

Regioselective synthesis and preliminary cytotoxic activity properties of tetrazole appendage N-substituted piperazine derivatives

Kommula Dileep¹, Mohana Rao Katiki¹, Busam Ramalingeswara Rao¹,
M. V. P. S. Vishnu Vardhan¹, Ramakrishna Sistla¹,
Jagadeesh Babu Nanubolu² and Madugula Sree Rama Murty^{1*}

¹Medicinal Chemistry & Pharmacology Division, Discovery Laboratory, CSIR–Indian Institute of Chemical Technology, Hyderabad, India

²Centre for X-Ray Crystallography, CSIR–Indian Institute of Chemical Technology, Hyderabad, India.

(Received April 08, 2017; Revised August 18, 2017; Accepted August 19, 2017)

Abstract: A series of 1-(4-substituted)-4-(3-((1-(substituted)-1H-tetrazol-5-yl)thio)propyl)piperazine derivatives were (**6a-t**) synthesized by KF-Al₂O₃ mediated S-alkylation of 5-thio-substituted tetrazole with 1-(3-chloropropyl)-4-(4-substituted)piperazine. The structures of the newly synthesized compounds were characterized by NMR and MS spectral data. Further, the regioselective formation of C-S bond was unambiguously confirmed by single crystal X-ray diffraction. All the synthesized compounds were screened for their *in vitro* cytotoxic activities against two cancer cell lines: DU-145 (prostate cancer) and HeLa (cervical cancer). These cell lines were utilized in MTT assays and the obtained results were compared to doxorubicin, and their IC₅₀ values were determined. Among the compounds tested, **6f**, **6j**, **6m**, **6n**, **6o**, **6q**, **6r** and **6t** showed considerable potent activity, while the compound **6p** exhibited significant potent activity against DU-145 and HeLa cancer cell lines compare to standard Doxorubicin.

Keywords: Tetrazole; cytotoxic activity; piperazine. © 2017 ACG Publications. All rights reserved.

1. Introduction

Cancer is one of the dreaded diseases of mankind and it is the major cause of mortality worldwide. Currently, one in 4 deaths in the United States are due to cancer and more than ten million new cancer cases occur annually.¹ Controlling growth of cancer cells is difficulty due to the many reasons, such as the genetic instability and poor

prognosis of cancer.² Main characteristic of cancer cells is their highly proliferative nature. Thus, inhibition of proliferative pathways is considered an effective approach to fight cancer. The main complicated problem of curing cancer is that side effect of drugs on the normal cells and makes some other abnormalities in our body. Hence, the development of efficient, selective and less toxic anticancer drug candidates is a challenge in cancer research.³

Tetrazole and its derivatives have received much attention in recent years due to their widespread applications in biology. For instance, sulfanyltetrazole derivative (**I**) was identified as a potent, broad

* Corresponding author: E-mail: msrmurty@ymail.com; Phone: +914027191654

spectrum NNRTI lead of HIV-1 replication^{4,5} and Cilostazol (**II**) which is a selective PDE3 phosphodiesterase inhibitor.

Similarly, a class of cephalosporin antibiotics and angiotensin II blocker (Losartan (**III**) and valsartan (**IV**)) contain tetrazole scaffold^{6,7} (Figure 1). Further, tetrazole derivatives have known to be resistant to metabolic processes, and their applications as in medicinal chemistry as metabolically stable surrogates for carboxylic acids.⁸ A well-known tetrazole is MTT, which is dimethyl thiazolyl diphenyl tetrazolium salt is used in MTT assay to quantify the respiratory activity of live cells in cell culture.⁹ Moreover, tetrazol-5-one and tetrazole-5-thione rings are a key frame work of many pesticides¹⁰ and herbicides,¹¹ and these derivatives exhibited diverse pharmacological activities such as antibacterial,¹² analgesic,¹³ anesthetic,¹⁴ anticancer and anticandida.¹⁵

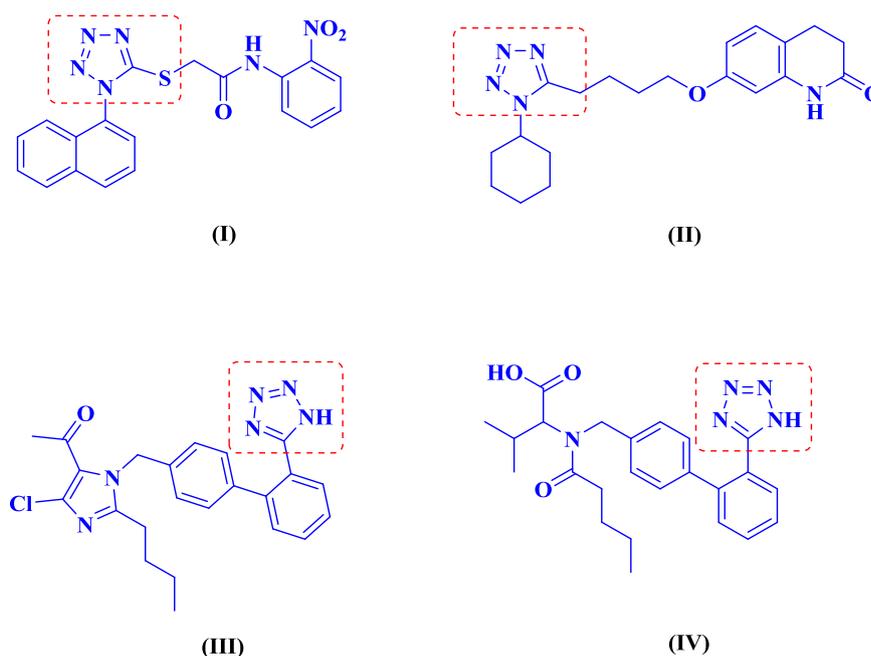


Figure 1. Some of the important bioactive tetrazole compounds

On the other hand, piperazine motif appears in many drugs encompassing a broad range of activities. Substituted piperazines are important pharmacophores that can be found in many marketed drugs, such as the Merck HIV protease inhibitor Crixivan.¹⁶ This motif is also displaying antimicrobial,¹⁷ anticancer,¹⁸ antidepressant and antioxidant activity.¹⁹ In recent years, alkyl chain arylpiperazine derivatives represent one of the important classes of building template in medicinal chemistry.¹⁶ Siracusa *et al.* reported that arylpiperazine moiety was linked to a benzoxazole, benzothiazole, or benzimidazole system by an ethylthio or propylthio unit showed to be high affinity towards 5-HT1A receptor.²⁰ Recently, as part of our on-going programme to discover and develop potential new anticancer agents, we have been reported alkyl chain arylpiperazine containing heterocyclic hybrids that includes benzoxazole/benzothiazole, triazoles and oxadiazoles moieties.^{21,22}

Thus, in continuation of our lasting interest in chemistry and pharmacological properties of tetrazole and substituted arylpiperazine, we incorporated the structural features of 5-thio substituted tetrazole and along with alkyl chain arylpiperazine by well-known using molecular hybridization approach^{23,24} for drug like molecules build-up, which allows achieving new pharmacological profile. Furthermore, after widespread literature search, it was observed that, till date no effort has been made to combine these vital moieties as a single molecular scaffold. Herein, we report a series novel of 1-(4-substituted)-4-(3-((1-(substituted)-1*H*-tetrazol-5-yl)thio)propyl)piperazine derivatives were evaluated for their preliminary cytotoxic activity.

