Regiospecific synthesis of some novel *N*-nucleosides of 4-amino-5-substituted-1,2,4-triazole-3-thiones and their in-vitro antimicrobial activity

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ABSTRACT

4-Amino-5-substituted-1,2,4-triazole-3-thiones were prepared by following two different reaction routes and comparing the effectiveness of using different reaction conditions. The coupling of aminotriazoles with acetylated α-bromo-*D*-glucose furnished protected *N*-nucleosides regiospecifically. The reagents used gave only one regioisomer *N*-glycosides not the other *S*-nucleosides. The protected nucleosides upon deacetylation using methanolic ammonia afforded deprotected products. The chemical structures of synthesized compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopic and elemental analysis data. All of the synthesized compounds were tested against ten different gram positive and gram negative bacterial strains which exhibited moderate to good antibacterial activity. The deprotected nucleosides portrayed high antibacterial activity than 4-amino-5-substituted-1,2,4-triazole-3-thiones and protected nucleosides against selected bacteria.

1. Introduction

Several five membered aromatic systems having three hetero atoms at symmetrical position have been studied because of their interesting physiological properties [1,2]. In the last few decades, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. For example, a large number of 1,2,4-triazole-containing ring system have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, central nervous system (CNS) stimulants, sedatives, anxyolytic, antimicrobial agents [3,4]. Derivatives of 1,2,4-triazole and 1,3,4-thiadiazole condensed nucleus systems (triazolothiadiazoles) found to have diverse pharmacological activities including unique anti-inflammatory, anti-edema, and analgesic properties [5-9]. Also, there are known drugs containing the 1,2,4-triazole group e.g. Triazolam, Alprazolam, Etizolam, and Furacylin [10].

Several triazolothiadiazole derivatives have been prepared from different non-steroidal anti-inflammatory agents and found to possess improved pharmacological profile [11-13]. In addition to these important biological applications, mercapto-1,2,4-triazoles are also of great utility in preparative organic chemistry, for example, in the presence of various reagents, undergo different types of reactions to yield other heterocyclic compounds, e.g. thiazolotriazoles, triazolothiadiazoles, triazolothiazines, triazolothiazepines and triazolothiadiazines.

The reaction of carboxylic acid chlorides and thiosemicarbazide gave acylthiosemicarbazide which without purification were cyclized in alkaline media to yield the corresponding 2,4-dihydro-3*H*-1,2,4-triazole-3-thiones [14-15]. Moreover, the 1,2,4-triazole-5-thiones were prepared by cyclization of the corresponding thiosemicarbazide [16].

5-Furan-2-yl-4*H*-1,2,4-triazole-3-thiol was prepared by the reaction of the appropriate 2-furoyl-thiosemicarbazide and potassium hydroxide in ethanol for 3 hours under reflux, followed by acidification with acetic acid [17,18].

In view of the above mentioned extensive applications of 1,2,4-triazoles and their nucleosides, we describe herein the synthesis of some novel 5-substituted-4-amino-1,2,4-triazole-3-thiones and their nucleosides and evaluation of their antimicrobial activities. The reaction sequence leading to the formation of desired heterocyclic compounds are outlined in Scheme 1. The structures of the compounds were assigned on the basis of FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.

2. Experimental

Melting points were recorded using a digital Gallenkamp (SANYO) model MPD.BM 3.5 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were determined in DMSO-d_6 at 300 MHz and 75 MHz, respectively, using a Bruker spectrometer. FT-IR spectra were recorded on Perkin Elmer Spectrum BX spectrophotometer as KBr pellets. Mass Spectra (EI, 70 eV) on a MAT 312 instrument, and elemental analyses were conducted using a LECO-183 CHNS analyzer. Antimicrobial activity was carried out at The Riphah Institute of Pharmaceutical Sciences, Riphah International University Islamabad, Pakistan. All chemicals were purchased from Merck and Aldrich, and were used without further purification.

2.1. Synthesis of 5-substituted-4-amino-1,2,4-triazole-3-thiones (2a-j)

Method A: A solution of KOH (0.022 moles) in water (20 mL) was added dropwise to a solution of hydrazide (0.022 moles) and carbondisulfide (0.028 moles) in ethanol (100-150 mL).
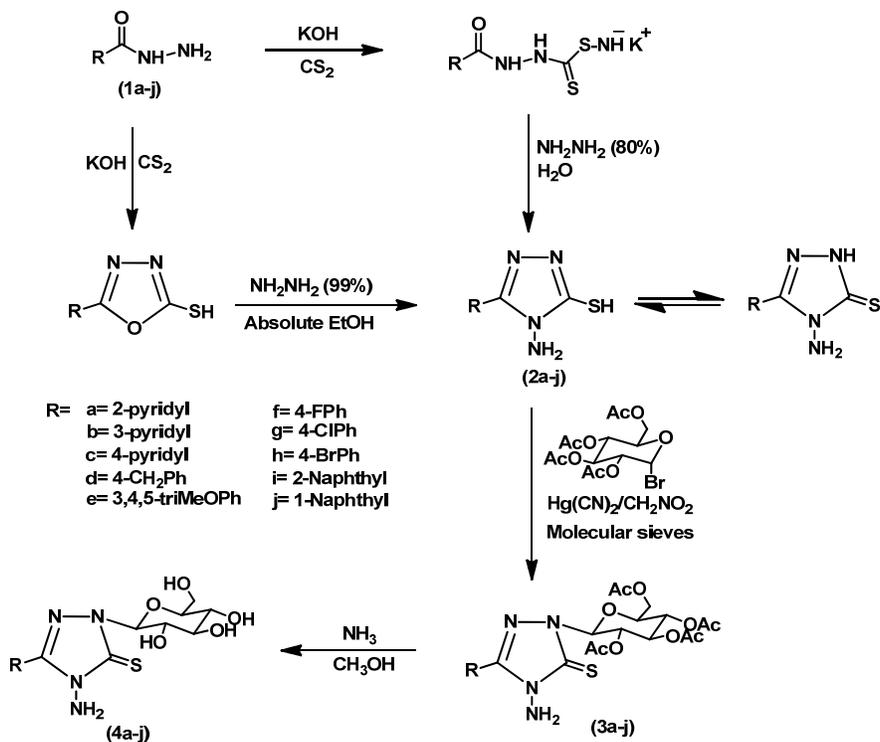
Scheme 1

The reaction mixture was refluxed until the evolution of hydrogen sulfide ceases. After completion of reaction it was concentrated. The residue was divided into two portions. One portion was treated with diethyl ether to give the potassium salt of corresponding oxadiazole, which was filtered. To the solution of potassium salt in water (4 mL) was added hydrazine hydrate (80%) under reflux. The reaction time was monitored through TLC (silica; ethylacetate:petroleum, ether 3:1). After completion of reaction, the reaction mixture was diluted with water and acidified with 4 N hydrochloric acid. The resulting solid was filtered, washed with water and recrystallized from ethanol (Scheme 1).

Method B: The residue from Method A was diluted with 200 mL of distilled water and acidified with 4 N HCl to pH = 2-3. The solid separated was filtered and recrystallized from ethanol. The resulting oxadiazole (0.01 moles) and hydrazine hydrate (99%, 0.03 moles) in absolute ethanol (20 mL) were refluxed for 6-8 hours until the reaction was completed. The solvent and excess hydrazine hydrate were removed under reduced pressure. The residue was washed with ether and recrystallized from ethanol to afford the desired pure product (Scheme 1).

4-Amino-5-(2-pyridyl)-1,2,4-triazole-3-thione (**2a**): Yield: 65% (Method A). M.p.: 210 °C. IR (KBr, ν, cm^{-1}): 3430-3330 (NH$_2$), 1630 (Ar C=C), 1567 (C=N), 1238 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.03 (d, 1H, J = 7.9 Hz, H-3''), 8.60 (d, 1H, J = 5.0 Hz, H-6''), 7.81 (t, 1H, J = 6.7 Hz, H-4''), 7.37 (t, 1H, J = 5.3 Hz, H-5''), 5.54 (s, 2H, NH$_2$), 13.8 (s, 1H, NH/SH). EI-MS (*m/z*, (%)): 193 (M$^+$, 100), 162 (6.2), 122 (10.3), 119 (33.2), 111 (3.0), 105 (20.3), 92 (10.1), 91 (4.0), 85 (15.9), 83 (25.3), 78 (36.6), 60 (34.6). UV (λ$_{max}$, EtOH): 324.61, 285.45.

4-Amino-5-(3-pyridyl)-1,2,4-triazole-3-thione (**2b**): Yield: 57% (Method B). M.p.: 218 °C. IR (KBr, ν, cm^{-1}): 3436-3334 (NH$_2$), 1627 (Ar C=C), 1570 (C=N), 1247(C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 9.13 (d, 1H, J = 1.5 Hz, H-2''), 8.68 (d, 1H, J = 3.5 Hz, H-6''), 8.36 (d, 1H, J = 7.9 Hz, H-4''), 7.56 (dd, 1H,

J = 7.9 Hz, J = 4.9 Hz, H-5''), 5.79 (s, 2H, NH$_2$), 12.6 (s, 1H, NH/SH). EI-MS (*m/z*, (%)): 193 (M$^+$, 100), 162 (4.46), 135 (8.74), 122 (12.80), 118 (8.96), 105 (19.37), 91 (5.39), 85 (13.15), 83 (20.2), 78 (23.4), 60 (34.9). UV (λ$_{max}$, EtOH): 326.91, 282.45.

