Synthesis, anticancer and molecular docking studies of 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazoles

Abstract:

Cancer a leading cause of human mortality worldwide, is characterized by untoward growth of cellular mass and signaled through the development of stress. Management of cancer treatment is still obscured and has been recently focused the need to discover a drug candidate with lesser side effects. The objective of present investigation is to explore 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazole derivatives synthesized by Huisgen cycloaddition of benzonitrile and sodium azide followed by acetylation then Aldol condensation reaction with aromatic aldehyde and further Michael addition reaction between nucleophile and α, β- unsaturated compound. Structures of synthesized compounds were characterized by MS, IR, 1H-NMR and elemental analysis. The synthesized compounds were evaluated for their in vitro anticancer activity under the drug discovery program of National Cancer Institute (NCI), USA. As per protocol of NCI seven compounds was selected and screened for anticancer activity at a single high dose (10^{-5} M) using NCI 60 cell lines. Among all synthesized compounds, **4b** and **4i** exhibited significant anticancer activity against Leukemia cell lines. Molecular docking studies for the 5-phenyl-1-(5-substituted phenylisoxazol-3-yl)-1H-tetrazole analogs was done with the help of Schrodinger software. Docking results also stated that the compounds **4b** and **4i** has good dock score among the other derivatives which shows good binding efficiency towards receptor.

Keywords: Sodium azide, isoxazole, tetrazole, renal cancer, breast cancer, molecular docking.

1. Introduction

Cancer is a disease of striking significance in the world today. It is the second leading cause of death in the world after cardiovascular diseases and is projected to be the primary cause within the coming years (Gibbs, 2000; KSY et al., 2014). The identification of novel structures that can be potentially useful in designing new, potent, selective and less toxic anticancer agents is still a major challenge to researchers (Heffeter et al., 2006).Despite of the

important advances achieved over recent decades in the research and development of various cancerostatic drugs, current antitumor chemotherapy still suffers from two major limitations- the first is the lack of selectivity of conventional chemotherapeutic agents for cancer tissues, bringing about unwanted side effects and the second is acquisition of multiple-drug resistance by cancer cells. Unwanted side effects of antitumor drugs could be overcome with agents capable of discriminating tumor cells from normal proliferative cells and the resistance is minimized using combined modality approach with different complementary mechanism of action (Menta and Palumbo, 1997).

The current scenario highlights the need for the discovery and development of new lead compounds of simple structure, exhibiting optimal in-vivo antitumor potency and new mechanism of action. Recent advances in clinical techniques, including large co-operative studies are allowing more rapid and reliable evaluation of new drugs. The combination of these advantages with improved preliminary screening systems is enhancing the emergence of newer and more potent compounds. In this regard, it should be emphasized that National Cancer Institute (NCI) in-vitro primary anticancer drug screen represents a valuable research tool to facilitate the drug discovery of new structural/ mechanistic types of antitumor agents (Boyd and Paull, 1995).

Tetrazole derivatives are well known compounds with a high level of biological activity (Schmidt and Schieffer, 2003) and screened for various biological activities such as antibacterial, antifungal (Bhaskar and Mohite, 2010), anticancer (Dhayanithi et al., 2011), analgesic (Bachar and Lahiri, 2004), anti-inflammatory (Mohite and Bhaskar, 2011), anti-diabetic, anti-hyperlipidemic (Momose et al., 2002) and anti-tubercular agents (Adamec et al., 2005). In drug design, tetrazoles are regarded as an isostere for the carboxylate group, and extensive work on tetrazoles has been carried out in the field of medicinal chemistry (Myznikov et al., 2007).

They are important ligands for many useful transformations and also precursors for a variety of nitrogen-containing heterocycles (Faucher et al., 2004). It was also noticed that toxic properties of a drug can decrease through the introduction of a tetrazole ring into the molecule (Bekhit et al., 2004). The tetrazole moiety is also generally accepted to exhibit stronger resistance to in vivo metabolization than the carboxylate group, thus conferring to the corresponding drug with longer bioavailability (Herr, 2002). The isoxazole moiety is a lead molecule in pharmaceutical development and has a wide range of biological activities like antimicrobial (Gautam and Singh, 2013), anti-inflammatory, analgesic

(Karabasanagouda et al., 2009), antitubercular (Tangallapally et al., 2007), anticancer (Yong et al., 2015), anti-hyperglycemic, lipid lowering activity (Kumar et al., 2009) and antihypertensive activity (Carenzi et al., 1989). The designed compounds were also subjected to molecular docking into the colchicines binding site of tubulin.

As part of our ongoing studies dealing with the synthesis of various derivatives of tetrazole containing isoxazole moiety, we describe here the synthesis, molecular docking studies of new derivatives and the outcome of preliminary evaluation of their anticancer activity.

2. Experimental

2.1. Chemistry

All of the reagents used were laboratory grade and purchased from commercial sources and were used after being purified by standard procedures. Melting point was determined by open capillary method and is uncorrected. The purity of synthesized compounds, commercial reagents used and monitoring of chemical reaction was done by thin layer chromatography. The spots were observed under iodine vapors and UV light. IR spectra were recorded by using KBr disk on Shimadzu FTIR-8400S. 1HNMR spectra were recorded on JEOL AL300 FTNMR 300 MHz spectrophotometer by using tetramethylsilane (TMS) as internal standard. The values of chemical shift (δ) are given in ppm. Mass spectrum was taken using Waters Micromass Q-Tof Micro. Mass spectrometer equipped with electrospray ionization (ESI).