2. Experimental

2.1. Materials and Apparatus

All the reagents were obtained from commercial sources. MTT was obtained from Sigma Chemicals (St. Louis, MO, USA). Melting points were determined on a Buchi capillary melting point apparatus. The NMR (300 and 500 MHz) spectra were recorded on Varian Gemini and Bruker Avance spectrometers. Chemical shifts are expressed in ppm down field with tetramethylsilane (TMS) as an internal standard. MS of the compounds were recorded on QSTAR XL hybrid MS/MS system, applied bio systems under electron spray ionization method conditions preparing sample solutions in methanol.

2.2. General Procedure for preparation of 1-substituted-1H-tetrazole-5-thiol (5a-e)²⁵: To the substituted isothiocyanates (**4a-e**) (3.7 g, 20 mmol) and NaN₃ 2.0 g (30 mmol) dissolved in 40 ml of water were stirred at reflux for 4 h. After this time, the reaction mixture was cooled, filtered and pH value of the filtrate was adjusted to 3.0 by adding concentrated hydrochloric acid, then white solid obtained and collected by filtration, washed with cold water, and dried under vacuum to give the crude product. The crude product was used directly or purified by crystallization using methanol to yield pure desired 1-substituted-1H-tetrazole-5-thiol (**5a-e**)

2.3. General procedure for preparation of 1-(3-chloropropyl)-4-substituted piperazine (2a-d): K₂CO₃ (15 mmol) is added to a solution of piperazine in dry acetonitrile (**1a-d**) (10 mmol), then 1, 3-dibromopropane was added drop wise (10 mmol) (10 mL) and stirred at room temperature for overnight. After the completion of reaction (confirmed by TLC), the solvent was evaporated. Extract the organic compound with ethyl acetate. The ethyl acetate layer is dried over anhydrous sodium sulphate. The compound was purified by column chromatography on silica eluting with EtOAc/n-hexane (1:2).

2.4. General procedure for preparation of 1-(3-((1-substituted-1H-tetrazol-5-yl)thio)propyl)-4-phenylpiperazine (6a-t): A mixture of 1-ethyl-1H-tetrazole-5-thiol (**5a-e**) (3.0 mmol) and KF·Al₂O₃ (4.5 mmol) in ethanol (15 mL) was stirred for 20 min under N₂ atmosphere. 1-(3-chloropropyl)-4-phenyl piperazines (**2a-d**) (3.2 mmol) was added to the above mixture and stirred for about 4 h. After the completion of reaction (confirmed by TLC), the solvent was evaporated and cold water was added to the reaction mixture and stirred for 30 min. The organic compound layer extracted with ethyl acetate. The ethyl acetate layer is dried over anhydrous sodium sulphate. The compound was purified by column chromatography on silica eluting with EtOAc/n-hexane (2:1).

1-(3-((1-ethyl-1H-tetrazol-5-yl)thio)propyl)-4-phenylpiperazine (6a): Light brown oil; ¹H NMR (300 MHz, CDCl₃) δ: 1.48 (t, 3H, *J* = 7.5 Hz), 2.05 (quint, 2H, *J* = 6.7 Hz), 2.49-2.70 (m, 6H), 3.14-3.26 (m, 4H), 3.41 (t, 2H, *J* = 6.7 Hz), 4.24 (q, 2H, *J* = 7.5 Hz), 6.80-6.99 (m, 3H), 7.21-7.34 (m, 2H); ¹³C NMR (75 MHz CDCl₃) δ = 14.1, 26.3, 29.6, 42.4, 48.9, 53.0, 56.4, 115.9, 119.6, 129.0, 151.0, 153.3; ESI-HRMS (*m/z*): [M+H]⁺. calcd. for C₁₆H₂₅N₆S: 333.18463, found: 333.18559.

1-benzyl-4-(3-((1-ethyl-1H-tetrazol-5-yl)thio)propyl)piperazine (6b): Light brown oil; ¹H NMR (300 MHz, CDCl₃) δ: 1.47 (t, 3H, *J* = 7.3 Hz), 1.99 (quint, 2H, *J* = 7.3 Hz), 2.42-2.49 (m, 10H), 3.37 (t, 2H, *J* = 6.4 Hz), 3.53 (s, 2H), 4.22 (q, 2H, *J* = 7.3 Hz), 7.27-7.33 (m, 5H); ¹³C NMR (75 MHz, CDCl₃) δ = 13.7, 25.8, 30.6, 42.0, 48.3, 52.4, 55.9, 115.4, 119.0, 128.5, 150.6, 152.5; ESI-HRMS (*m/z*): [M+H]⁺. calcd. for C₁₇H₂₇N₆S: 347.28473, found: 347.28503.

1-(3-((1-ethyl-1H-tetrazol-5-yl)thio)propyl)-4-(pyridin-2-yl)piperazine (6c): Light Yellow oil; ¹H NMR (300 MHz, CDCl₃) δ: 1.49 (t, 3H, *J* = 7.0 Hz), 2.02 (quint, 2H, *J* = 7.3 Hz), 2.42- 2.49 (m, 4H), 2.53-2.55 (m, 6H), 3.40 (t, 2H, *J* = 7.3 Hz), 3.52 (q, 2H, *J* = 7.0 Hz), 6.58-6.63 (m, 2H), 7.43-7.47 (m, 1H), 8.13-8.18 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ = 13.8, 26.0, 30.7, 42.1, 44.7, 52.5, 56.2, 106.6, 112.8, 137.0, 147.4, 153.1, 159; ESI-HRMS (*m/z*): [M+H]⁺. calcd. for C₁₅H₂₄N₇S: 334.28443, found: 334.28530.

1-(benzo[d][1,3]dioxol-5-ylmethyl)-4-(3-((1-ethyl-1H-tetrazol-5-yl)thio)propyl)piperazine (6d): Light brown oil; ¹H NMR (300 MHz, CDCl₃) δ: 1.49 (t, 3H, *J* = 7.3 Hz), 2.05 (quint, 2H, *J* = 7.1 Hz), 2.56-2.71

(m, 10H), 3.38 (t, 2H, $J = 7.1$ Hz), 3.55 (s, 2H), 4.24 (q, 2H, $J = 7.3$ Hz), 5.94 (s, 2H), 6.72-6.75 (m, 2H), 6.85 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 14.0, 25.8, 30.7, 42.3, 51.7, 52.7, 61.8, 100.7, 107.7, 109.4, 122.5, 129.8, 146.7, 147.4, 153.1$; ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$. calcd. for $\text{C}_{18}\text{H}_{27}\text{O}_2\text{N}_6\text{S}$: 391.20117, found: 391.20124.

1-phenyl-4-(3-((1-phenyl-1H-tetrazol-5-yl)thio)propyl)piperazine (6e): Light yellow solid, m.p 80 – 82 °C; ^1H NMR (300 MHz, CDCl_3) δ : 1.97 (quint, 2H, $J = 7.0$ Hz), 2.25-2.68 (m, 10H), 3.36 (t, 2H, $J = 7.0$ Hz), 7.20-7.41 (m, 10H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 25.6, 30.8, 48.4, 52.4, 56.0, 115.4, 119.2, 123.5, 124.6, 128.5, 132.1, 132.4, 150.6, 153.8$; ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$. calcd. for $\text{C}_{20}\text{H}_{25}\text{N}_6\text{S}$: 380.18563, found: 380.18659.