5-(4-Pyridyl)-4-amino-1,2,4-triazole-3-thione (**2c**): Yield: 70% (Method A). M.p.: 226 °C. IR (KBr, ν, cm^{-1}): 3272 (NH$_2$), 1629 (Ar C=C), 1566 (C=N), 1218 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.82 (d, 2H, J = 5.9 Hz, H-3'', H-5''), 7.83 (d, 2H, J = 6.0 Hz, H-2'', H-6''), 5.53 (s, 2H, NH$_2$), 13.7 (s, 1H, NH/SH). EI-MS (*m/z*, (%)): 193 (M$^+$, 100), 162 (7.2), 122 (6.3), 119 (57.0), 111 (3.0), 105 (27.3), 91 (2.0), 85 (46.9), 83 (5.3), 78 (36.6), 60 (6.7), 56 (18.6). UV (λ$_{max}$, EtOH): 328.24, 275.48.

4-Amino-5-(4-methylphenyl)-1,2,4-triazole-3-thione (**2d**): Yield: 68% (Method B). M.p.: 208 °C. IR (KBr, ν, cm^{-1}): 3427-3309 (NH$_2$), 1630 (Ar C=C), 1588 (C=N), 1236 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.23-7.39 (m, 4H, Ar-H), 5.49 (s, 2H, NH$_2$), 2.71 (s, 3H, CH$_3$), 12.8 (s, 1H, NH/SH). EI-MS (*m/z*, (%)): 206 (M$^+$, 100), 191 (1.94), 175 (2.94), 160 (1.83), 135 (47.33), 132 (7.79), 118 (46.92), 117 (29.92), 102 (7.31), 91 (26.47), 78 (2.66), 77 (4.89), 65 (14.47), 60 (29.14). UV (λ$_{max}$, EtOH): 327.32, 280.42.

4-Amino-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole-3-thione (**2e**): Yield: 60% (Method B). M.p.: 220 °C. IR (KBr, ν, cm^{-1}): 3438-3325 (NH$_2$), 1630 (Ar C=C), 1578 (C=N), 1268 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): δ 7.33 (s, 2H, H-6''), 3.71 (s, 9H, -OCH$_3$), 5.81 (s, 2H, NH$_2$), 13.2 (s, 1H, NH/SH). EI-MS (*m/z*, (%)): 296 (M$^+$, 100), 281 (36.70), 235 (5.31), 249 (1.72), 218 (2.16), 193 (21.49), 178 (19.34), 150 (10.23), 135 (10.35), 120 (11.69), 118 (6.57), 105 (1.51), 103 (15.50), 93 (2.80), 90 (3.23), 77 (3.39), 74 (8.62), 64 (8.43), 60 (75.88). UV (λ$_{max}$, EtOH): 325.46, 280.75.

4-Amino-5-(4-fluorophenyl)-1,2,4-triazole-3-thione (**2f**): Yield: 58% (Method A). M.p.: 205 °C. IR (KBr, ν, cm^{-1}): 3456-3310 (NH$_2$), 1637 (Ar C=C), 1585 (C=N), 1238 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.93 (m, 2H, H -2'', H-6''), 7.20 (m,

2H, H-3'', H-5''), 5.75 (s, 2H, NH$_2$), 12.5 (s, 1H, NH/SH). EI-MS (*m/z*, (%)): 210 (M$^+$, 100), 209 (1.1), 194 (2.8), 180 (4.1), 178 (2.7), 138 (33.4), 121 (39.51), 108 (10.2), 102 (20.3), 89 (7.8), 75 (32.1), 60 (56.4). UV (λ$_{max}$, EtOH): 320.26, 276.35.

4-Amino-5-(4-chlorophenyl)-1,2,4-triazole-3-thione (**2g**): Yield: 78% (Method A). M.p.: 208 ºC. IR (KBr, ν, cm^{-1}): 3410-3310 (NH$_2$), 1636 (Ar C=C), 1595 (C=N), 1233 (C=S). ^{1}H NMR (300 MHz, DMSO-*d$_6$*, δ, ppm): 8.05 (d, 2H, *J* = 8.1 Hz, H -2'', H-6''), 7.59 (d, 2H, *J* = 8.1 Hz, H-3'', H-5''), 5.77 (bs, 2H, NH$_2$), 13.9 (bs, 1H, NH/SH). EI-MS (*m/z*, (%)): 226 (M$^+$,100), 211 (1.16), 195 (3.70), 157 (14.56), 155 (40.92), 138 (38.91), 137 (35.47), 127 (2.17), 125 (7.04), 113 (11.14), 111 (22.80), 102 (26.85), 95 (3.68), 89 (9.52), 76 (11.01), 75 (29.27), 60 (54.81). UV (λ$_{max}$, EtOH): 327.43, 281.95.

4-Amino-5-(4-Bromophenyl)-1,2,4-triazole-3-thione (**2h**): Yield: 80% (Method B). M.p.: 202 ºC. IR (KBr, ν, cm^{-1}): 3455-3318 (NH$_2$), 1613 (Ar C=C), 1594 (C=N), 1235 (C=S). ^{1}H NMR (300 MHz, DMSO-*d$_6$*, δ, ppm): 8.05 (d, 2H, *J* = 8.3 Hz, H-2'', H-6''), 7.63 (d, 2H, *J* = 8.4 Hz, H-3'', H-5''), 5.25 (s, 2H, NH$_2$), 13.6 (bs, 1H, NH/SH). EI-MS (*m/z*, (%)): 271 (M$^+$, 38), 270 (M$^+$-1, 100), 255 (2.6), 241 (1.2), 239 (4.5), 199 (42.7), 182 (15.3), 142 (16.8), 155 (2.8), 102 (31.7), 75 (67.8). UV (λ$_{max}$, EtOH): 326.45, 288.15, 245.41.

4-Amino-5-(2-naphthyl)-1,2,4-triazole-3-thione (**2i**): Yield: 81% (Method B). M.p.: 205-206 ºC. IR (KBr, ν, cm^{-1}): 3420-3305 (NH$_2$), 1625 (Ar C=C), 1526 (C=N), 1232 (C=S). ^{1}H NMR (300 MHz, DMSO-*d$_6$*, δ, ppm): 8.80 (s, 1H, H-1''), 8.10 (dd, 1H, *J*=7.5 Hz, 4.6 Hz, Naphthyl-H), 7.86-7.98 (m, 3H, Naphthyl-H), 7.54-7.62 (m, 2H, Naphthyl-H), 5.15 (s, 2H, NH$_2$), 13.5 (bs, 1H, NH/SH). EI-MS (*m/z*, (%)): 242 (M$^+$, 100), 241 (M$^+$-1, 70), 209 (5.4), 208 (3.2), 183 (32.7), 182 (11.3), 168 (13.8), 154 (27.8), 140 (46.7), 127 (32.2). UV (λ$_{max}$, EtOH): 364.33, 298.17.

4-Amino-5-(1-Naphthyl)-1,2,4-triazole-3-thione (**2j**): Yield: 65% (Method B). M.p.: 200 ºC. IR (KBr, ν, cm^{-1}): 3429-3307 (NH$_2$), 1624 (Ar C=C), 1526 (C=N), 1272 (C=S). ^{1}H NMR (300 MHz, DMSO-*d$_6$*, δ, ppm): 8.95 (d, 1H, *J* = 8.5 Hz, H-2''), 8.20 (d, 1H, *J* = 7.3 Hz, H-8''), 8.06 (d, 1H, *J* = 8.1 Hz, H-4''), 7.56 (m, 4H, H-3'', 5'', 6'', 7''), 5.61 (s, 2H, NH$_2$), 13.6 (s, 1H, NH/SH). EI-MS (*m/z*, (%)): 242 (M$^+$, 100), 241 (M$^+$-1, 65), 209 (3.4), 208 (4.2), 183 (32.4), 182 (7.3), 168 (12.8), 154 (42.8), 140 (54.7), 127 (68.2). UV (λ$_{max}$, EtOH): 360.40, 290.15.

2.2. Synthesis of acetylated nucleosides bearing 4-amino-5-substituted-1,2,4-triazole-3-thiones (3a-j)

General procedure: To a mixture of 2 mmoles of acetobromo sugar (2,3,4,6-tetra-*O*-acetyl-*D*-glucopyranosyl bromide), mercuric cyanide (2 mmoles) and anhydrous calcium sulfate or molecular sieves (2 g), was added 1 mmole of the corresponding heterocyclic compound. In each case the reaction mixture was refluxed and monitored by TLC (Silica; Ethylacetate:Petroleum ether, 3:1). Generally, the completion of reaction occurred within 5-6 hours. Then the reaction mixture was filtered while hot in order to remove any insoluble residue which was washed thoroughly with hotter nitromethane. The filterate was evaporated to dryness in vacuo. The product, thus obtained, was treated with dichloromethane (50-70 mL) and filtered to remove an undesired solid (a complex formed by mercuric halide and the corresponding heteroyclic compound). The dichloromethane extract was washed with 30% potassium iodide (50 mL), then with water (30 mL) and dried over anhydrous sodium sulfate. After filteration the solvent was evaporated to dryness. The crude solid obtained was purified either by recrystallization from aqueous ethanol or by column chromatography (silica gel (230-400 mesh); petroleum ether:ethylacetate, 4:1) to get pure products (Scheme 1).