*2.2. General procedure for the synthesis of 5-phenyl tetrazole,**1***

The synthetic procedure and spectral characterization of synthesized compounds described in earlier reports by author (Kaushik et al., 2015). The equimolar quantities of sodium azide, ammonium chloride and benzonitrile were refluxed with dimethyl formamide at 125 °C for 7-8 hours, reaction mixture was poured in 100 ml of water; a milky solution was obtained which was acidified with concentrated hydrochloric acid to get the precipitate. The solution was cooled to 5°C, filtered, dried and recrystallized by using ethanol to obtain the 5-phenyl tetrazole (1).

White solid, Yield: 82%; m.p.(210-212°C).IR (KBr disk, cm^{-1}):ν3348 (NH), 3062 (Ar-CH), 1628 (C=N), 1292 (N-N=N-). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 8.72(s, 1H, NH), 7.56 (m, 5H, Ar-H).

*2.3. General procedure for the synthesis of 5-phenyl-1-acetyl tetrazole,**2***

5-phenyl tetrazole (1), (10 mmol) was mixed with acetic anhydride (10 mmol) and 2-3 drops of concentrated sulphuric acid. The reaction mixture was warmed at 60-70 °C for 15-20

minutes on water bath. The content was cooled to room temperature and poured into ice cold water to obtain a white colored precipitate. The precipitate was filtered, washed, dried and recrystallized from ethanol.

White solid, Yield: 78%; m.p. (218-220°C).IR (KBr disk, cm^{-1}):ν3058 (Ar-CH), 1730 (C=O), 1638 (C=N), 1285 (N-N=N-). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.51 (m, 5H, Ar-H), 2.28 (s, 3H, CH_3).

*2.4. General procedure for the synthesis of 3-(substituted phenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one,**3a-3j***

A solution of 5-phenyl-1-acetyl tetrazole (2), (10 mmol) and different substituted aromatic aldehyde (10 mmol) in ethanol (20 ml) was cooled to 5 to 10 °C in an ice bath. The cooled solution was treated with drop wise addition of 40% sodium hydroxide solution. The reaction mixture was magnetically stirred for 30 minutes and then left over night in the refrigerator. The resulting dark solution was diluted with ice water and acidified using hydrochloric acid. The solution was filtered, washed with water and recrystallized with ethanol.

*2.4.1. Synthesis of 3-(3-bromo-4-nitrophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one,**3a***

Brown solid, Yield: 86%; m.p.(260-262°C).IR (KBr disk, cm^{-1}):ν3034 (Ar-CH), 1746 (C=O), 1618 (C=C), 1593 (C=N), 1570 (NO_2), 1284 (N-N=N-), 646 (C-Br). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.94 (d, 1H, Ar-H), 7.53 (s, 1H, Ar-H), 7.47 (d, 1H, Ar-H), 7.40 (m, 5H, Ar-H), 7.23 (d, 1H, CH), 6.80 (d, 1H, CH). Anal. Calcd. (%) for $C_{16}H_{10}BrN_5O_3$: C, 48.02; H, 2.52; Br, 19.97; N, 17.50; O, 11.99.

*2.4.2. Synthesis of 3-(3, 4, 5-trimethoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one.**3b***

Yellowish Brown solid, Yield: 82%; m.p.(230-232°C).IR (KBr disk, cm^{-1}):ν3054 (Ar-CH), 1740 (C=O), 1630 (C=C), 1600 (C=N), 1248 (N-N=N-), 1221 (OCH_3). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.47 (d, 1H, CH), 7.45 (m, 5H, Ar-H), 6.56 (s, 2H, Ar-H), 6.40 (d, 1H, CH), 3.66 (s, 9H, OCH_3). Anal. Calcd. (%) for $C_{19}H_{18}N_4O_4$: C, 62.29; H, 4.95; N, 15.29; O, 17.47.

*2.4.3. Synthesis of 3-(2, 4-difluorophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one, **3c***

Creamy solid, Yield: 79%; m.p.(234-236°C).IR (KBr disk, cm^{-1}):ν3059 (Ar-CH), 1712 (C=O), 1614 (C=C), 1608 (C=N), 1268 (N-N=N-), 1163 (C-F). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.68 (d, 1H, CH), 7.51 (m, 5H, Ar-H), 7.18 (d, 1H, Ar-H), 6.77 (s, 1H, Ar-H), 6.58

(s, 1H, Ar-H), 6.43 (d, 1H, CH). Anal. Calcd. (%) for $C_{16}H_{10}F_2N_4O$: C, 61.54; H, 3.23; F, 12.17; N, 17.94; O, 5.12.

2.4.4. Synthesis of 3-(4-fluorophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one, ***3d***

Brown solid, Yield: 68%; m.p.(224-226°C).IR (KBr disk, cm^{-1}):ν3060 (Ar-CH),1764 (C=O), 1620 (C=C), 1606 (C=N), 1310 (N-N=N-), 1166 (C-F). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.48 (d, 1H, CH), 7.42 (m, 5H, Ar-H), 7.21 (d, 2H, Ar-H), 7.04 (d, 2H, Ar-H), 6.56 (d, 1H, CH). Anal. Calcd. (%) for $C_{16}H_{11}FN_4O$: C, 65.30; H, 3.77; F, 6.46; N, 19.04; O, 5.44.

*2.4.5. Synthesis of 3-(2-hydroxy-5-nitrophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one,****3e***

Brown solid, Yield: 63%; m.p.(252-254°C).IR (KBr disk, cm^{-1}):ν3583 (OH), 3055 (Ar-CH), 1680 (C=O), 1630 (C=C), 1610 (C=N), 1564 (NO_2), 1344 (N-N=N-). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.76 (s, 1H, Ar-H), 7.57 (d, 1H, CH), 7.54 (d, 1H, Ar-H), 7.48 (m, 5H, Ar-H), 6.81 (d, 1H, Ar-H), 6.76 (d, 1H, CH), 4.92 (s, 1H, OH). Anal. Calcd. (%) for C16H11N5O4: C, 56.98; H, 3.29; N, 20.76; O, 18.97.