1-benzyl-4-(3-((1-phenyl-1H-tetrazol-5-yl) thio) propyl) piperazine (6f): Brown oil; ^1H NMR (300 MHz, CDCl_3) δ : 1.94 (quint, 2H, $J = 6.6$ Hz), 2.28-2.48 (m, 10H), 3.33 (t, 2H, $J = 6.6$ Hz), 3.51 (s, 2H), 7.22-7.42 (m, 10H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 25.6, 31.1, 50.8, 51.7, 52.0, 55.9, 62.2, 127.3, 127.9, 128.2, 128.8, 128.9, 129.4, 132.8, 136.3, 153.8$; ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$. calcd. for $\text{C}_{21}\text{H}_{27}\text{N}_6\text{S}$: 395.20019, found: 395.20124.

1-(3-((1-phenyl-1H-tetrazol-5-yl) thio) propyl)-4-(pyridin-2-yl) piperazine (6g): Light yellow oil; ^1H NMR (300 MHz, CDCl_3) δ : 2.07 (quint, 2H, $J = 7.5$ Hz), 2.47-2.58 (m, 6H), 3.48 (t, 2H, $J = 7.5$ Hz), 3.82 (t, 3H, $J = 5.2$ Hz), 6.46-6.51 (m, 1H), 7.52-7.62 (m, 6H), 8.28-8.34 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 25.8, 30.8, 44.6, 48.3, 52.0, 56.2, 106.6, 112.8, 123.3, 129.4, 129.7, 133.2, 137.0, 147.4, 154.0, 159.0$; ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$. calcd. for $\text{C}_{16}\text{H}_{25}\text{O}_4\text{N}_3\text{PS}$: 382.20117, found: 382.20194.

1-(benzo[d][1,3]dioxol-5-ylmethyl)-4-(3-((1-phenyl-1H-tetrazol-5-yl)thio)propyl)piperazine (6h): Light brown oil; ^1H NMR (400 MHz, CDCl_3) δ : 2.03 (quint, 2H, $J = 6.9$ Hz), 2.45-2.74 (m, 10H), 3.38 (t, 2H, $J = 6.9$ Hz), 3.49 (s, 2H), 5.88 (s, 2H), 6.63-6.73 (m, 2H), 6.79-6.84 (m, 1H), 7.41-7.59 (m, 5H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 25.7, 31.0, 52.2, 52.5, 56.0, 62.1, 100.4, 107.3, 109.0, 121.8, 123.6, 124.7, 132.5, 146.1, 147.1, 154.0$; ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$. calcd. for $\text{C}_{22}\text{H}_{27}\text{O}_2\text{N}_6\text{S}$: 439.21214, found: 439.21288.

1-(3-((1-benzyl-1H-tetrazol-5-yl)thio)propyl)-4-phenylpiperazine (6i): Brown oil; ^1H NMR (300 MHz, CDCl_3) δ : 1.92 (quint, 2H, $J = 6.6$ Hz), 2.29-2.69 (m, 10H), 3.32 (t, 2H, $J = 6.6$ Hz), 5.39 (s, 2H), 6.70-6.95 (m, 4H), 7.20-7.49 (m, 6H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 27.8, 29.3, 48.2, 51.3, 53.2, 56.1, 117.0, 121.2, 128.0, 129.2, 129.9, 137.0, 150.1, 154.2$; ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$. calcd. for $\text{C}_{21}\text{H}_{27}\text{N}_6\text{S}$: 395.20017, found: 395.20124.

1-benzyl-4-(3-((1-benzyl-1H-tetrazol-5-yl) thio) propyl) piperazine (6j): Light brown oil; ^1H NMR (300 MHz, CDCl_3) δ : 1.93 (quint, 2H, $J = 6.6$ Hz), 2.30-2.70 (m, 10H), 3.33 (t, 2H, $J = 6.6$ Hz), 3.52 (s, 2H), 5.40 (s, 2H), 7.72-7.40 (m, 10H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 25.4, 30.9, 50.6, 51.5, 55.7, 62.0, 127.2, 127.8, 128.1, 128.6, 129.3, 132.6, 136.0, 153.7$; ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$. calcd. for $\text{C}_{22}\text{H}_{29}\text{N}_6\text{S}$: 409.20117, found: 409.20134.

1-(3-((1-benzyl-1H-tetrazol-5-yl) thio) propyl)-4-(pyridin-2-yl) piperazine (6k): Light yellow oil; ^1H NMR (300 MHz, CDCl_3) δ : 2.01 (quint, 2H, $J = 7.5$ Hz), 2.50 (t, 2H, $J = 5.2$ Hz), 3.31 (t, 2H, $J = 7.5$ Hz), 3.80 (t, 2H, $J = 5.2$ Hz), 5.41 (s, 2H), 6.46-6.52 (m, 1H), 7.25-7.31 (m, 2H), 7.33-7.40 (m, 3H), 8.28-8.33 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 25.9, 31.1, 44.6, 50.5, 52.4, 56.1, 106.8, 113.0, 127.7, 128.7, 132.6, 137.1, 147.5, 153.8, 159.0$; ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$. calcd. for $\text{C}_{20}\text{H}_{26}\text{N}_7\text{S}$: 396.21217, found: 396.21314.

1-(benzo[d][1,3]dioxol-5-ylmethyl)-4-(3-((1-benzyl-1H-tetrazol-5-yl)thio)propyl)piperazine (6l): Light brown oil; ^1H NMR (300 MHz, CDCl_3) δ : 1.92 (quint, 2H, $J = 7.5$ Hz), 2.37-2.52 (m, 10H), 3.42 (s, 2H), 3.32 (t, 2H, $J = 7.5$ Hz), 5.39 (s, 2H), 5.91 (s, 2H), 6.70-6.73 (m, 2H), 6.82-6.84 (m, 1H), 7.24-7.84 (m, 5H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 26.0, 31.1, 50.5, 52.4, 56.1, 62.3, 100.5, 107.5, 109.25, 122.0, 127.7, 128.7, 131.4, 132.7, 146.3, 147.2, 153.8$; ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$. calcd. for $\text{C}_{23}\text{H}_{29}\text{O}_2\text{N}_6\text{S}$: 453.22614, found: 453.22689.

1-(3-((1-(4-bromophenyl)-1H-tetrazol-5-yl)thio)propyl)-4-phenylpiperazine (6m): Pale yellow crystalline solid, m.p 94 – 96 °C; ¹H NMR (400 MHz, CDCl₃) δ: 1.96-2.19 (quint, 2H, *J* = 6.9 Hz), 2.22-2.34 (m, 6H), 3.11-3.27 (m, 4H), 3.42-3.56 (t, 2H, *J* = 6.9 Hz), 6.76-7.0 (m, 3H), 7.18-7.33 (m, 2H), 7.45-7.58 (m, 2H), 7.66-7.80 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ = 26.1, 29.6, 31.4, 48.9, 53.0, 56.5, 116.0, 119.7, 124.0, 125.1, 129.0, 132.9, 151.1, 154.4 ; ESI–HRMS (*m/z*): [M+H]⁺. calcd. for C₂₀H₂₄N₆BrS: 459.09285, found: 459.01378.