(2R,3R,4S,5R)-(acetoxymethyl)-6-(4-amino-3-(pyridin-2-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3a**): Yield: 67.6%. M.p.: 174 ºC. IR (KBr, ν, cm^{-1}): 3432 (NH$_2$), 1745 (COCH$_3$), 1629 (Ar C=C), 1592 (C=N), 1226 (C=S). ^{1}H NMR (300 MHz, DMSO-*d$_6$*, δ, ppm): 1.88, 2.01,

2.04, 2.10 (4s, 4 x 3H, 4-CH$_3$), 4.12 (m, 1H, H-5'), 4.27 (dd, 1H, *J* = 2.0 Hz, 12.8 Hz, H-6'), 4.50 (dd, 1H, *J* = 4.5 Hz, 12.8 Hz, H-6'a), 5.31 (t, 1H, *J* = 9.8 Hz, H-4'), 5.49 (s, 2H, NH$_2$), 5.65 (t, 1H, *J* = 9.6 Hz, H-3'), 5.88 (t, 1H, *J* = 9.3 Hz, H-2'), 6.15 (d, 1H, *J* = 9.2 Hz, H-1'), 7.41 (dt, 1H, *J* = 2.1 Hz, 7.6 Hz, H-5''), 7.62 (t, 1H, *J* = 7.2 Hz, H-4''), 7.81 (d, 1H, *J* = 4.8Hz, H-4''), 8.05 (d, 1H, *J* = 7.1 Hz, H-3''). ^{13}C NMR (75 MHz, DMSO-*d$_6$*, δ, ppm): 20.6, 20.7, 20.8, 20.9 (4-CH$_3$), 61.2 (C-6'), 67.6 (C-2'), 69.9 (C-3'), 71.1 (C-4'), 73.2 (C-5'), 83.0 (C-1'), 130.8 (C-5''), 133.5 (C-6''), 136.4 (C-4''), 145.6 (C-3''), 151.4 (C-5), 169.3, 170.0, 170.5, 170.8 (4-CO), 171.3 (C-3). EI-MS (*m/z*, (%)): 523 (M$^+$ 5), 193 (2), 456 (2), 453 (1), 331 (12), 271 (2), 229 (2), 211 (4), 194 (5), 169 (100), 145 (6), 127 (30), 109 (80), 109 (80), 98 (12), 78 (5). Anal. calcd. for C$_{21}$H$_{25}$N$_5$O$_9$S: C, 48.18; H, 4.78; N, 13.38. Found: C, 48.08; H, 4.69; N, 13.40%.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(4-amino-3-(pyridin-3-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3b**): Yield: 57.2%. M.p.: 180 ºC. IR (KBr, ν, cm^{-1}): 3430 (NH$_2$), 1752 (COCH$_3$), 1588 (C=N), 1224 (C=S). ^{1}H NMR (300 MHz, DMSO-*d$_6$*, δ, ppm): 1.90, 2.02, 2.06, 2.12(4s, 4x3H, 4-CH$_3$), 4.14 (m, 1H, H-5'), 4.30 (m, 1H, H-6'), 4.51 (dd, 1H, *J* = 4.6 Hz, 12.6 Hz, H-6'a), 5.30 (t, 1H, *J* = 9.5 Hz, H-4'), 5.50 (s, 2H, NH$_2$), 5.67 (t, 1H, *J* = 9.3 Hz, H-3'), 5.89 (t, 1H, *J* = 9.3 Hz, H-2'), 6.15 (d, 1H, *J* = 9.2 Hz, H-1'), 7.49 (dd, 1H, *J* = 5.6, 7.2 Hz, H-5''), 8.25 (m, 1H, H-6''), 8.60 (d, 1H, *J* = 5.0Hz, H-4''), 9.10 (d, 1H, *J* = 1.7 Hz, H-2''). ^{13}C NMR (75 MHz, DMSO-*d$_6$*, δ, ppm): 20.3, 20.6, 20.8, 20.9 (4-CH$_3$), 61.5 (C-6'), 67.6 (C-2'), 69.9 (C-3'), 71.1 (C-4'), 73.2 (C-5'), 83.0 (C-1'), 130.8 (C-5''), 133.5 (C-6''), 136.4 (C-4''), 148.6 (C-2''), 151.4 (C-5), 169.3, 170.0, 170.5, 170.8 (4-CO), 171.3 (C-3). EI-MS (*m/z*, (%)): 523 (M$^+$ 2), 456 (1), 331 (15), 271 (5), 236 (7), 211 (9), 202 (4), 193 (12), 169 (100), 145 (1), 128 (9), 109 (77), 99 (4), 78 (5), 60 (21). Anal. calcd. for C$_{21}$H$_{25}$N$_5$O$_9$S: C, 48.18; H, 4.78; N, 13.38. Found: C, 48.12; H, 4.73; N, 13.35%.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(4-amino-3-(pyridin-4-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3c**): Yield: 69.4%. M.p.: 176 ºC. IR (KBr, ν, cm^{-1}): 3435 (NH$_2$), 1750 (COCH$_3$), 1588 (C=N), 1224 (C=S). ^{1}H NMR (300 MHz, DMSO-*d$_6$*, δ, ppm): 1.89, 2.02, 2.06, 2.12 (4s, 4 x 3H, 4-CH$_3$), 4.02-4.55 (m, 3H, H-5', 6', 6'a), 5.33 (t, 1H, *J* = 9.5 Hz, H-4'), 5.42 (bs, 2H, NH$_2$), 5.69 (t, 1H, *J* = 9.4 Hz, H-3'), 5.87 (t, 1H, *J* = 9.4 Hz, H-2'), 6.17 (d, 1H, *J* = 9.4 Hz, H-1'), 6.68 (d, 2H, *J* = 8.9 Hz, H-2'', 6''), 9.21 (d, 2H, *J* = 8.9 Hz, H-3'', 5''). ^{13}C NMR (75 MHz, DMSO-*d$_6$*, δ, ppm): 20.4, 20.5,20.7,20.9 (4–CH$_3$), 61.2 (C-6'), 67.5 (C-2'), 69.2 (C-3'), 73.4 (C-4'), 74.5 (C-5'), 82.0 (C-1'), 126.1 (C-2'', 6''), 142.6 (C-1''), 143.7 (C-3'', C-5''), 149.2 (C-5), 169.1, 169.3, 169.5, 169.9 (4-CO), 170.5 (C-3). EI-MS (*m/z*, (%)): 523 (M$^+$, 3), 456 (2), 453 (1), 331 (14), 271 (3), 236 (4), 229 (2), 211 (3), 202 (3), 194 (20), 193 (8), 169 (100), 145 (7), 128 (32), 109 (92), 98 (13), 78 (3), 60 (20). Anal. calcd. for C$_{21}$H$_{25}$N$_5$O$_9$S (523): C, 48.18; H, 4.78; N, 13.38. Found: 48.20; H, 4.76; N, 13.32%.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(4-amino-5-thioxo-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3d**): Yield: 62.9%. M.p.: 168-170 ºC. IR (KBr, ν, cm^{-1}): 3468 (NH$_2$), 1749 (COCH$_3$), 1620 (Ar C=C), 1588 (C=N), 1225 (C=S). ^{1}H NMR (300 MHz, DMSO-*d$_6$*, δ, ppm): 1.95, 2.01, 2.05, 2.09 (4s, 4 x 3H, 4-CH$_3$), 2.4 (s, 3H, CH$_3$-Ar), 4.01 (ddd, 1H, *J* = 1.9 Hz, 4.5 Hz, 10.0 Hz, H-5'), 4.14 (dd, 1H, *J* = 1.6 Hz, 12.3 Hz, H-6'), 4.29 (dd, *J* = 4.5, 12.3 Hz, 1H, H-6'a), 4.92 (bs, 2H, NH$_2$), 5.27 (t, 1H, *J* = 9.8 Hz, H-4'), 5.42 (t, *J* = 9.4 Hz, 1H, H-3'), 5.88 (t, *J* = 9.3 Hz, 1H, H-2'), 6.15 (d, 1H, *J* = 9.2 Hz, H-1'), 7.05 (d, 2H, *J* = 8.4 Hz, H-3'', 5''), 8.01 (d, 2H, *J* = 7.9 Hz, H-2'', 6''). ^{13}C NMR (75 MHz, DMSO-*d$_6$*, δ, ppm): 20.4, 20.5, 20.6, 20.8 (4-CH$_3$), 21.3 (CH$_3$-Ar), 61.6 (C-6), 67.6 (C-2), 69.4 (C-3), 73.3 (C-4), 74.5 (C-5), 83.1 (C-1), 121.9 (C-1), 128.4 (C-3, 5), 129.1 (C-2, 6), 137.2 (C-4), 159.6 (C-5), 168.8, 169.5, 170.1, 170.4 (4-CO), 178.0 (C-3). EI-MS (*m/z*, (%)): 535 (M$^+$ 2), 331 (16), 271 (5), 229 (3), 211 (3), 205 (13), 204 (20), 189 (5), 169 (100), 146 (7), 127 (32), 116 (16), 109 (92), 85 (13), 68 (4). Anal. calcd. for C$_{23}$H$_{28}$N$_4$O$_9$S: C, 51.49; H, 5.22; N, 10.44. Found: C, 51.38; H, 5.20; N, 10.50%.