*2.4.6. Synthesis of 3-(2-chloro-4-fluorophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one,****3f***

Reddish brown solid, Yield: 74%; m.p.(220-222°C).IR (KBr disk, cm^{-1}):ν3054 (Ar-CH), 1735 (C=O), 1630 (C=C), 1608 (C=N), 1285 (N-N=N-), 1178 (C-F), 786 (C-Cl). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.72 (d, 1H, CH), 7.44 (m, 5H, Ar-H), 7.17 (d, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 6.74 (d, 1H, Ar-H), 6.37 (d, 1H, CH). Anal. Calcd. (%) for $C_{16}H_{10}ClFN_4O$: C, 58.46; H, 3.07; Cl, 10.78; F, 5.78; N, 17.04; O, 4.87.

*2.4.7. Synthesis of 3-(2, 4-dimethoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one,****3g***

Brown solid, Yield: 78%; m.p.(246-248°C).IR (KBr disk, cm^{-1}):ν3054 (Ar-CH), 1666 (C=O), 1640 (C=C), 1606 (C=N), 1275 (N-N=N-), 1248 (OCH_3). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.62 (d, 1H, CH), 7.41 (m, 5H, Ar-H), 7.16 (d, 1H, Ar-H), 6.71 (d, 1H, CH), 6.53 (d, 1H, Ar-H), 6.47 (d, 1H, Ar-H), 3.68 (s, 6H, OCH_3). Anal. Calcd. (%) for $C_{18}H_{16}N_4O_3$: C, 64.28; H, 4.79; N, 16.66; O, 14.27.

2.4.8. Synthesis of 3-(2, 4, 6-trimethoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one, ***3h***

Yellowish brown solid, Yield: 82%; m.p.(212-214°C).IR (KBr disk, cm^{-1}):ν3064 (Ar-CH), 1666 (C=O), 1652 (C=C), 1606 (C=N), 1290 (N-N=N-), 1186 (OCH_3). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.63 (d, 1H, CH), 7.45 (m, 5H, Ar-H), 6.67 (s, 2H, Ar-H), 6.63 (d, 1H, CH), 3.66 (s, 9H, OCH_3). Anal. Calcd. (%) for $C_{19}H_{18}N_4O_4$; C, 62.29; H, 4.95; N, 15.29; O, 17.47.

*2.4.9 Synthesis of 3-(2-nitrophenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one,**3i***

Yellow solid, Yield: 87%; m.p.(228-230°C).IR (KBr disk, cm^{-1}):ν3074 (Ar-CH), 1726 (C=O), 1620 (C=C), 1608 (C=N), 1578 (NO_2), 1248 (N-N=N-). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.93 (d, 1H, CH), 7.78 (d, 1H, Ar-H), 7.58 (t, 1H, Ar-H), 7.61 (d, 1H, Ar-H), 7.53 (m, 5H, Ar-H), 7.28 (t, 1H, Ar-H),6.65 (d, 1H, CH). Anal. Calcd. (%) for $C_{16}H_{11}N_5O_3$: C, 59.81; H, 3.45; N, 21.80; O, 14.94.

*2.4.10.Synthesis of 3-(3-bromo-4-methoxyphenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one,**3j***

Yellowish brown solid, Yield: 78%; m.p.(164-166°C).IR (KBr disk, cm^{-1}):ν3034 (Ar-CH), 1660 (C=O), 1615 (C=C), 1608 (C=N), 1255 (N-N=N-), 1170 (OCH_3), 640 (C-Br); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.47 (d, 1H, CH), 7.41 (m, 5H, Ar-H), 7.27 (s, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 6.58 (d, 1H, CH), 6.64 (d, 1H, Ar-H); 3.77 (s, 3H, OCH_3). Anal. Calcd. (%) for $C_{17}H_{13}BrN_4O_2$: C, 53.00; H, 3.40; Br, 20.74; N, 14.54; O, 8.31.

*2.5. General procedure for the synthesis of 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazole,**4a-4j***

A mixture of 3-(substituted phenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one (3a-3j, 10 mmol), hydroxylamine hydrochloride (10 mmol) and 40% potassium hydroxide in ethanol were refluxed on water bath for 4-5 hours. On completion of reaction, reaction mixture was cooled at room temperature and poured into crushed ice. The precipitate was separated out which than filtered, dried and recrystallized from ethanol.

*2.5.1. Synthesis of 1-(5-(3-bromo-4-nitrophenyl) isoxazol-3-yl)-5-phenyl-1H-tetarzole,**4a***

Yellow solid, Yield: 76%; m.p.(158-160°C). IR (KBr disk, cm^{-1}):ν 3072 (Ar-CH), 2896 (CH), 1625 (C=N), 1560 (NO_2), 1502 (C=C), 1438 (N-O), 1261 (N-N=N-), 617 (C-Br). ^{1}H-NMR (300 MHz, DMSO,ppm)δ: 7.96 (d, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.85 (d, 1H, Ar-H), 7.71 (d, 5H, Ar-H), 7.04 (s, 1H, isoxazole). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.4, 153.7, 152.4, 136.2, 134.8, 131.5, 130.4, 128.6, 122.7, 102.6. MS m/z: 411 [M^+], Anal. Calcd. (%)

for $C_{16}H_9BrN_6O_3$: C, 46.51; H, 2.20; Br, 19.34; N, 20.54; O, 11.62%. Found: C, 46.63; H, 2.23; N, 20.48.