1-benzyl-4-(3-((1-(4-bromophenyl)-1H-tetrazol-5-yl)thio)propyl)piperazine (6n): Light brown oil; ¹H NMR (400 MHz, CDCl₃) δ: 2.01 (quint, 2H, *J* = 7.1 Hz), 2.41-2.55 (m, 10H), 3.42 (t, 2H, *J* = 7.1 Hz), 3.52 (s, 2H), 7.18-7.23 (m, 5H), 7.45-7.58 (m, 2H), 7.66-7.74 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ = 27.4, 30.2, 50.0, 52.0, 56.2, 64.2, 117.2, 120.3, 122.5, 127.0, 127.7, 130.6, 133.8, 135.6, 154.4; ESI–HRMS (*m/z*): [M+H]⁺. calcd. for C₂₁H₂₅N₆BrS: 474.09208, found: 474.09299.

1-(3-((1-(4-bromophenyl)-1H-tetrazol-5-yl)thio)propyl)-4-(pyridin-2-yl)piperazine (6o): Light yellow oil; ¹H NMR (400 MHz, CDCl₃) δ: 2.19 (quint, 2H, *J* = 7.1 Hz), 2.20-2.34 (m, 6H), 3.11-3.26 (m, 4H), 3.48 (t, 2H, *J* = 7.1 Hz), 6.46-6.52 (m, 1H), 7.25-7.31 (m, 2H), 7.33-7.40 (m, 3H), 8.28-8.33 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ = 26.1, 31.6, 45.0, 52.7, 56.5, 106.9, 113.2, 123.9, 125.0, 132.8, 137.3, 147.7, 154.3, 159.3; ESI–HRMS (*m/z*): [M+H]⁺. calcd. for C₁₉H₂₃N₇BrS: 460.09037, found: 460.09135.

1-(benzo[d][1,3]dioxol-5-ylmethyl)-4-(3-((1-(4-bromophenyl)-1H-tetrazol-5-yl)thio)propyl)piperazine (6p): Brown oil; ¹H NMR (300 MHz, CDCl₃) δ: 2.08 (quint, 2H, *J* = 6.9 Hz), 2.32-3.61 (m, 10H), 3.48 (s, 2H), 3.52 (t, 2H, *J* = 6.9 Hz), 5.94 (s, 2H), 6.67-6.90 (m, 3H), 7.18-7.33 (m, 5H); ¹³C NMR (75 MHz, CDCl₃) δ = 26.1, 31.3, 52.6, 52.8, 56.4, 62.5, 100.7, 107.7, 109.4, 122.1, 124.0, 125.1, 131.5, 132.9, 146.5, 147.5, 154.3; ESI–HRMS (*m/z*): [M+H]⁺. calcd. for C₂₂H₂₆O₂N₆BrS: 517.10065, found: 517.09845.

1-phenyl-4-(3-((1-(3,4,5-trimethoxyphenyl)-1H-tetrazol-5-yl)thio)propyl)piperazine (6q): Yellow solid, m.p 76 – 78 °C; ¹H NMR (300 MHz, CDCl₃) δ: 2.03 (quint, 2H, *J* = 6.9 Hz), 2.57 (t, 2H, *J* = 6.9 Hz), 2.62 (t, 4H, *J* = 4.5 Hz), 3.18 (t, 3H, *J* = 4.5 Hz), 3.48 (t, 3H, *J* = 6.9 Hz), 3.89 (s, 9H), 6.79 (s, 2H), 6.82-6.87 (m, 1H), 6.89-6.95 (m, 2H), 7.21-7.29 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ = 25.9, 30.9, 48.6, 52.7, 56.0, 57.2, 60.5, 101.5, 115.5, 128.6, 138.7, 150.8, 153.4, 154.1; ESI–HRMS (*m/z*): [M+H]⁺. calcd. for C₂₃H₃₁O₃N₆S: 471.21444, found: 471.21488.

1-benzyl-4-(3-((1-(3,4,5-trimethoxyphenyl)-1H-tetrazol-5-yl)thio)propyl)piperazine (6r): Light yellow oil; ¹H NMR (300 MHz, CDCl₃) δ: 2.03 (quint, 2H, *J* = 7.5 Hz), 2.42-2.54 (m, 10H), 3.44 (t, 2H, *J* = 7.5 Hz), 3.51 (s, 2H), 3.91 (s, 9H), 6.77-6.79 (m, 2H), 7.24-7.34 (m, 5H); ¹³C NMR (75 MHz, CDCl₃) δ = 25.7, 30.8, 52.4, 56.0, 60.4, 62.3, 101.1, 126.6, 127.7, 128.7, 137.1, 138.6, 153.3, 154.3; ESI–HRMS (*m/z*): [M+H]⁺. calcd. for C₂₃H₃₃O₃N₆S: 485.21414, found: 485.21498.

1-(pyridin-2-yl)-4-(3-((1-(3,4,5-trimethoxyphenyl)-1H-tetrazol-5-yl)thio)propyl)piperazine (6s): Yellow oil; ¹H NMR (400 MHz, CDCl₃) δ: 2.12 (quint, 2H, *J* = 6.9 Hz), 2.20-2.34 (m, 6H), 3.50 (t, 3H, *J* = 6.9 Hz), 3.75-3.86 (m, 4H), 3.91 (s, 9H), 6.75-6.83 (s, 2H), 7.33-7.40 (m, 2H), 8.20-8.28 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ = 25.6, 30.8, 51.7, 52.1, 56.0, 61.9, 100.7, 107.7, 109.5, 122.5, 123.5, 129.5, 133.3, 146.7, 147.4, 154.1. ESI–HRMS (*m/z*): [M+H]⁺. calcd. for C₂₂H₃₀O₃N₇S: 472.21614, found: 472.21688.

1-(benzo[d][1,3]dioxol-5-ylmethyl)-4-(3-((1-(3,4,5-trimethoxyphenyl)-1H-tetrazol-5-yl)thio)propyl)piperazine (6t): Light brown oil; ¹H NMR (400 MHz, CDCl₃) δ: 2.02 (quint, 2H, *J* = 7.1 Hz), 2.36-2.60 (m, 10H), 3.41 (s, 2H), 3.44 (t, 2H, *J* = 7.17 Hz), 3.92 (s, 9H), 5.94 (s, 2H), 6.71-6.88 (m, 5H); ¹³C NMR (75 MHz, CDCl₃) δ = 25.7, 30.8, 52.2, 52.5, 60.4, 62.1, 100.0, 101.9, 107.2, 121.5, 128.5, 131.2, 138.5, 139.0, 146.0, 147.0, 153.2, 152.0; ESI–HRMS (*m/z*): [M+H]⁺. calcd. for C₂₅H₃₃O₅N₆S: 529.10165, found: 529.10228.

2.4 Crystallographic data for the compound 1-(3-((1-(4-bromophenyl)-1H-tetrazol-5-yl)thio)propyl)-4-phenylpiperazine (**6m**):

X-ray data for the compound **6m** were collected at room temperature using a Bruker Smart Apex CCD diffract meter with graphite monochromatic Mo-K α radiation ($\lambda=0.71073\text{\AA}$) with ω -scan method.²⁶ Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Unit cell dimensions were determined using 8415 reflections for **6m** compound data. Integration and scaling of intensity data was accomplished using SAINT program.²⁷ The structures were solved by Direct Methods using SHELXS97 and refinement was carried out by full-matrix least-squares technique using SHELXL97.²⁷ Anisotropic displacement parameters were included for all non-hydrogen atoms. All H atoms were positioned geometrically and treated as riding on their parent C atoms, with C-H distances of 0.93-0.97 \AA , and with $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C})$ or $1.5U_{\text{eq}}(\text{C})$ for methyl atoms.