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(4-amino-5-thioxo-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3e**): Yield: 59.7%. M.p.: 193 ºC. IR (KBr, ν, cm⁻¹): 3455 (NH₂), 1750 (COCH₃), 1622 (Ar C=C), 1580 (C=N), 1226 (C=S). ¹H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.89, 2.01, 2.04, 2.06 (4s, 4 x 3H , 4-CH₃), 3.81 (s, 3H, OCH₃), 3.85(s, 6H, 2-OCH₃), 4.05 (ddd, 1H, J = 2.1 Hz, 9.9 Hz, H-5'), 4.16 (1H, dd, J = 2.0 Hz, 12.5 Hz, H-6'), 4.32 (1H, dd, J = 4.8, 12.5 Hz, H-6'a), 5.27 (1H, t, J = 9.8 Hz, H-4'), 5.39 (1H, t, J = 9.5 Hz, H-3'), 5.80 (s, 2H, NH₂), 5.89 (t, 1H, J = 9.4 Hz, H-2'), 6.09 (d, 1H, J = 9.2 Hz, H-1'), 7.33 (s, 2H, H-2″, 6″). ¹³C NMR (75 MHz, DMSO-d_6, δ, ppm): 20.46, 20.48, 20.49, 20.66 (4-CH₃), 56.41 (OCH₃), 61.6 (C-6), 67.6 (C-2), 69.3 (C-3), 73.1 (C-4), 74.7 (C-5), 83.0 (C-1), 104.0 (C-2, 6), 116.7 (C-1), 142.0 (C-4), 153.7 (C-3, 5), 159.4 (C-5), 168.9, 169.3, 169.9, 170.4 (4-CO), 177.8 (C-3). EI-MS (*m/z*, (%)): 611 (1), 331 (14), 271 (4), 229 (2), 211 (1), 281 (10), 280 (2), 266 (9), 265 (5), 233 (5), 222 (8), 206 (4), 203 (8), 169 (100), 167 (5), 144 (7), 127 (23), 116 (16), 109 (92). Anal. calcd. for C₂₅H₃₂N₄O₁₂S: C, 49.01; H, 5.22; N, 9.15. Found: C, 48.92; H, 5.15; N, 9.08%.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(4-amino-3-(4-fluoro-phenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3f**): Yield: 48.6%. M.p.: 173-174 ºC. IR (KBr, ν, cm⁻¹): 3420 (NH₂), 1750 (COCH₃), 1620 (Ar C=C), 1554 (C=N), 1230 (C=S). ¹H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.89, 2.02, 2.04, 2.06 (4s, 4 x 3H, 4-CH₃), 4.01 (m, 1H, H-5'), 4.19 (dd, 1H, J = 1.8, 12.6 Hz, H-6'), 4.30 (m, 1H, H-6'a), 4.90 (s, 2H, NH₂), 5.25 (t, 1H, J = 9.3 Hz, H-4'), 5.40 (t, 1H, J = 9.4 Hz, H-3'), 5.88 (t, 1H, J = 9.3 Hz, H-2'), 6.09 (d, 1H, J = 9.2 Hz, H-1'), 7.50 (d, 2H, J = 7.8 Hz, H-3″, 5″), 8.05 (d, 2H, J = 7.8 Hz, H- 2″, 6″). ¹³C NMR (75 MHz, DMSO-d_6, δ, ppm): 20.4, 20.5, 20.6, 20.7 (4-CH₃), 61.5 (C-6), 67.6 (C-2), 69.2 (C-3), 73.2 (C-4), 74.7 (C-5), 83.0 (C-1), 116.5 (C-3, 5), 129.2 (C-2, 6), 158.7 (C-5), 164.0 (C-4), 168.8, 169.3, 169.9, 170.4 (4-CO), 177.8 (C-3). EI-MS (*m/z*, (%)): 540 (3), 331 (29), 271 (6), 211 (4), 229 (13), 187 (1), 169 (100), 209 (5), 210 (7), 138 (10), 151 (5), 128 (46), 111 (2), 95 (1), 75 (3). Anal. calcd. for C₂₂H₂₅N₄O₉SF: C, 48.88; H, 4.62; N, 10.37. Found: C, 48.20; H, 4.59; N, 10.08%.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(4-amino-3-(4-chloro-phenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3g**): Yield: 70.8%. M.p.: 182 ºC. IR (KBr, ν, cm⁻¹): 3420 (NH₂), 1748 (COCH₃), 1592 (Ar C=C), 1549 (C=N), 1226 (C=S). ¹H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.88, 2.01, 2.03, 2.04 (4s, 4 x 3H, 4–CH₃), 3.97 (m, 1H, H-5'), 4.13 (dd, 1H, J = 1.1 Hz, 12.3 Hz, H-6'), 4.27 (dd, 1H, J = 4.8 Hz, 12.5 Hz, H-6'a), 4.83 (bs, 2H, NH₂), 5.23 (t, 1H, J = 9.7 Hz, H-4'), 5.39 (t, 1H, J = 9.4 Hz, H-3'), 5.85 (t, 1H, J = 9.4 Hz, H-2'), 6.10 (d, 1H, J=9.3 Hz, H-1'), 7.43 (d, 2H, J = 8.5 Hz, H-3″, 5″), 8.08 (d, 2H, J = 8.5 Hz, H-2″, 6″). ¹³C NMR (75 MHz, DMSO-d_6, δ, ppm): 20.2, 20.4, 20.5, 21.1 (4-CH₃),62.2 (C-6), 68.2 (C-2), 69.5 (C-3'), 74.2 (C-4'), 75.1 (C-5'), 83.5 (C-1'), 123.7 (C-1″), 129.6 (C-2″, 6″), 130.3 (C-3,″ 5″), 138.2 (C-4″), 149.2,(C-5), 169.5, 170.0, 170.8 (4-CO), 171.3 (C-3). EI-MS (*m/z*, (%)): 556 (1), 331 (24), 269 (7), 229 (13), 227 (27), 226 (12), 211 (4), 169 (100), 155 (2), 145 (6), 127 (21), 109 (64), 99 (2). Anal. calcd. for C₂₂H₂₅N₄O₉SCl: C, 46.68; H, 4.42; N, 9.90. Found: C, 46.34; H, 4.40; N, 9.78%.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(4-amino-3-(4-bromo phenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3h**): Yield: 72.4%. M.p.: 180 ºC. IR (KBr, ν, cm⁻¹): 3477 (NH₂), 1752 (COCH₃), 1622 (Ar C=C), 1555 (C=N), 1223 (C=S). ¹H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.93, 2.04, 2.08, 2.10 (4s, 4 x 3H, 4-CH₃), 4.05 (ddd, 1H, J = 2.1 Hz, 4.5 Hz, 10.2 Hz, H-5'), 4.16 (dd, 1H, J = 1.8 Hz, 12.9 Hz, H-6'), 4.31 (dd, 1H, J = 5.1 Hz, 12.9 Hz, H-6'a), 4.97 (s, 2H, NH₂), 5.28 (t, 1H, J = 9.9 Hz, H-4'), 5.44 (t, 1H, J = 9.6 Hz, H-3'), 5.90 (t, 1H, J = 9.3 Hz, H-2'), 6.17 (d, 1H, J = 9.3 Hz, H-1'), 7.63 (d, 2H, J = 8.4 Hz, H 3″, 5″), 8.05 (d, 2H, J = 8.4 Hz, H-2″, 6″). ¹³C NMR (75 MHz, DMSO-d_6, δ, ppm): 20.4, 20.5, 20.61, 20.65 (4-CH₃), 61.5(C-6'), 67.6 (C-2'), 68.8 (C-3'), 73.5 (C-4'), 74.7 (C-5'), 83.0 (C-1'), 123.3 (C-1″), 126.1 (C-4″), 129.9 (C-2″, 6″), 132.0 (C-3″,5″), 149.0 (C-5),

