihydro-[1,2,4]triazole-3-thione. All the synthesized compounds are screened for *in-vivo* analgesic and anti-inflammatory activities. The given data proved that synthesized derivatives showed promising pharmacological activities.

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