2.5.2. Synthesis of 1- (5-(3, 4, 5-trimethoxypheny)-isoxazol-3-yl)-5-phenyl-1H-tetarzole, ***4b***

Yellow brown solid, Yield: 78%; m.p.(136-138°C).IR (KBr disk, cm^{-1}):ν3051 (Ar-CH), 2918 (CH), 1691 (C=N), 1564 (C=C), 1485 (N-O), 1288 (N-N=N-), 1163 (OCH_3). ^{1}H-NMR (300 MHz, DMSO, ppm) δ: 7.98 (m, 5H, Ar-H), 6.91 (s, 1H, isoxazole), 6.88 (d, 2H, Ar-H) 3.60 (s, 9H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 152.2, 150.4, 140.7, 132.7, 130.2, 127.5, 126.3, 125.8, 102.7, 101.4, 57.3. MS m/z: 379 [M^+], Anal. Calcd. (%) for $C_{19}H_{17}N_5O_4$: C, 60.15; H, 4.52; N, 18.46; O, 16.87%. Found: C, 60.28; H, 4.56; N, 18.52.

2.5.3. Synthesis of 1- (5-(2, 4-difluorophenyl)-isoxazol-3-yl)-5-phenyl-1H-tetarzole, ***4c***

Brown solid, Yield: 68%; m.p.(144-146°C).IR (KBr disk, cm^{-1}):ν3093 (Ar-CH), 2921 (CH), 1610 (C=N), 1561 (C=C), 1474 (N-O), 1282 (N-N=N-), 1118 (C-F). ^{1}H-NMR (300 MHz, DMSO, ppm) δ: 7.48 (d, 1H, Ar-H), 7.97 (m, 5H, Ar-H), 6.90 (s, 1H, isoxazole), 6.87 (d, 1H, Ar-H), 6.86 (s, 1H, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.3, 165.5, 162.5, 152.5, 131.8, 130.7, 128.5, 127.4, 120.5, 113.6, 102.1, 101.2. MS m/z: 325 [M^+], Anal. Calcd. (%) for $C_{16}H_9F_2N_5O$: C, 59.08; H, 2.79; F, 11.68; N, 21.53; O, 4.92%. Found: C, 59.20; H, 2.83; N, 21.48.

2.5.4. Synthesis of 1-(5-(4-fluorophenyl)-isoxazol-3-yl)-5-phenyl-1H-tetarzole, ***4d***

Yellowish white solid, Yield: 74%; m.p.(153-155°C).IR (KBr disk, cm^{-1}):ν3063 (Ar-CH), 2896 (CH), 1682 (C=N), 1544 (C=C), 1485 (N-O), 1288 (N-N=N-), 1163 (C-F). ^{1}H-NMR (300 MHz, DMSO, ppm) δ: 7.84 (d, 2H, Ar-H), 7.65 (m, 5H, Ar-H), 6.90 (d, 2H, Ar-H), 6.86 (s, 1H, isoxazole). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.8, 163.7, 151.2, 132.5, 130.2, 129.6, 128.1, 115.2, 101.5. MS m/z: 308 [M^+], Anal. Calcd. (%) for $C_{16}H_{10}FN_5O$: C, 62.54; H, 3.28; F, 06.18; N, 22.79; O, 4.92.

2.5.5. Synthesis of 4-nitro-2-(3-(5-phenyl-1H-tetrazol-1-yl)-isoxazol-5-yl) phenol, ***4e***

Reddish brown solid, Yield: 73%; m.p.(139-141°C).IR (KBr disk, cm^{-1}):ν3577 (OH), 3053 (Ar-CH), 2902 (CH), 1689 (C=N), 1564 (NO_2), 1514 (C=C), 1485 (N-O), 1284 (N-N=N). ^{1}H-NMR (300 MHz, DMSO, ppm) δ: 8.05 (s, 1H, Ar-H), 7.84 (d, 1H, Ar-H), 7.70 (m, 5H, Ar-H), 7.09 (d, 1H, Ar-H), 6.89 (s, 1H, isoxazole), 4.11 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.7, 163.8, 152.5, 140.6, 132.2, 130.8, 128.6, 127.8, 122.4, 120.9, 101.2. MS m/z: 350 [M^+], Anal. Calcd. (%) for $C_{16}H_{10}N_6O_4$: C, 54.86; H, 2.88; N, 23.99; O, 18.27.

*2.5.6. Synthesis of 1- (5-(2-chloro-4-fluorophenyl)-isoxazol-3-yl)-5-phenyl-1H-tetrazol**, 4f***

White solid, Yield: 68%; m.p.(145-147°C).IR (KBr disk, cm^{-1}):ν3095 (Ar-CH), 2848 (CH), 1610 (C=N), 1508 (C=C), 1465 (N-O), 1288 (N-N=N-), 1120 (C-F), 727 (C-Cl). ^{1}H-NMR (300 MHz, DMSO, ppm) δ:8.09 (d, 1H, Ar-H), 7.88 (m, 5H, Ar-H), 7.44 (d, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 6.99 (s, 1H, isoxazole). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.7, 165.8, 153.5, 135.2, 134.0, 132.5, 130.4, 131.7, 128.1, 115.7, 102.6. MS m/z: 341 [M^+], Anal. Calcd. (%) for $C_{16}H_9ClFN_5O$: C, 56.24; H, 2.65; Cl, 10.37; F, 5.56; N, 20.49.

*2.5.7. Synthesis of 1- (5-(2, 4-dimethoxyphenyl)-isoxazol-3-yl)-5-phenyl-1H-tetrazol**, 4g***

Brown solid, Yield: 72%; m.p.(172-174°C).IR (KBr disk, cm^{-1}):ν3056 (Ar-CH), 2880 (CH), 1608 (C=N), 1560 (C=C), 1485 (N-O), 1288 (N-N=N-), 1163 (OCH_3). ^{1}H-NMR (300 MHz, DMSO, ppm) δ: 7.93 (m, 5H, Ar-H), 7.18(d, 1H, Ar-H), 6.89 (s, 1H, isoxazole), 6.87 (d, 1H, Ar-H), 6.85 (s, 1H, Ar-H), 3.96 (s, 6H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.6, 163.2, 160.3, 157.4, 153.2, 132.4, 130.5, 128.5, 127.2, 109.2, 106.8, 102.6, 56.4. MS m/z: 349 [M^+], Anal. Calcd. (%) for $C_{18}H_{15}N_5O_3$: C, 61.89; H, 4.33; N, 20.05; O, 13.74.