168.9, 169.4, 169.8, 170.1 (4-CO), 170.6 (C-3). EI-MS (*m/z*, (%)): 601 (3), 331 (8), 271 (10), 211 (2), 229 (3), 199 (4), 212 (2), 156 (7), 169 (100), 127 (90), 145 (1), 109 (60), 99 (3), 75 (2). Anal. calcd. for C₂₂H₂₅N₄O₉SBr: C, 43.92; H, 4.15; N, 9.31. Found: C, 43.46; H, 4.10; N, 9.28%.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(4-amino-3-(naphthalen-2-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3i**): Yield: 72.1%. M.p.: 197 ºC. IR (KBr, ν, cm⁻¹): 3423 (NH₂), 1748 (COCH₃), 1624 (Ar C=C), 1533 (C=N), 1228 (C=S). ¹H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.94, 1.97, 2.06, 2.09 (4s, 4 x 3H, 4CH₃), 4.07 (ddd, 1H, J = 2.1 Hz, 4.8 Hz, 10.1 Hz, H-5'), 4.19 (dd, 1H, J = 2.4 Hz, 12.6 Hz, H -6'), 4.33 (dd, 1H, J = 5.1 Hz, 12.6 Hz, H-6'a), 4.99 (s, 2H, NH₂), 5.31 (t, 1H, J = 9.9 Hz, H- 4'), 5.46 (t, 1H, J = 9.3 Hz, H-3'), 5.99 (t, 1H, J = 9.3 Hz, H-2'), 6.19 (d, 1H, J = 9.6 Hz, H-1'), 7.59 (m, 2H, Naphthyl-H⁺), 7.93 (m, 3H, Naphthyl-H), 8.12 (dd, 1H, J = 1.3 Hz, 8.7 Hz, H-8″), 8.80 (s, 1H, H-1″). ¹³C NMR (75 MHz, DMSO-d_6, δ, ppm): 20.4, 20.5, 20.6, 20.7 (4-CH₃), 61.6 (C-6'), 67.8 (C-2'), 69.0 (C-3'), 73.7 (C-4'), 74.5 (C-5'), 83.0 (C-1'), 124.6(C-3″), 127.9 (C-2″, 10″), 128.4 (C-5'), 126.8 (C-7″), 128.9 (C -8″), 129.0 (C-6'), 132.5 (C-1″), 134.3 (C-4″, 9″), 149.4 (C-5), 168.8, 169.3, 170.1, 170.3 (4-CO), 170.6 (C-3). EI-MS (*m/z*, (%)): 572 (M⁺,6), 512 (3), 469 (2), 331 (16), 271 (4), 288 (9), 242 (5), 229 (2), 226 (4), 211 (4), 194 (1), 169 (100), 153 (8), 127 (33), 126 (4), 139 (2), 109 (73), 103 (1). Anal. calcd. for C₂₆H₂₈N₄O₉S: C, 54.54; H, 4.89; N, 9.79. Found: C, 53.98; H, 4.59; N, 9.60%.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(4-amino-3-(naphthalen-1-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**3j**): Yield: 69.2%. M.p.: 189 ºC. IR (KBr, ν, cm⁻¹): 3425 (NH₂), 1750 (COCH₃), 1622 (Ar C=C), 1552 (C=N), 1232 (C=S). ¹H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.95, 1.98, 2.02, 2.07 (4s, 4 x 3H, 4CH₃), 4.02 (ddd, 1H, J = 2.1 Hz, 4.8 Hz, 10.1 Hz, H-5'), 4.15 (dd, 1H, J = 2.1 Hz, 12.6 Hz, H-6'), 4.29 (dd, 1H, J = 4.8 Hz, 12.6 Hz, H-6'a), 5.03 (s, 2H, NH₂), 5.37 (t, 1H, J = 7.6 Hz, H- 4'), 5.45 (t, 1H, J = 9.6 Hz, H-3'), 5.85 (t, 1H, J = 9.4 Hz, H-2'), 6.12 (d, 1H, J = 9.2 Hz, H-1'), 7.60 (m, 4H, Naphthyl H), 8.01 (d, 1H, J = 7.2 Hz, H-4'), 8.17 (d, 1H, J = 8.0 Hz, H-8″), 8.80 (d, 1H, J = 8.1 Hz, H-2″). ¹³C NMR (75 MHz, DMSO-d_6, δ, ppm): 20.2, 20.4, 20.6, 20.7 (4-CH₃), 60.9 (C–6/), 65.8 (C-2'), 68.3 (C-3'), 71.7 (C-4'), 73.5 (C-5'), 82.0 (C-1'), 124.6(C-3″), 128.5 (C-1″), 128.4 (C-5″), 126.8 (C-7″), 127.9 (C-2″, 10″), 128.9 (C-8″), 129.0 (C-6″), 134.3 (C-4″, 9″), 149.4 (C-5), 168.8, 169.3, 170.1, 170.3 (4-CO), 170.6 (C-3). EI-MS (*m/z*, (%)): 572 (M⁺, 3), 512 (1), 469 (2), 331 (19), 288 (3), 271 (6), 242 (4), 229 (3), 211 (7), 194 (3), 169 (100), 153 (16), 127 (92), 126 (62), 109 (85), 103 (2). Anal. calcd. for C₂₆H₂₈N₄O₉S: C, 54.54; H, 4.89; N, 9.79. Found: C, 54.68; H, 4.75; N, 9.72%.

2.3. Synthesis of deprotected nucleosides (4a-j)

General procedure: Each of the protected nucleoside (1 mmole) was dissolved in dry methanol and a fairly rapid stream of dry ammonia was passed into the solution for 3-4 hours. During this period the reaction mixture was kept stirred. The completion of reaction was checked by TLC. The solution was kept for 24 hours at 0-4 ºC. Removal of the solvent in vacuo yielded a sirup which was dissolved in dry ethanol and recrystallised by adding a few drops of ethyl acetate or the product was purified by column chromatography (silica gel (230-400 mesh); chloroform:methanol, 3:1) to get pure products (Scheme 1).

4-Amino-3-(pyridin-2-yl)-1-((3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole-5(4H)-thione (**4a**): Yield: 46.6%. M.p.: 142 ºC (Decomp.). IR (KBr, ν, cm⁻¹): 3410 (NH₂), 2970-3100 (br peak OH), 1586 (C=N), 1225 (C=S). ¹H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.78-4.08 (m, 3H, H-5′, H-6′/6′a), 4.58 (d, 1H, J = 8.5 Hz, H-3′), 4.62 (m, 1H, H-4′), 5.02 (d, 1H, J = 9.1 Hz, H-2′), 5.43 (s, 2H, NH₂), 5.51 (bs, 4H, 4-OH), 6.02 (d, 1H, J = 9.0 Hz, H-1′), 7.52 (d, 1H, J = 7.5 Hz, H-4″), 7.75 (m, 1H, H-5″), 7.95 (d, 1H, J = 7.6 Hz, H-6″), 8.20 (s, 1H, H-3″). FAB-MS (*m/z*, (%)): 355 ((M⁺ + H, 30), 193

(67). Anal. calcd. for $C_{13}H_{17}N_5O_5S$: C, 43.94; H, 4.78; N, 19.71. Found: C, 43.85; H, 4.65; N, 19.80%.

4-Amino-3-(pyridin-3-yl)-1-((3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole-5(4H)-thione (**4b**): Yield: 41.3%. M.p.: 142 °C (Decomp.). IR (KBr, ν, cm^{-1}): 3372 (NH$_2$), 2980-3055 (br peak OH), 1584 (C=N), 1228 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.98-4.18 (m, 3H, H-5', H-6'/6'a), 4.52 (m, 1H, H-4'), 4.78 (d, 1H, *J* = 8.5 Hz, H-3'), 4.98 (d, 1H, *J* = 9.1 Hz, H-2'), 5.49 (s, 2H, NH$_2$), 5.30 (bs, 4H, 4-OH), 5.95 (d, 1H, *J* = 9.0 Hz, H-1'), 7.43 (m, 1H, H-5''), 7.65 (d, 1H, *J* = 7.6 Hz, H-6''), 7.91 (s, 1H, H-2''), 8.09 (d, 1H, 7.5 Hz, H-4''). FAB-MS (*m/z*, (%)): 355 (M$^+$ + H, 17), 193 (100). Anal. calcd. for $C_{13}H_{17}N_5O_5S$: C, 43.94; H, 4.78; N, 19.71. Found: C, 43.89; H, 4.69; N, 19.71%.

4-Amino-3-(pyridin-4-yl)-1-((3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole-5(4H)-thione (**4c**): Yield: 49.1%. M.p.: 161-163 °C (Decomp.). IR (KBr, ν, cm^{-1}): 3410 (NH$_2$), 2980-3254 (br peak OH), 1584 (C=N), 1224 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.76-3.84 (m, 3H, H-5', H-6'/6'a), 4.24 (d, 1H, *J* = 7.8 Hz, H-4'), 4.52 (d, 1H, *J* = 6.5, 8.1 Hz, H-3'), 4.98 (bs, 2H, NH$_2$), 5.41 (bs, 4H, 4-OH), 5.83 (d, 1H, *J* = 8.6 Hz, H-2'), 6.12 (d, 1H, *J* = 8.3 Hz, H-1'), 7.38 (dd, 2H, *J* = 2.1 Hz, 7.8 Hz, H-2'', 6''), 8.29 (dd, 2H, *J* = 2.3 Hz, 7.6 Hz, H-3'', 5''). FAB-MS (*m/z*, (%)): 355 (M$^+$ + H, 60), 193 (88). Anal. calcd. for $C_{13}H_{17}N_5O_5S$: C, 43.94; H, 4.78; N, 19.71. Found: C, 43.87; H, 4.75; N, 19.66%.