Crystal data for 6m: $\text{C}_{19}\text{H}_{22}\text{BrN}_7\text{S}$, $M = 460.41$, colourless plate, $0.31 \times 0.26 \times 0.08 \text{ mm}^3$, triclinic, space group $P-1$ (No. 2), $a = 10.8203(5)$, $b = 14.0199(6)$, $c = 15.0893(7) \text{ \AA}$, $\alpha = 105.2830(10)$, $\beta = 103.8490(10)$, $\gamma = 103.3070(10)^\circ$, $V = 2036.09(16) \text{ \AA}^3$, $Z = 4$, $D_c = 1.502 \text{ g/cm}^3$, $F_{000} = 944$, CCD area detector, Mo-K α radiation, $\lambda = 0.71073 \text{ \AA}$, $T = 293(2)\text{K}$, $2\theta_{\text{max}} = 50.0^\circ$, 19783 reflections collected, 7155 unique ($R_{\text{int}} = 0.0223$). Final $\text{Goof} = 1.033$, $R_1 = 0.0294$, $wR_2 = 0.0764$, R indices based on 5876 reflections with $I > 2\sigma(I)$ (refinement on F^2), 505 parameters, $\mu = 2.142 \text{ mm}^{-1}$. CCDC 954133 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0) 1223 336 033; email: deposit@ccdc.cam.ac.uk].

2.5. Biochemistry

HeLa (Cervical Cancer) and DU-145(Prostrate Cancer) cell line was obtained from National center for Cell science (NCCS), Pune, India. MEM (Minimum essential Medium), MTT [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], Trypsin, EDTA were purchased from Sigma Chemicals Co (st. Louis, MO), Fetal bovine serum were purchased from Arrow labs, 96 well flat bottom tissue culture plates were purchased from Tarson

HeLa (Cervical Cancer) and DU-145(Prostrate Cancer) cell line were grown in MEM and DMEM, media respectively, supplemented with 10% fetal bovine serum, 100 $\mu\text{g/mL}$ penicillin, 200 $\mu\text{g/mL}$ streptomycin, 2 μM L-glutamine, and culture was maintained in a humidified atmosphere with 5% CO_2 . Stock solution of 10 mg/mL stock solution in DMSO, from the above stock various dilutions was made with sterile water to get required concentration. Toxicity of test compound in cells was determined by MTT assay²⁸ based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue formazan product. 1×10^4 Cells (counted by Trypan blue exclusion dye method) in 96- well plates were incubated with compounds with series of concentrations tested for 48 hrs at 37°C in RPMI/DMEM/MEM with 10% FBS medium. Then the above media was replaced with 90 μL of fresh serum free media and 10 μL of MTT reagent (5 mg/mL) and plates were incubated at 37°C for 4 h, there after the above media was replaced with 200 μL of DMSO and incubated at 37°C for 10 min. The absorbance at 570 nm was measured on a spectrophotometer (spectra max, Molecular devices) IC_{50} values were determined from plot: % inhibition (from control) versus concentration.

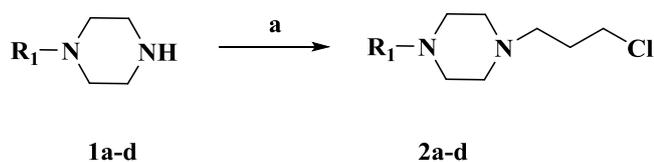
3. Results and discussion

3.1. Chemistry

In the present work, a series novel 1-(4-substituted)-4-(3-((1-(substituted)-1H-tetrazol-5-yl) thio) propyl) piperazine derivatives (**6a-t**) were prepared. The synthetic route for the preparation of target compounds (**6a-t**) is summarized in Scheme 2. Initially, substituted isothiocyanates²⁹ (**4a-e**) were prepared from substituted anilines following the procedure shown in Scheme 2. As depicted in Scheme 2, reaction

between substituted isothiocyanates (**4a-e**) and sodium azide in water provided 1-substituted -1*H*-tetrazole-5-thiol²⁵ (**5a-e**) in good yield. The mercapto tetrazole (**5a-e**) formed can exist in thione-thiol tautomeric forms shown in scheme 2 (**I** and **II**).

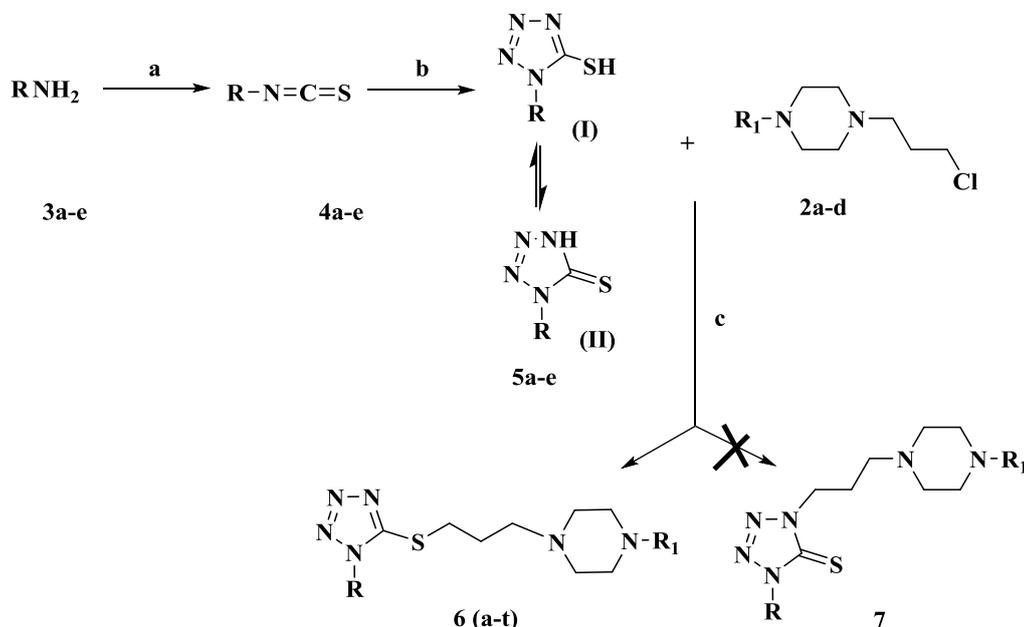
On the other hand, 1-(3-chloropropyl)-4-(4-substituted)piperazines were prepared from substituted arylpiperazines with 1-bromo-3-chloropropane using K_2CO_3 in acetonitrile solvent (scheme 1). 1-substituted -1*H*-tetrazole-5-thiol compounds (**3a-e**) were then treated with 1-(3-chloropropyl)-4-(4-substituted) piperazines (**2a-d**) using $KF-Al_2O_3$ in ethanol solvent at 80 °C to afford the final target compounds (**6a-t**, Scheme 2). All the synthesized compounds were characterized by 1H NMR, ^{13}C NMR and MS spectral data. The 1H NMR spectra of the target compounds (**6a-t**) $S-CH_2$ peak appeared as a triplet at 3.14 – 3.54 ppm for methylene protons attached to sulfur atom of the tetrazole ring. The ^{13}C NMR spectra of tetrazole derivatives showed $S-CH_2$ peak appearance at 32.7 – 32.4 ppm. The presence of these signals in 1H NMR and ^{13}C NMR spectra of the synthesized compounds is strong evidence of C–S bond formation.