*2.5.8. Synthesis of 1- (5-(2, 4, 6-trimethoxyphenyl)-isoxazol-3-yl)-5-phenyl-1H-tetarzole, **4h***

Brown solid, Yield: 76%; m.p.(131-133°C).IR (KBr disk, cm^{-1}):ν3055 (Ar-CH), 2877 (CH), 1606 (C=N), 1564 (C=C), 1485 (N-O), 1288 (N-N=N-), 1163 (OCH_3). ^{1}H-NMR (300 MHz, DMSO, ppm) δ: 8.25 (m, 5H, Ar-H), 7.79 (s, 1H, isoxazole), 6.86 (s, 2H, Ar-H), 3.83 (s, 9H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.8, 164.5, 152.2, 132.4, 131.2, 129.1, 128.7, 102.3, 94.2, 57.5. MS m/z: 379 [M^+], Anal. Calcd. (%) for $C_{19}H_{17}N_5O_4$: C, 60.15; H, 4.52; N, 18.46; O, 16.87.

*2.5.9. Synthesis of 1- (5-(2-nitrophenyl)-isoxazol-3-yl)-5-phenyl-1H-tetarzole, **4i***

Yellowish brown solid, Yield: 68%; m.p.(174-176°C).IR (KBr disk, cm^{-1}):ν3086 (Ar-CH), 2893 (CH), 1623 (C=N), 1548 (NO_2), 1517 (C=C), 1485 (N-O), 1276 (N-N=N-). ^{1}H-NMR (300 MHz, DMSO, ppm) δ: 8.01 (m, 4H, Ar-H), 7.35 (m, 5H, Ar-H), 6.89 (s, 1H, isoxazole). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.2, 152.6, 146.8, 136.4, 132.4, 131.3, 130.5, 129.2, 128.4, 127.3, 120.6, 103.2. MS m/z: 334 [M^+], Anal. Calcd. (%) for $C_{16}H_{10}N_6O_3$: C, 57.49; H, 3.02; N, 25.14; O, 14.36.

*2.5.10. Synthesis of 1- (5- (3-bromo-4-methoxyphenyl)-isoxazol-3-yl)-5-phenyl-1H-tetarzole, **4j***

Creamy white solid, Yield: 73%; m.p.(136-138°C).IR (KBr disk, cm^{-1}):ν3066 (Ar-CH), 2877 (CH) 1602 (C=N), 1564 (C=C), 1465 (N-O), 1261 (N-N=N-), 1114 (OCH_3), 676 (C-Br). ^{1}H-NMR (300 MHz, DMSO, ppm) δ:8.21 (s, 1H, Ar-H), 7.43 (m, 5H, Ar-H), 6.96 (d, 1H, Ar-H), 7.79 (s, 1H, isoxazole), 6.50 (d, 1H, Ar-H), 3.68 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ170.2, 158.9, 151.7, 135.2, 132.2, 129.8, 128.2, 127.2, 125.4, 118.0, 101.4, 56.4. MS m/z: 397 [M^+], Anal. Calcd. (%) for $C_{17}H_{12}BrN_5O_2$: C, 51.27; H, 3.04; Br, 20.07; N, 17.59; O, 08.04.

2.6. Anticancer activity

Preliminary anticancer assay was performed according to the protocol of National Cancer Institute, USA (Bekircan et al., 2006). The compounds were evaluated at single concentration of 10-5 M towards the panel of 60 cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers. All synthesized compounds were registered on its website and seven compounds 4b, 4c, 4d, 4f, 4g, 4h, and 4i were selected. All the selected compounds were added to a previously prepared cell culture at a single concentration. The cell culture was incubated for 48 h. End point determination was made with a protein binding dye, sulforhodamine B (SRB). The result of each compound reported as the percent growth of treated cell lines or panel when compared to untreated control cells.

Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels.

Percentage growth inhibition is calculated as:

For concentrations when Ti > / = Tz:

[(Ti-Tz)/(C-Tz)]x 100

For concentrations when Ti < Tz:

[(Ti-Tz)/Tz]x 100

2.7. Molecular Docking studies

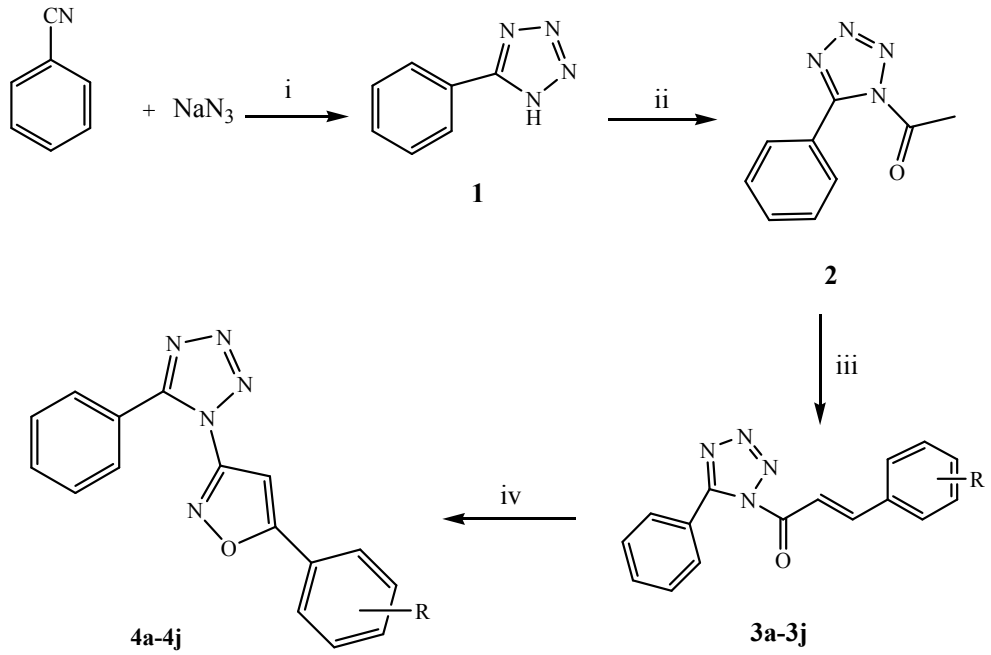
Molecular docking studies were done to check out interaction between the synthesized compounds and the active site of the receptor. The computation was carried out using Schrodinger molecular modeling software package. Docking was performed by using the Glide integrated with Maestro (Schrodinger, LLC, 2011) interface on the Linux operating