4-Amino-3-(p-tolyl)-1-((3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole-5(4H)-thione (**4d**): Yield: 48.5%. M.p.: 177-179 °C (Decomp.). IR (KBr, ν, cm^{-1}): 3398 (NH$_2$), 2980-3054 (br peak OH), 1554 (C=N), 1228 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.3 (s, 3H, CH$_3$), 3.80-4.05 (m, 3H, H-5', H-6'/6'a), 4.49 (dd, 1H, *J* = 6.5 Hz, 8.1 Hz, H-3'), 4.64 (d, 1H, *J* = 7.8 Hz, H-4'), 4.98 (bs, 2H, NH$_2$), 5.33 (bs, 4H, 4-OH), 5.69 (d, 1H, *J* = 8.6 Hz, H-2'), 6.12 (d, 1H, *J* = 8.3 Hz, H-1'), 7.28-7.34 (m, 4H, Ar-H). FAB-MS (*m/z*, (%)): 369 (M$^+$ + H, 26), 207 (100). Anal. calcd. for $C_{15}H_{20}N_4O_5S$: C, 48.91; H, 5.43; N, 15.21. Found: C, 48.87; H, 5.41; N, 15.06%.

4-Amino-1-((3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxyl methyl)tetrahydro-2H-pyran-2-yl)-3-(3,4,5-trimethoxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (**4e**): Yield: 43.5%. M.p.: 142-145 °C (Decomp.). IR (KBr, ν, cm^{-1}): 3408 (NH$_2$), 2980-3024 (br peak OH), 1556 (C=N), 1225 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.92 (s, 9H, 3 x OCH$_3$), 3.73-3.80 (m, 3H, H-5', H-6'/6'a), 4.32 (d, 1H, *J* =, 8.1 Hz, H-4'), 4.41 (m, 1H, H-3'), 4.89 (d, 1H, *J* = 8.5 Hz, H-2'), 5.10 (bs, 2H, NH$_2$), 5.47 (bs, 4H, 4-OH), 5.98 (d, 1H, *J* = 8.6 Hz, H-1'), 6.95 (s, 2H, H-2'',6''). FAB-MS (*m/z*, (%)): 445 ((M$^+$ + H, 76), 283 (94). Anal. calcd. for $C_{17}H_{24}N_4O_8S$: C, 45.94; H, 5.40; N, 12.6. Found: C, 45.87; H, 5.38; N, 12.45%.

4-Amino-3-(4-fluorophenyl)-1-((3R,4S,5S,6R)-3,4,5-tri-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole-5(4H)-thione (**4f**): Yield: 50.2%. M.p.: 145-147 °C (Decomp.). IR (KBr, ν, cm^{-1}): 3879 (NH$_2$), 2960-3150 (br peak OH), 1588 (C=N), 1227 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.70-3.98 (m, 3H, H-5', H-6'/6'a), 4.52 (d, 1H, *J* = 7.8 Hz, H-4'), 4.75 (m, 1H, H-3'), 5.09 (d, 1H, *J* = 8.3 Hz, H-2'), 5.13 (bs, 2H, NH$_2$), 5.32 (bs, 4H, 4-OH), 5.94 (d, 1H, *J* = 8.4 Hz, H-1'), 7.18-7.38 (m, 4H, Ar-H). FAB-MS (*m/z*, (%)): 373 (M$^+$ + H, 36), 211 (77). Anal. calcd. for $C_{14}H_{17}N_4O_5SF$: C, 45.16; H, 4.56; N, 15.06. Found: C, 45.07; H, 4.52; N, 15.03%.

4-Amino-3-(4-chlorophenyl)-1-((3R,4S,5S,6R)-3,4,5-tri-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole-5(4H)-thione (**4g**): Yield: 48.2%. M.p.: 150-153°C (Decomp.). IR (KBr, ν, cm^{-1}): 3409 (NH$_2$), 2960-3250 (br peak OH), 1585 (C=N), 1225 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.95-4.03 (m, 3H, H-5', H-6'/6'a), 4.51 (d, 1H, *J* = 7.6 Hz, H-4'), 4.65 (d, 1H, *J* = 8.5 Hz, H-3'), 5.05 (bs, 2H, NH$_2$), 5.29 (d, 1H, *J* = 8.1 Hz, H-2'), 5.41 (bs, 4H, 4-OH), 5.98 (d, 1H, *J* = 8.2 Hz, H-1'), 7.12-7.26 (m, 4H, Ar-H). FAB-MS (*m/z*, (%)): 388 (M$^+$ + H, 46), 226 (100). Anal. calcd. for $C_{14}H_{17}N_4O_5SCl$: C, 43.24; H, 4.37; N, 14.41. Found: C, 43.20; H, 4.30; N, 14.50%.

4-Amino-3-(4-bromophenyl)-1-((3R,4S,5S,6R)-3,4,5-tri-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole-5(4H)-thione (**4h**): Yield: 51.2%. M.p.: 153-156 °C (Decomp.). IR (KBr, ν, cm^{-1}): 3408 (NH$_2$), 2960-3252 (br peak OH), 1580 (C=N), 1224 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.70-3.86 (m, 3H, H-5', H-6'/6'a), 4.20 (d, 1H, *J* = 7.2 Hz, H-4'), 4.48 (m, 1H, H-3'), 4.90 (s, 2H, NH$_2$), 5.39 (bs, 4H, 4-OH), 5.52 (d, 1H, *J* = 8.9 Hz, H-2'), 6.20 (d, 1H, *J* = 9.0 Hz, H-1'), 7.19-7.32 (m, 4H, Ar-H). FAB-MS (*m/z*, (%)): 433 (M$^+$ + H, 21), 271 (56). Anal. calcd. for $C_{14}H_{17}N_4O_5SBr$: C, 38.79; H, 3.92; N, 12.93. Found: C, 38.75; H, 3.95; N, 3.95%.

4-Amino-3-(naphthalen-2-yl)-1-((3R,4S,5S,6R)-3,4,5-tri-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole-5(4H)-thione (**4i**): Yield: 47.8%. M.p.: 150-151 °C (Decomp.). IR (KBr, ν, cm^{-1}): 3418 (NH$_2$), 2908-3272 (br peak OH), 1580 (C=N), 1240 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.67-3.85 (m, 3H, 3H, H-5', H-6', H-6'a), 4.35 (m, 1H, H-4'), 4.58 (d, 1H, *J* = 7.8 Hz, H-3'), 4.96 (s, 2H, NH$_2$), 5.38 (d, 1H, *J* = 8.0 Hz, H-2'), 5.43 (bs, 4H, 4-OH), 5.91 (d, 1H, *J* = 8.0 Hz, H-1'), 7.80-8.21 (m, 7H, Naphthyl-H). FAB-MS (*m/z*, (%)): 405 (M$^+$ +H, 32), 243 (68). Anal. calcd. for $C_{18}H_{20}N_4O_5S$: C, 53.46; H, 4.95; N, 13.86. Found: C, 53.51; H, 3.91; N, 13.84%.

4-Amino-3-(naphthalen-1-yl)-1-((3R,4S,5S,6R)-3,4,5-tri-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole-5(4H)-thione (**4j**): Yield: 53.8%. M.p.: 156 °C (Decomp.). IR (KBr, ν, cm^{-1}): 3409 (NH$_2$), 2932-3268 (br peak OH), 1585 (C=N), 1238 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.73-3.95 (m, 3H, H-5', H-6', H-6'a), 4.42 (m, 1H, H-4'), 4.60 (d, 1H, *J* = 7.5 Hz, H-3'), 5.01 (s, 2H, NH$_2$), 5.28 (d, 1H, *J* = 8.4 Hz, H-2'), 5.35 (bs, 4H, 4-OH), 5.78 (d, 1H, *J* = 8.5 Hz, H-1'), 7.85-8.20 (m, 7H, Naphthyl-H). FAB-MS, *m/z* (%): 405 (M$^+$ + H, 26), 243 (83). Anal. calcd. for $C_{18}H_{20}N_4O_5S$: C, 53.46; H, 4.95; N, 13.86. Found: C, 53.45; H, 3.90; N, 13.82%.