Reagents and conditions: a) 1-Bromo-3-chloropropane, K_2CO_3 , Acetonitrile, rt, 12 h

Scheme 1. Preparation of 1-(3-chloropropyl)-4-substituted piperazines (**2a-d**).

Thus, under the present reaction condition compound **6** is formed as the only product in a regioselective manner and the compound **7** is not formed (Scheme 2). Theoretically two tautomeric forms are possible for the synthesized 1-substituted-1*H*-tetrazole-5-thiol derivatives (Figure 2). Considering **6m**



Reagents and conditions: a) i) CS_2 , Et_3N , THF, 1 h, ii) $TsCl$, 1 h, rt b) NaN_3 , H_2O , 80 °C. c) $KF-Al_2O_3$, ethanol, reflux, 4 h

Scheme 2. Preparation of 1-(4-Substituted)-4-(3-((1-(substituted)-1*H*-tetrazol-5-yl)thio)propyl) piperazine (**6a-t**).

as an example, we investigated the tautomerism of these mercapto tetrazole through single X-ray crystal diffraction analysis. Crystallographic data for **6m** have been deposited with the Cambridge Crystallographic Data Centre with the deposition number CCDC 954133. As shown in ORTEP diagram (Figure 3), the compound **6m** exhibits an S–H tautomer.

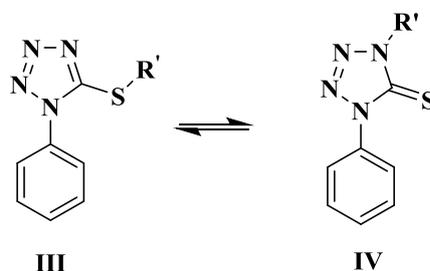


Figure 2. Tautomeric forms for the 1- Substituted -1*H*-tetrazole-5-thiol

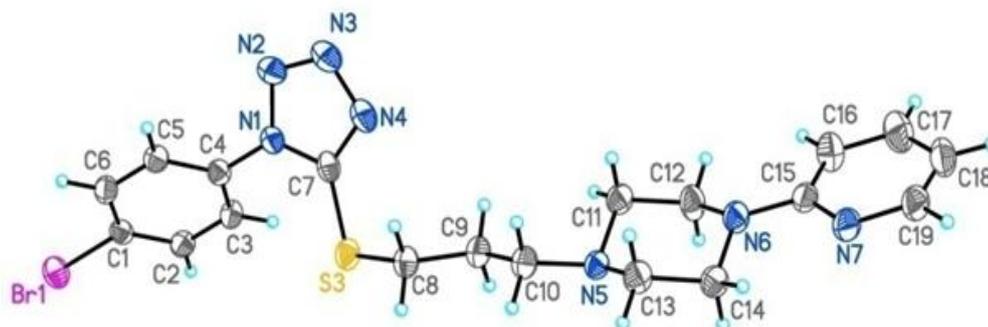


Figure 3. The molecular structure of 1-(3-((1-(4-bromophenyl)-1*H*-tetrazol-5-yl)thio)propyl)-4-phenylpiperazine (**6m**) with the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radius. There are two molecules in the asymmetric unit ($Z'=2$) of the crystal structure, however, only one molecule is shown for clarity.

3.2. Cytotoxic Activity

All the title compounds **6a-t** were screened for their *in vitro* cytotoxic activity against two cancer cell lines DU-145 (prostate cancer) and HeLa (Cervical) by using MTT bioassay, at 48 h of drug administration. Doxorubicin was used as a positive control. After treatment of DU-145 and HeLa cell lines for 48 h with compounds **6a-t** in the range of concentration 9.2 to >100 $\mu\text{g}/\text{mL}$. The cytotoxicity of the compounds was found to be commonly concentration-dependent. The IC_{50} values of the compounds were calculated and listed in Table 1.

The Compound **6p** was found as the significant potent compound against DU-145 and HeLa cell lines with IC_{50} values 9.2 and 19.9 $\mu\text{g}/\text{mL}$ compared to standard doxorubicin had IC_{50} values of 1.5 and 0.9 $\mu\text{g}/\text{mL}$ against DU-145 and HeLa cells lines, respectively. Moreover, compounds, **6j**, **6m**, **6n**, **6o**, **6q**, **6r** and **6t** showed good cytotoxic activity against DU-145 and HeLa cell lines. Further, compounds **6e** and **6s** showed moderate activity against DU-145. The results in DU-145 and HeLa cell lines demonstrated that after **6p**, the compound **6n** was found to be the second significant potent active compound. All the compounds exhibited percentage inhibitory activities at different concentrations of 5, 10 and 20 $\mu\text{g}/\text{mL}$

were plotted against DU-145 and HeLa cell lines in Figure 4 and Figure 5 respectively, among all the tested compounds, the compound **6p** displayed highest percentage inhibition followed by compound **6n**.

Table 1 synthesis and *In vitro* cytotoxic activity of the synthesized compounds (**6a-t**).

Compounds ^a	R	R ₁	%Yield ^b	Log P ^c	HeLa	DU-145
6a	-ethyl	N-phenyl	88	3.29	NA	96.9
6b	-ethyl	N-benzyl	90	3.09	96.8	63.6
6c	-ethyl	N-pyridyl	92	2.81	66.2	>100
6d	-ethyl	N-piperonyl	88	2.86	>100	63
6e	-phenyl	N-phenyl	90	4.75	>100	33.1
6f	-phenyl	N-benzyl	90	4.41	26.6	37.1
6g	-phenyl	N-pyridyl	92	4.13	>100	>100
6h	-phenyl	N-piperonyl	90	4.19	>100	>100
6i	-benzyl	N-phenyl	88	3.09	79.4	>100
6j	-benzyl	N-benzyl	90	4.48	31.6	39.8
6k	-benzyl	N-pyridyl	90	3.81	63.0	>100
6l	-benzyl	N-piperonyl	86	4.26	>100	>100
6m	-4-bromophenyl	N-phenyl	90	5.58	39.8	31.6
6n	-4-bromophenyl	N-benzyl	88	5.24	22.9	27
6o	-4-bromophenyl	N-pyridyl	90	4.96	31.6	63
6p	-4-bromophenyl	N-piperonyl	85	5.02	9.2	19.9
6q	-3,4,5-trimethoxyphenyl	N-phenyl	92	3.45	34.7	>100
6r	-3,4,5-trimethoxyphenyl	N-benzyl	90	4.03	31.6	50.1
6s	-3,4,5-trimethoxyphenyl	N-pyridyl	94	3.75	79.4	56.0
6t	-3,4,5-trimethoxyphenyl	N-piperonyl	92	3.81	37.7	>100
Doxorubicin					1.5	0.9

^aAll the compounds were characterized by NMR and Mass spectroscopy. ^bIsolated yields after column chromatography. ^cLog P values are calculated using chemdraw 14. IC₅₀ values are reported in micro molar concentration of the compound required to effect 50% inhibition of the tumor cell growth. NA- not active

The tetrazole moiety with different substituents was applied to investigate cytotoxicity i.e., alkyl, phenyl, benzyl, substituted phenyl groups (Electron-withdrawing (Br) and electron-donating (OMe) were introduced on the tetrazole). Similarly, substituted piperazines were employed for enhance the cytotoxic

effects. According to the substituent difference alkyl, phenyl, benzyl groups on tetrazole showed moderate to good activity. But the Electron-withdrawing (Br) substituted phenyl tetrazole of all target compounds shown enhanced the cytotoxic activity against DU-145 and HeLa cell lines, while the electron-donating group (OMe) cause a poor enhancement in potency. Probably, the increase in electron-withdrawing activity of phenyl substituted tetrazole is beneficial for activity. The utilization of an electron-donating (OMe) phenyl substituted tetrazole cause a poor enhancement in potency.