system. The starting coordinates of the human Tubulin-Colchicine: Stathmin-Like Domain Complex [PDB:1SA0] was taken from the Protein Data Bank (www.rcsb.org) and further modified for docking calculations. A compound library of synthesized 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazole derivatives was built on Maestro build panel and minimized in Schrodinger and optimized by means of the OPLS molecular mechanics force field using default settings. For Glide (Schrodinger) calculations, the protein Tubulin-Colchicine: Stathmin-Like Domain Complex [PDB: 1SA0] was optimized with the ‘‘protein preparation wizard’’ workflow by subjecting a cycle of constrained minimization steps allowing a maximum root mean square deviation (RMSD) from the original structure. For Glide (Schrodinger) calculations, Tubulin-Colchicine: Stathmin-Like Domain Complex was imported to Maestro (Schrodinger), the co-crystallized ligands were identified and removed from the structure. Docking was performed using Glide.

3. Results and discussions

3.1. Chemistry

The reaction sequences used for the synthesis of titled compounds are shown in Scheme 1.The equimolar quantities of sodium azide, benzonitrile, ammonium chloride were refluxed with dimethylformamide to obtained 5-phenyltetrazole 1, which after acetylation yield 5-phenyl-1-acetyl tetrazole 2. The compound 2 was treated with substituted aromatic aldehyde in ethanol and sodium hydroxide under ice cooled condition then acidified to form 3-(substituted phenyl)-1-(5-phenyl-1H-tetrazol-1-yl) prop-2-en-1-one 3a-3j. The compounds 3a-3j was refluxed with hydrazine hydrate and acetic acid to form titled compounds 4a-4j. Structures of all synthesized compounds were confirmed by their spectral data interpretation. The IR spectral data of compounds 4a-4j showed characteristic absorption band of methene group of isoxazole ring at 2877-2921 cm-1. The absorption bands of the C=N group and C=C group appeared at 1602-1691 cm-1and 1502-1564 cm-1 respectively. The presence of N-O group and N=N- group was confirmed by characteristic absorption band at 1438-1485 cm-1 and 1261-1288 cm-1. In 1H-NMR spectra of compounds 4a-4j, the one proton of isoxazole ring appeared as singlet in the range of δ 6.84-7.12 ppm. Protons of methoxy group showed as singlet at δ 3.68-3.78 ppm. The one proton of OH group of compound 4e showed a singlet at δ 4.92. All the aromatic protons were observed in the expected regions. Mass spectra of the compounds showed M+1 in agreement with their molecular formula.



Scheme 1 Synthetic routes to titled compounds (4a-4j).
Reagents and conditions:(i) NH_4Cl, DMF, 125 °C, 7-8 h; (ii) $(CH_3CO)_2O$, H_2SO_4; 60-70 °C, 15-20 min; (iii) R-CHO/NaOH, C_2H_5OH; (iv) $NH_2OH.HCl$, KOH, C_2H_5OH, 130 °C, 4-5h.

3.2. Anticancer activity

All the synthesized compounds were submitted to National Cancer Institute (NCI, USA), for anticancer activity. Seven compounds were selected according to NCI protocoland screened for *in vitro* anticancer activity at a single high dose 10^{-5} M in full NCI 60 cell lines panel. Anticancer activity data of synthesized compounds on NCI cancer cell lines were presented in Table 1.The synthesized compounds displayed moderate to low activity in the *in vitro* screening in all tested cancer cell line. The compound **4b** and **4i** were found to be most active compounds by showing 65.50 and 58.95 growth percent and highly active on MOLT-4 (Leukemia) and CCRF-CEM (Leukemia) cell line respectively while rest of the compounds showed less activity.

The possible mechanism of action of synthesized compounds would be inhibition of non-covalent polymerization of tubulin into microtubules. Tubulin, the major structural component of microtubules, is a target for the development of anticancer agents. Microtubules are a key component of cytoskeleton, and they are involved in a wide range of cellular functions, including regulation of motility, cell division, organelle transport, maintenance of cell morphology, and signal transduction. The essential role of microtubules in mitotic spindle

formation and proper chromosomal separation makes them one of the most attractive targets for the design and development of synthetic antitumor drugs.