2.4. Antibacterial activity

In vitro evaluation of antibacterial activity of the 4-amino-5-substituted-1,2,4-triazole-3-thiones (**2a-j**) and there corresponding protected (**3a-j**) and deprotected nucleosides (**4a-j**) was carried out by agar well diffusion assay against ten different Gram positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) and Gram negative (*Proteus mirabilis, Escherichia coli, Pseudomonas putida, Pseudomonas aeruginosa, Salmonalla typhi, Shigella flexineri* and *Klebsiella pneumoniae*) bacteria [19]. Antibacterial activity was determined by using the Mueller Hinton Agar (MHA). The fresh inoculums of these bacteria were prepared and diluted by sterilized normal saline. The turbidity of these cultures was adjusted by using 0.5 Mc-Farland. A homogeneous bacterial lawn was developed by sterile cotton swabs. The inoculated plates were bored by 6 mm sized borer to make the wells. The sample dilutions were prepared by dissolving each sample (1.0 mg) in 1.0 mL of DMSO used as negative control in this bioassay. The equimolar concentration of Levofloxacin (1.0 mg/mL), a broad spectrum antibiotic (positive control) was prepared. These plates were incubated at 37 °C for 24 hours. Antibacterial activity of these three series of compounds was determined by measuring the diameter of zone of inhibition (mm, ± standard deviation) and presented by subtracting the activity of the negative control.

3. Results and discussion

3.1. Synthesis

Ten 5-substituted-4-amino-1,2,4-triazole-3-thiones (**2a-g**) were synthesized by the reaction of either potassium salts of oxadiazoles or the corresponding oxadiazoles with hydrazine hydrate.
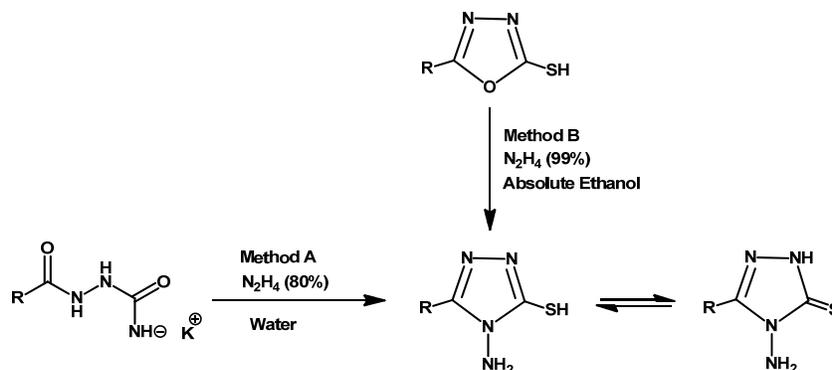
Scheme 2

Two different reaction routes are followed for the synthesis of said compounds (Scheme 1 and 2). The yield in case of hydrazine hydrate (99%) with oxadiazoles (Method B) was good as compared to Method A.

Most of the methods reported for the preparations of nucleosides afforded the mixture of α and β-glycosides. But the method adopted for the synthesis of target nucleosides furnished only β-isomer. The stereochemistry of the final products was confirmed by 1,2-diaxial coupling constants in ^{1}H NMR spectra. The reaction was found to be quite convenient giving moderate to high yields. Each one of the synthesized triazoles was subjected to an identification test with ninhydrin solution and appearance of yellow colour gave the indication of 4-aminotriazole. The structures of the synthesized amino triazoles (**2a-j**) were confirmed by spectroscopic data. A broad peak appeared at 3330-3430 cm^{-1} indicated the presence of -NH$_2$ group in the molecule. The absorption at 1642-1630 cm^{-1} was attributed to C=N and C=S stretching vibrations were observed at 1218-1268 cm^{-1}.

In ^{1}H NMR a singlet appeared at 5.43-5.81 ppm integrating to 2H for -NH$_2$ protons. In the pyridyl bearing triazoles (**2a-c**), the pyridyl protons resonated as expected for these protons e.g. H-5'' exhibited a doublet of a doublet in the region 7.37-7.83 ppm. In compounds (**2f-h**), the aromatic protons H-2'', 6'' resonated downfield at 7.93-8.05 ppm as doublet due to electron withdrawing effects of C=N group while doublet for H-3'', 5'' was observed at 7.20-7.63 ppm due to electron donating effect of halogen atoms. In all the amino triazoles (**2a-j**), the molecular ion peak was the base peak with 100% intensity. The fragment ions are also in good agreement with the structures.

The substituted aminotriazoles (**2a-j**) were coupled with acetylated α-bromoglucose in presence of mercuric cyanide and molecular sieves using nitromethane as solvent to afford the corresponding nucleosides (**3a-j**). The IR spectra of nucleosides (**3a-j**) exhibited a band at 1745-1752 cm^{-1} for the carbonyl stretching of acetyl groups and the NH$_2$ group stretchings vibration observed in the region 3310-3432 cm^{-1}. The protons of acetyl groups appeared as singlets in the region 1.88-2.12 ppm in ^{1}H NMR spectra of amino triazole nucleosides (**3a-j**). The anomeric proton resonated as doublet in the range 6.09-6.19 ppm with a coupling constant of 9-10 Hz which clearly indicates the diaxial orientation of H-1' and H-2' confirming the β-configuration of these acetylated nucleosides. The triplets for H-2' were observed in the region 5.85-5.99 ppm. In all the compounds (**3a-j**), a singlet of two protons was observed in the region 4.83-5.50, corresponding to the protons of amino group.

In ^{13}C NMR spectra of compounds (**3a-j**) the methyl carbon atoms of acetyl groups resonated in the region 20.2-21.2 ppm. The carbonyl carbon atoms resonated downfield at 168.8-170.8 ppm. The anomeric carbon atom in all these compounds

appeared at 82.0-83.5 ppm indicating its linkage to nitrogen atom of heterocyclic moiety. The C-3 carbon (C=S) resonated downfield at 170.5-171.3 ppm. Mass spectral data of these compounds further confirmed the structure of the compounds. In the mass spectra of compound (**3a-j**), the molecular ion peaks appeared with low intensities (1-6%). In compounds (**3a-c**), the loss of heterocyclic moiety from the molecular ion yielded a peak at *m/z* 331, and the successive loss of CH$_3$COOH and CH$_2$=C=O molecules from this peak furnished the base peak at *m/z* 169. The loss of sugar molecule from the molecular ion yielded the stable triazole cation radical at *m/z* 193.

Acetylated 4-amino-5-substituted-1,2,4-triazole glucosides (**3a-j**) were deprotected successfully using methanolic ammonia giving *N*-(β-*D*-Glucopyranosyl)-4-amino-5-substituted-1,2,4-triazole-3-thiones (**4a-j**). The deacetylation of nucleosides was confirmed by FT-IR, ^{1}H NMR, FAB-MS and elemental analysis data for these compounds.

The FT-IR spectra showed no peak for carbonyl stretching indicating complete deaceylation of acetylated nucleosides. The N-H stretching vibrations of NH$_2$ group were observed at 3100-3410 cm^{-1}. Also a peak for C=S stretchings was found at 1224-1225 cm^{-1}. The absence of methyl protons signal of acetyl groups in ^{1}H NMR spectra of deprotected nucleosides (**4a-j**) confirm the complete removal of acetyl groups. Anomeric proton H-1' also resonated as doublet with coupling constant of 8-9 Hz, which indicated diaxial orientation of H-1' and H-2' and confirmed β-configuration of these nucleosides. The ^{13}C NMR spectral data also supported the deacetylation of nucleosides (**4a-j**). No signals for methyl carbon atoms and carbonyl carbon atoms of acetyl groups were observed. The remaining sugar carbon atoms resonated in the range 61.9-87.2 ppm, similar to acetylated glucosides. The elemental analysis data also proved the deacetylation of blocked nucleosides. This data showed good agreement between the calculated and found percentages of carbon, hydrogen and nitrogen for the deacetylated products.

3.2. Antimicrobial bioassay

Biological activity of a compound is a function of the nature and extent of interactions between the target site and functional groups of the compound. A range of structural features present in the molecule like electronegativity and hydrophobicity are the important determining factors. Molecules with high HOMO (highest occupied molecular orbital) energies are more proficient to donate their electrons and are good hydrogen bond donors than molecules with low-lying HOMOs; thus HOMO is a measure of the ability to develop hydrogen bonding with target site. However, structure activity relationship cannot be established on the basis of single structural feature.

Table 1. Anti-bacterial activity results of aminotriazoles (**2a-j**) *.

Compound	Anti-bacterial activity, mm									
	Proteus mirabilis	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas putida*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Micrococcus luteus*	*Shigella flexneri*	*Klebsiella pneumoniae*
2a	10	12	-	13	06	-	-	12	-	-
2b	-	10	11	17	-	06	08	13	04	07
2c	05	19	07	15	-	-	-	15	-	-
2d	-	09	-	11	11	-	-	11	07	11
2e	11	18	-	22	-	08	-	21	-	-
2f	-	17	-	20	-	-	05	20	-	-
2g	-	15	06	18	10	-	-	17	10	08
2h	-	14	-	16	-	05	-	16	-	-
2i	08	08	06	10	10	-	-	09	11	-
2j	-	11	-	07	05	09	-	10	-	-
Standard	30	20	30	25	30	28	30	25	30	25

* Standard= Levofloxacin; (-) No activity.

Table 2. Anti-bacterial activity results of acetylated *N*-nucleosides (**3a-j**) *.