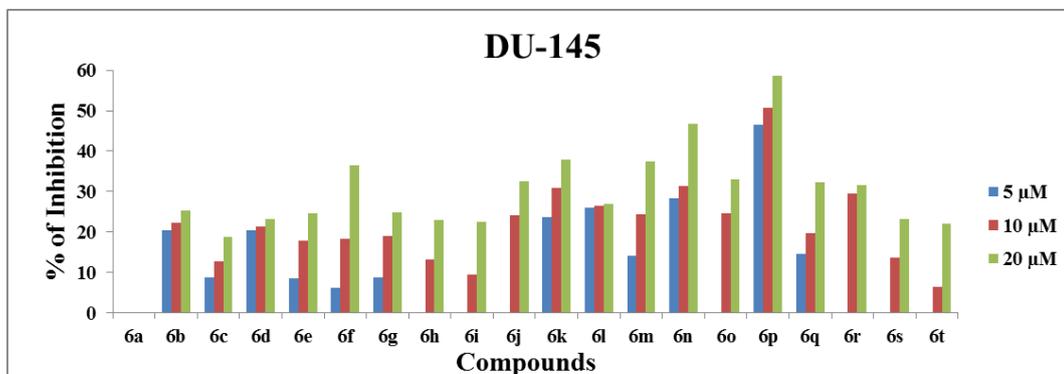


Figure 4. Percentage viability of cells upon treatment with different concentrations of sequences based on MTT assay for the compounds (**6a-t**).

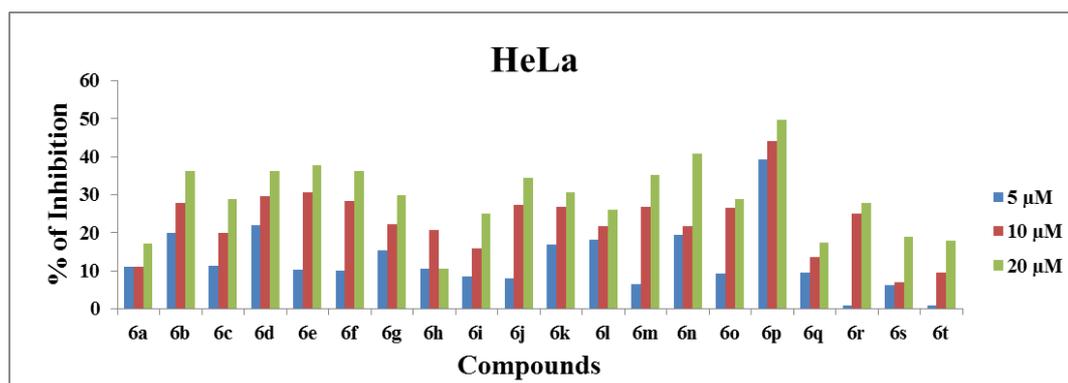


Figure 5. Percentage viability of cells upon treatment with different concentrations of sequences based on MTT assay for the compounds (**6a-t**).

4. Conclusion

In conclusion, a novel series of 1-(4-substituted)-4-(3-((1-(substituted)-1*H*-tetrazol-5-yl)thio)propyl)piperazine compounds were synthesized and their cytotoxic activity were evaluated. The cytotoxicity results showed that most of the synthesized tetrazole based piperazine derivatives (**6a-t**) exhibited moderate to significant cytotoxic activities *in vitro*. Especially, the compounds **6f**, **6j**, **6m**, **6n**, **6o**, **6q**, **6r** and **6t** showed moderate to good activity, while the compound **6p** exhibited significant potent activity against DU-145 compared to standard Doxorubicin. The cytotoxic activity results of the tested compounds indicated that the target compounds bearing electron withdrawing substituent on phenyl ring showed superior inhibitory effect compared electron donating substituent on phenyl ring attached to tetrazole against all the tested cancer cell lines. These findings demonstrated that tetrazole analogs are of biological significance, which have the perspective to become new members of cytotoxic agents.

Acknowledgements

The authors are thankful to the Director, Indian Institute of Chemical Technology, Hyderabad for the encouragement, the authors KD, RRB, and KRR are thankful to CSIR New Delhi, India, for the award of research fellowships. We thank CSIR for financial support under the 12th Five Year plan projects “Affordable Cancer Therapeutics (ACT)” (CSC 0301) and “Small Molecules in Lead Exploration (SMiLE)” (CSC0111).

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/OC>

Kommula Dileep: [0000-0003-1195-665X](http://orcid.org/0000-0003-1195-665X)

Mohana Rao Katiki: [0000-0003-0432-5374](http://orcid.org/0000-0003-0432-5374)

Busam Ramaligeswara Rao: [0000-0002-9864-0289](http://orcid.org/0000-0002-9864-0289)

M.V.P.S Vishnu Vardhan: [0000-0003-0087-4640](http://orcid.org/0000-0003-0087-4640)

Ramakrishna Sistla: [0000-0001-6268-2762](http://orcid.org/0000-0001-6268-2762)

Jagadeesh Babu Nanubolu: [0000-0002-1677-2975](http://orcid.org/0000-0002-1677-2975)

Madugula Sree Rama Murty: [0000-0001-6479-5162](http://orcid.org/0000-0001-6479-5162)