Table 1. Anticancer screening data of title compounds

Compound	60 cell line assay in one dose 10^{-5} M conc.				
	NSC code	Mean growth %	Range of growth %	The most sensitive cell line	Growth % of most sensitive cell line
4b	761443	95.42	65.50 - 152.35	MOLT-4 (Leukemia)	65.5
				SR (Leukemia)	69.34
4c	778577	100.12	75.41 - 116.41	A498 (Renal cancer)	75.41
				HOP-92 (Non small cell lung cancer)	81.22
4d	761444	95.48	73.81 - 131.39	HL-60 (Leukemia)	73.81
				UO-31 (Renal cancer)	78.96
4f	778579	99.11	68.97-117.02	UO-31 (Renal cancer)	68.97
				BT-459 (Breast cancer)	69.64
4g	778578	100.95	80.52-121.84	UO-31 (Renal cancer)	80.52
				A498 (Renal cancer)	84.9
4h	778580	101.81	79.84-118.88	A498 (Renal Cancer)	79.84
				BT-459 (Breast cancer)	80.62
4i	761445	93.18	58.95 - 119.00	CCRF-CEM (Leukemia)	58.95
				SR (Leukemia)	74.13

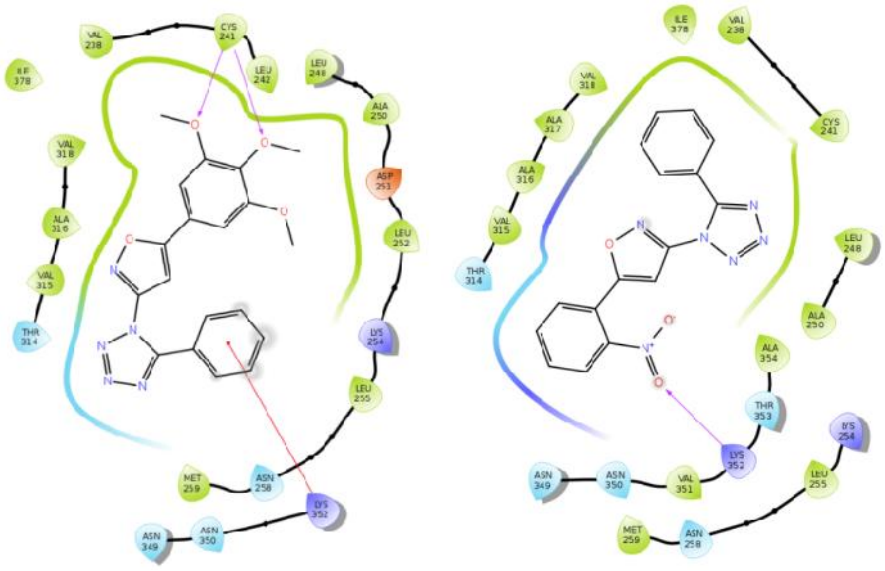
3.3. Molecular Docking studies

To check the molecular interaction and affinity of binding of Tubulin-Colchicine: Stathmin-Like Domain Complex [PDB: 1SA0] of synthesized 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazolederivatives, all the ligands were docked into the domain of Tubulin-Colchicine: Stathmin-Like Domain Complex [PDB: 1SA0]. Docking was done using Glide module of Schrodinger software. Docking results of these ligands are given in Table 2. Compounds **4b** and **4i** showed good interaction with Tubulin-Colchicine: Stathmin-Like Domain Complex and these results matched with wet laboratory findings. Compound **4b** was found to have a glide score of **-6.551** with two strong H bonds between Cys-241 and methoxy groups and one Pi-cation bond with Lys-352and compound **4i** was having have a glide score of **-6.421** with strong H bond between Lys-352 and nitro group of the compound. They were also found to have strong hydrophobic contacts with the residues of active site. This signifies

a strong binding of the molecules to the receptor. The ligand receptor interactions of compound **4b** and **4i** are shown in Fig 1.

Table 2 Docking results of title compound with tubulin-colchicine: stathmin-like domain complex [PDB: 1SA0]

Comp.	Glide score/docking score	Glide ligand efficiency	Glide rotable bonds	Glide RMSD to input
4b	-6.551	-0.273	3	146.118
4c	-4.711	-0.168	6	148.736
4d	-4.623	-0.173	5	148.863
4f	-4.832	-0.165	6	147.362
4g	-4.594	-0.177	5	147.86
4h	-5.876	-0.21	6	148.944
4i	-6.421	-0.256	4	146.391



4b **4i**

Fig. 1 Schematic representation of interaction between compounds **4b** and **4i** with theTubulin-Colchicine: Stathmin-Like Domain Complex (1SA0), active site amino acid residues, i.e., Cys-241 and Lys-352.

These compounds shows an increased anticancer activity and hence these are an ideally suited for further modifications to obtain more effective anticancer compounds. Hence, our study has identified some of 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazole derivatives that are important for cytotoxic activity against a panel of human cancer cell lines, and these findings indicates the need for additional investigations with respect to some new 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazolederivatives as anticancer agents.

4. Conclusions

In summary, 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazole have been synthesized and characterized. In vitro anticancer activity of synthesized compounds was performed by the National Cancer Institute (NCI), USA. The compound **4b** and **4i** were found to be most active compounds by showing 65.50 and 58.95 growth percent on MOLT-4 (Leukemia) and CCRF-CEM (Leukemia) cell line respectively. Molecular docking of these compounds was done by using Glide module of Schrodinger software which state that compounds **4b** and **4i** showed good interaction with Tubulin-Colchicine: Stathmin-Like Domain Complex and obtained results matched with wet laboratory findings.

References

Adamec, J., Waisser, K., Kunes, J., Kaustová, J., 2005. A note on the antitubercular activities of 1-aryl-5-benzylsulfanyltetrazoles. Arch. Pharm. (Weinheim). 338, 385–9.

Bachar, S.C., Lahiri, S.C., 2004. Synthesis of chloro and bromo substituted 5-(indan-1'-yl)tetrazoles and 5-(indan-1'-yl)methyltetrazoles as possible analgesic agents. Pharmazie 59, 435–8.