Compound	Anti-bacterial activity, mm									
	Proteus mirabilis	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas putida*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Micrococcus luteus*	*Shigella flexneri*	*Klebsiella pneumoniae*
3a	19	-	18	-	16	12	19	-	16	10
3b	23	08	21	07	18	15	21	04	20	12
3c	27	-	25	-	26	19	25	-	24	17
3d	-	-	08	11	-	-	-	07	05	-
3e	02	-	-	-	-	-	-	-	-	-
3f	16	05	14	-	10	10	05	-	15	12
3g	18	-	19	08	13	13	10	10	17	18
3h	20	-	22	-	17	16	18	-	21	21
3i	10	07	-	-	-	-	-	11	-	-
3j	07	-	05	-	-	06	-	-	05	-
Standard	30	20	30	25	30	28	30	25	30	25

* Standard= Levofloxacin; (-) No activity.

Table 3. Anti-bacterial activity results of deacetylated *N*-nucleosides (**4a-j**) *.

Compound	Anti-bacterial activity, mm									
	Proteus mirabilis	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas putida*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Micrococcus luteus*	*Shigella flexneri*	*Klebsiella pneumoniae*
4a	19	-	20	-	16	12	19	-	16	10
4b	21	08	23	07	18	15	21	04	20	12
4c	24	-	26	-	26	19	25	-	24	17
4d	-	-	-	11	-	-	-	07	05	-
4e	28	18	27	22	25	22	27	22	26	20
4f	-	16	04	18	10	10	05	17	15	-
4g	-	18	-	20	13	13	10	19	17	08
4h	-	20	-	22	17	16	18	21	21	-
4i	10	07	-	-	-	-	-	11	-	-
4j	07	-	05	-	-	06	-	-	05	-
Standard	30	20	30	25	30	28	30	25	30	25

* Standard= Levofloxacin; (-) No activity.

3.3. Antibacterial activity

The antibacterial activity results revealed that 4-amino-5-substituted-1,2,4-triazole-3-thiones (**2a-j**) exhibited moderate to potent bacterial growth inhibition against gram positive bacterial strains but most of them are inactive against gram negative strains (Table 1). It was also found that anti-bacterial activity was dependant on the type and position of the substituent present on 5-aromatic ring. 4-Amino-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole-3-thiones (**2e**) is the most active among this series. The compounds which possess halogen substituted 5-phenyl ring are more active than methyl substituted and also naphthyl. The antibacterial activity of the halogen substituted analogues is directly related to the electronegativity of the halogens. 4-Amino-5-(4-flourophenyl)-1,2,4-triazole-3-thiones (**2f**) exhibited excellent bacterial growth inhibition than chloro substituted and bromo substituted 5-phenyl ring. It may be due to the greater electronegativity of halogen make the carbon-halogen bond more polar which help in receptor binding.

The acetylated nucleoside analogues (**3a-j**) of 4-amino-5-substituted-1,2,4-triazole-3-thiones have high potential towards bacterial growth inhibition than 4-amino-5-substituted-1,2,4-triazole-3-thiones (**2a-j**). The protected nucleosides (**3a-j**) unlike aminotriazoles (**2a-j**) are more active against gram negative bacteria than gram positive (Table 2). The acetylated nucleosides which possess 5-pyridyl ring (**3a-c**) showed potent antibacterial activity and the compounds having halogen substituted 5-phenyl ring (**3f-h**) also portray good bacterial growth inhibition. In these compounds the protected sugar moiety and heterocyclic pyridine ring is critical for antibacterial activity against gram negative microorganisms. The nucleoside (**3c**) possess 4-pyridyl ring is most active against tested gram negative bacteria than compound **3a** and **3b** having 2-pyridine and 3-pyridine ring respectively. The nucleoside (**3c**) actually have less sterically crowded pyridine ring than compounds (**3a**) and (**3b**) which play important role in antibacterial activity. The activity of the halogen substituted nucleosides can again be correlated to the electronegativity of the halogens i.e. greater the electronegativity of the halogen higher will be the antibacterial activity.

The deprotection of the acetylated nucleosides (**3a-j**) results in increase of the antimicrobial activity. Some of these deprotected nucleosides (**4a-j**) represented excellent antibacterial activity results against both gram positive and gram negative bacteria (Table 3). The compounds (**4a-c**) possess 5-pyridyl ring are more effective against gram negative strains than gram positive but the nucleosides (**4f-h**) having halogen substituted 5-phenyl ring exhibited high potential against gram positive than gram negative bacteria. The most

active deprotected nucleoside in this series is (**4e**) which possess trimethoxy substituted 5-phenyl ring. The increase in antibacterial activity after deprotection is because of the presence of free hydroxyl groups which may involve in formation of hydrogen bonding with the polar receptor functionalities.

4. Conclusion

Two different reaction routes are adopted for the preparation of 4-amino-5-substituted-1,2,4-triazole-5-thiones. The method in which absolute ethanol was used along with hydrazine furnished the desired products with excellent yield compared to the other method. The glycosides of 4-amino-5-substituted-1,2,4-triazole-5-thiones (**2a-j**) are formed region-specifically as the reaction give only *N*-glycosides not *S*-glycosides. The aminotriazloes (**2a-j**) exhibited high anti-bacterial activity against gram positive than gram negative but the protected nucleosides (**3a-j**) are more active against gram negative than gram positive bacteria. The deprotected nucleosides (**4a-j**) in general showed high antimicrobial activity than 4-amino-5-substituted-1,2,4-triazole-5-thiones (**2a-j**) and protected nucleosides (**3a-j**). The hydrophobicity of 5-aryl ring system and electronegativity of the halogens play important role in antimicrobial activity.

References

[1]. Silvia, S.; Chiara, B.; Olga, B.; Francesco, B.; Angelo, R.; Walter, F.; Barbara, R.; Annalisa, C.; Giuseppe, F. *Bioorg. Med. Chem.* **2006**, *14*, 1698-1705.
[2]. Kucukguzel, S. G.; Kucukguzel, I.; Tatar, E.; Rollas, S.; Sahin, F.; Gulluce, M.; DeClercq, E.; Kabasakal, L. *Eur. J. Med. Chem.* **2007**, *42*, 893-901.
[3]. Heindel, N. D.; Reid, J. R. *J. Heterocycl. Chem.* **1980**, *17*, 1087-1088.
[4]. Holla, B. S.; Kalluraya, B.; Sridhar, K. R.; Drake, E.; Thomas, L. M.; Bhandary, K. K.; Levine, M. *Eur. J. Med. Chem.* **1994**, *29*, 301-308.
[5]. Mathew, V.; Keshavayya, J.; Vaidya, V. P. *Eur. J. Med. Chem.* **2006**, *41*, 1048-1058.
[6]. Mathew, V.; Giles, D.; Keshavayya, J.; Vaidya, V. P. *Arch. Pharm.* **2009**, *342*, 210-222.
[7]. Karthikeyan, M. S.; Holla, B. S.; Kulkuraya, B.; Kumari, N. S. *Monatsh. Chem.* **2007**, *138*, 1309-1316.
[8]. Sharma, R.; Sainy, J.; Chaturvedi, S. C. *Acta. Pharm.* **2008**, *58*, 317-326.
[9]. Prasad, D. J.; Ashok, M.; Karegoudar, P.; Poojary, B.; Holla, B. S.; Kumari, N. S. *Eur. J. Med. Chem.* **2009**, *44*, 551-557.
[10]. Karegoudar, P.; Prasad, D. J.; Ashok, M.; Mahalinga, M.; Poojary, B.; Holla, B. S.; *Eur. J. Med. Chem.* **2008**, *43*, 808-815
[11]. Udupi, R. H.; Rajeeva, B.; Srinivasulu, N.; Pasha, T. Y.; Setty, S. R.; Bhat, A. R. *Indian J. Heterocycl. Chem.* **2004**, *13*, 233-236.
[12]. Metwally, K. A.; Yaseen, S. H.; Lashine, S. M.; El-Fayomi, H. M.; El-Sadek, M. E. *Eur. J. Med. Chem.* **2007**, *42*, 152-160.
[13]. Amir, M.; Kumar, H.; Javed, S. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4504-4508.
[14]. Golovlyova, S. M.; Moskvichev, Yu. A.; Alov, E. M.; Kobylinsky, D. B.; Ermolaeva, V. V. *Chem. Heterocycl. Compd.* **2001**, *37*, 1102-1106.
[15]. Labanauskas, L.; Udrenaite, E.; Gaidelis, P.; Brukstus, A. *Farmaco* **2004**, *59*, 255-259.
[16]. Tozkoparan, B.; Gokhan, N.; Aktay, G.; Yesilada, E.; Ertan, M. *Eur. J. Med. Chem.* **2000**, *35*, 743-750.
[17]. Ozturk, S.; Akkurt, M.; Causiz, A.; Koparir, M.; Sekerci, M.; Heinemann, F. W. *Acta Cryst. E* **2004**, *60*, o425-o427.
[18]. Koparir, M.; Cetin, A.; Causiz, A. *Molecules* **2005**, *10*, 475-480.
[19]. Okeke, M. I.; Iroegbu, C. U.; Eze, E. N.; Okoli, A. S.; Esimone, C. O. *J. Ethnopharmacol.* **2001**, *78*, 119-127.