References

- [1] Siegel, R.; Ma, J.; Zou, Z.; Jemal, A. Cancer statistics. *Ca. Cancer J. Clin.* **2014**, *64*, 9–29.
- [2] Despaigne, A. A.; Parrilha, G. L.; Izidoro, J. B.; da Costa, P. R.; dos Santos, R.G.; Piro, O. E.; Castellano, E.E.; Rocha, W. R.; Beraldo, H. 2-Acetylpyridine- and 2-benzoylpyridine-derived hydrazones and their gallium(III) complexes are highly cytotoxic to glioma cells. *Eur. J. Med. Chem.* **2012**, *50*, 163–172.
- [3] Jemal, A.; Bray, F.; Center, M.; Ferlay, M.; Ward, J. E.; Forman, D. Global cancer statistics. *Ca Cancer J. Clin.* **2011**, *61*, 69–90.
- [4] OMeara, J.A.; Jakalian, A.; LaPlante, S.; Bonneau, P.R.; Coulombe, R.; Faucher, A.M.; Guse, I.; Landry, S.; Racine, J.; Simoneau, B.; Thavonekham, B.; Yoakim, C. Scaffold hopping in the rational design of novel HIV-1 non-nucleoside reverse transcriptase inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3362–3366.
- [5] Muraglia, E.; Kinzel, O.D.; Laufer, R.; Miller, M.D.; Moyer, G.; Munshi, V.; Orvieto, F.; Palumbi, M.C.; Pescatore, G.; Rowley, M.; Williams, P.D.; Summa, V. Tetrazole thioacetanilides: potent non-nucleoside inhibitors of WT HIV reverse transcriptase and its K103N mutant. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2748–2752.
- [6] Herr, R. J. 5-Substituted-1*H*-tetrazoles as carboxylic acid isosteres: medicinal chemistry and synthetic methods. *Bioorg. Med. Chem.* **2002**, *10*, 3379–3393.
- [7] Luo, Y.P.; Yang, G.F. Discovery of a new insecticide lead by optimizing a target-diverse scaffold: tetrazolinone derivatives. *Bioorg. Med. Chem.* **2007**, *15*, 1716–1724.
- [8] Nishi, Y.; Miyachi, H. New formulation and application method with fentrazamide in transplanted rice. *Pflanzenschutz-Nachr. Bayer (Engl. Ed.)* **2001**, *54*, (1), 43–50.
- [9] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Meth.* **1983**, *65*, 55–63.
- [10] Shenk, T.; Rabinowitz, J. D.; Munger, J.; Bennett, B.; U.S Patent 6959 2009023059, 2009.
- [11] Theodoridis, G. Herbicidal 1-aryl-4-substituted-1,4-dihydro-5*H*-tetrazol-5-ones and sulfur analogs thereof. 1984-US17998501939, 19841102. 1985.
- [12] Rami, Y. M.; Nisreen, H. A.; Omar, S. A.; Maschke, U.; Adel M. A.; Ahmed, M. M.; Abdelraouf, A. E.; James, R.; Fakh, M. A.; Ziyad, J. Y.; Ammar, F. E.; John, M. G. Synthesis, antibacterial and QSAR evaluation of 5-oxo and 5-thio derivatives of 1,4-disubstituted tetrazoles. *Bioorg. Med. Chem.* **2015**, *25*, 4024–4028.
- [13] Natalini, C. C.; Robinson, E. P. Evaluation of the analgesic effects of epidurally administered morphine, alfentanil, butorphanol, tramadol, and U50488H in horses. *Am. J. Vet. Res.* **2000**, *61*, 1579–1586.
- [14] Abou-Arab, M. H.; Heier, T.; Caldwell, J. E. Dose of alfentanil needed to obtain optimal intubation conditions during rapid-sequence induction of anaesthesia with thiopentone and rocuronium. *Br. J. Anaesth.* **2007**, *98*, 604–610.
- [15] Altıntop, M. D.; Özdemir, A.; Turan-Zitouni, G.; Ilgın, S.; Atlı, O.; Iscan, G.; Kaplancıklı, Z.M. Synthesis and biological evaluation of some hydrazone derivatives as new anticandidal and anticancer agents. *Eur. J. Med. Chem.* **2012**, *58*, 299–307.

- [16] Anuj, K. R.; Riyaz, S.; Han-Seung, S.; Rahul, V. P. Piperazine derivatives for therapeutic use: a patent review (2010-present). *Expert Opinion on Therapeutic Patents*. **2016**, *16*, 777–797.
- [17] Chaudhary, P.; Kumar, R.; Verma, A. K.; Singh, D.; Yadav, V.; Chhillar, A. K.; Sharmab, G. L.; Chandraa, R.; Chaudhary, P. Synthesis and antimicrobial activity of N-alkyl and N-aryl piperazine derivatives. *Bioorg. Med. Chem.* **2006**, *14*, 1819–1826.
- [18] Kumar, C.S. A.; Prasad, S. B. B.; Vinaya, K.; Chandrappa, S.; Thimmegowda, N. R.; Kumar, Y.C. S.; Swarup, S.; Rangappa, K.S. Synthesis and in vitro antiproliferative activity of novel 1-benzhydrylpiperazine derivatives against human cancer cell lines. *Eur. J. Med. Chem.* **2009**, *44*, 1223–1229.
- [19] Prashanth M. K.; Hosakere R.D.; Rai L.K. M.; Veeresh, B. Synthesis, characterization, antidepressant and antioxidant activity of novel piperamides bearing piperidine and piperazine analogues *Bioorg. Med. Chem.* **2012**, *22*, 7065.
- [20] Siracusa, M. A.; Salerno, L.; Modica, M. N.; Pittala, V.; Romeo, G.; Amato, M. E.; Nowak, M.; Bojarski, A.J.; Mereghetti, I.; Cagnotto, A.; Mennini, T. Synthesis of new arylpiperazinyl alkylthio benzimidazole, benzothiazole, or benzoxazole derivatives as potent and selective 5-HT_{1A} serotonin receptor ligands. *J. Med. Chem.* **2008**, *51*, 4529–4538.
- [21] Murty, M. S. R.; Rao, B. R.; Katiki, M. R.; Nath, L. R.; Anto, R. J. Synthesis of piperazinyl benzothiazole/benzoxazole derivatives coupled with 1,3,4-oxadiazole-2-thiol: Novel hybrid heterocycles as anticancer agents. *Med. Chem. Res.* **2013**, *22*, 4980–4991.
- [22] Murty, M. S. R.; Rao, B. R.; Ram, K. R.; Yadav, J. S.; Antony, J.; John, A. R. Synthesis and preliminary evaluation activity studies of novel 4-(aryl/heteroaryl-2-ylmethyl)-6-phenyl-2-[3-(4-substituted piperazine-1-yl)propyl]pyridazin-3(2H)-one derivatives as anticancer agents. *Med. Chem. Res.* **2012**, *21*, 3161–3169.
- [23] Murty, M. S. R.; Raju, P.; Nath, L. R.; Anto, R. J. Synthesis of salicylic acid-based 1,3,4-oxadiazole derivatives coupled with chiral oxazolidinones: Novel hybrid heterocycles as antitumor agents. *Lett. Drug. Des. Discov.* **2014**, *11*, 1133–1142.
- [24] Murty, M. S. R.; Katiki, M. R.; Babu, N. J.; Garimella, S.; Polepalli, S.; Jain, N.; Buddana, S. K.; Prakasham, R. S. Synthesis and biological evaluation of novel tamoxifen-1,2,4-triazole conjugates. *Mol. Divers.* **2016**, *20*, 687–703.
- [25] Altland, H. W. The Smiles rearrangement of 2-tetrazolylthio-3-aminopyridines. *J. Org. Chem.* **1976**, *41*, 3395–3398.
- [26] SMART & SAINT. Software Reference manuals. Versions 6.28a & 5.625, *Bruker Analytical X-ray Systems Inc.*, Madison, Wisconsin, U.S.A., 2001.
- [27] Sheldrick, G. M. SHELXS97 and SHELXL97, *Programs for crystal structure solution and refinement*; University of Gottingen: Germany, 1997.
- [28] Mosmann, T. J. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Immunol. Methods* **1983**, *65*, 55–63.
- [29] Wong, R.; Dolman, S. J. Isothiocyanates from tosyl chloride mediated decomposition of in situ Generated dithiocarbamic acid salts. *J. Org. Chem.* **2007**, *72*, 3969–3971.

ACG
publications

© 2017 ACG Publications