Bekhit, A.A., El-Sayed, O.A., Al-Allaf, T.A.., Aboul-Enein, Y.H., Kunhi, M., Pulicat, S.M., Khalid, A.-H., Fahad, A.-K., Arifc, J., 2004. Synthesis, characterization and cytotoxicity evaluation of some new platinum(II) complexes of tetrazolo[1,5-a]quinolines. Eur. J. Med. Chem. 39, 499–505.

Bekircan, O., Kahvec, B., Kucuk, M., 2006. Synthesis and anticancer evaluation of some new unsymmetrical 3,5-diaryl-4H-1,2,4-triazole derivatives. Turk J Chem 30, 29–40.

Bhaskar, V.H., Mohite, P.B., 2010. Synthesis, characterization and evaluation of anticancer activity of some tetrazole derivatives. J. Optoelectron. Biomed. Mater. 2, 249–259.

392 Boyd, M.R., Paull, K.D., 1995. Some practical considerations and applications of the national
393 cancer institute in vitro anticancer drug discovery screen. Drug Dev. Res. 34, 91–109.

394 Carenzi, A., Chiarino, D., Napoletano, M., Reggiani, A., Sala, A., Sala, R., 1989. New
395 isoxazole derivatives provided with antihypertensive activity. Arzneimittelforschung.
396 39, 642–6.

397 Dhayanithi, V., Syed, S.S., Kumaran, K., Reguraman, K., Sankar, J., Ragavan, R.V., Sanath,
398 P., Goud, K., Kumari, S., Pati, H.N., 2011. Synthesis of selected 5-thio-substituted
399 tetrazole derivatives and evaluation of their antibacterial and antifungal activities. J.
400 Serbian Chem. Soc. 76, 165–175.

401 Faucher, A.-M., White, P.W., Brochu, C., Grand-Maitre, C., JRancourt, J., Fazal, G., 2004.
402 Discovery of small-molecule inhibitors of the ATPase activity of human papillomavirus
403 E1 helicase. J. Med. Chem. 47, 18–21.

404 Gautam, K.C., Singh, D.P., 2013. Synthesis and antimicrobial activity of some isoxazole
405 derivatives of thiophene. Chem. Sci. Trans. 2, 992–996.

406 Gibbs, J.B., 2000. Mechanism-based target identification and drug discovery in cancer
407 research. Science 287, 1969–73.

408 Heffeter, P., Jakupec, M.A., Körner, W., Wild, S., von Keyserlingk, N.G., Elbling, L.,
409 Zorbas, H., Korynevska, A., Knasmüller, S., Sutterlüty, H., Micksche, M., Keppler,
410 B.K., Berger, W., 2006. Anticancer activity of the lanthanum compound [tris(1,10-
411 phenanthroline)lanthanum(III)]trithiocyanate (KP772; FFC24). Biochem. Pharmacol.
412 71, 426–440.

413 Herr, R.J., 2002. 5-Substituted-1H-tetrazoles as carboxylic acid isosteres: medicinal
414 chemistry and synthetic methods. Bioorg. Med. Chem. 10, 3379–93.

415 Karabasanagouda, T., Adhikari, A.V., Girisha, M., 2009. Synthesis of some new pyrazolines
416 and isoxazoles carrying 4-methylthiophenyl moiety as potential analgesic and anti-
417 inflammatory agents. Indian J. Chem. 48B, 430–437.

418 Kaushik, N., Kumar, N., Kumar, A., 2015. Synthesis of substituted 5-phenyl-1-(5-phenyl)-
419 isoxazol-3-yl)-1H-tetrazole as antioxidant agents. J. Adv. Sci. Res. 6, 14–19.

420 KSY, H., Raizaday, A., Kasina, S., 2014. New therapeutic approaches in treating cancer.

421 Pharm. Regul. Aff. Open Access 03, 1–2.

422 Kumar, A., Maurya, R.A., Sharma, S., Ahmad, P., Singh, A.B., Tamrakar, A.K., Srivastava,
423 A.K., 2009. Design and synthesis of 3,5-diarylisoxazole derivatives as novel class of
424 anti-hyperglycemic and lipid lowering agents. Bioorg. Med. Chem. 17, 5285–5292.

425 Menta, E., Palumbo, M., 1997. Novel antineoplastic agents. Expert Opin. Ther. Pat. 7, 1401–
426 1426.

427 Mohite, P.B., Bhaskar, V.H., 2011. A facile synthesis, characterisation and in-vitro anti-
428 inflammatory activity of novel N-substituted tetrazoles. J. Optoelectron. Biomed. Mater.
429 3, 87–93.

430 Momose, Y., Maekawa, T., Odaka, H., Ikeda, H., Sohda, T., 2002. Novel 5-substituted-1H-
431 tetrazole derivatives as potent glucose and lipid lowering agents. Chem. Pharm. Bull.
432 (Tokyo). 50, 100–11.

433 Myznikov, L. V., Hrabalek, A., Koldobskii, G.I., 2007. Drugs in the tetrazole series. Chem.
434 Heterocycl. Compd. 43, 1–9.

435 Schmidt, B., Schieffer, B., 2003. Angiotensin II AT1 receptor antagonists. Clinical
436 implications of active metabolites. J. Med. Chem. 46, 2261–2270.

437 Tangallapally, R.P., Sun, D., Rakesh, Budha, N., Lee, R.E.B., Lenaerts, A.J.M., Meibohm,
438 B., Lee, R.E., 2007. Discovery of novel isoxazolines as anti-tuberculosis agents. Bioorg.
439 Med. Chem. Lett. 17, 6638–6642.

440 Yong, J.-P., Lu, C.-Z., Wu, X., 2015. Potential anticancer agents. I. Synthesis of isoxazole
441 moiety containing quinazoline derivatives and preliminarily in vitro anticancer activity.
442 Anticancer. Agents Med. Chem. 15, 131–6.