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 unseemly growth of cellular mass and signaled through the enlargement of stress. Management of cancer treatment is still buried and has been recently alert the need to discover a drug molecule with lesser side effects. The objective of present study is to explore the anticancer activity and docking studies of 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazole derivatives. The compounds were evaluated for *in-vitro* anticancer activity under the drug discovery program of National Cancer Institute (NCI), USA. Only seven compounds was selected and screened for anticancer activity at a single high dose (10^{-5} M) using NCI 60 cancer cell lines. Among all the selected 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 by Schrodinger software. Docking results 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 prominent significance in the world today. It is the second foremost cause of death in the world after cardiovascular diseases and is predictable 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 development of new, potent, selective and less toxic anticancer agents is a major challenge to researchers till date (Heffeter et al., 2006, Lokhande et al., 2013). Although important advances achieved over recent decades in the development of various cancerostatic drugs, current antitumor chemotherapy still face two major limitations-the first one is lack of selectivity of conservative chemotherapeutic agents for cancer tissues, leading to undesired effects and the second is attainment of multiple-drug

resistance by cancer cells. Undesired effects of anticancer drugs could be overcome with the agents capable of astute tumor cells from normal proliferative cells and the resistance is minimized by using combined modality approach with different complementary mode 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 act as lead molecule in the drug development and has a broad 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 of synthesized compounds were recorded on Shimadzu FTIR-8400S by using KBr disk. ^{1}HNMR spectra were recorded on JEOL AL300 FTNMR 300 MHz spectrophotometer and tetramethylsilane (TMS) is used as internal standard. Chemical shift (δ) values 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, Kaushik et al., 2016,). The equal quantity 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), 7.56-7.18 (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 few drops of concentrated sulphuric acid was added to the solution and warmed this solution at 60-70 °C for 15-20 minutes on water bath. The reaction mixture was cooled to room temperature and poured into ice cold water. The white precipitate was filtered, washed, dried and recrystallized from ethanol.

White solid, Yield: 78%; m.p. (218-220°C).IR (KBr disk, cm^{-1}):v3058 (Ar-CH), 1730 (C=O), 1638 (C=N), 1285 (N-N=N-). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 7.51-7.28 (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***

5-phenyl-1-acetyl tetrazole (2), (10 mmol) was mixed with different substituted aromatic aldehyde (10 mmol) in presence of ethanol (20 ml), the reaction mixture was cooled to 5 to 10 °C in an ice bath. The ice cooled solution was treated with 40% sodium hydroxide solution. The reaction mixture was magnetically stirred for 30 minutes and left over night in the refrigerator. The resulting dark solution was diluted with ice cold water and acidified by 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}):v3034 (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), 7.40-7.28 (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}):v3054 (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), 7.45-7.28 (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}):v3059 (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), 7.51-7.34 (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), 7.42-7.29 (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), 7.48-7.25 (m, 5H, Ar-H), 6.81 (d, 1H, Ar-H), 6.76 (d, 1H, CH), 4.92 (s, 1H, OH). Anal. Calcd. (%) for $C_{16}H_{11}N_5O_4$: 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), 7.44-7.28 (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), 7.41-7.30 (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), 7.45-7.30 (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), 7.53-7.32 (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), 7.41-7.29 (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. After that, reaction mixture was cooled at room temperature and poured into crushed ice to form the precipitate which was 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: 7.92 (d, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 7.71 (d, 1H, Ar-H), 7.56-7.28 (m, 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: 7.59-7.25 (m, 5H, Ar-H), 7.04 (s, 1H, isoxazole), 6.82 (d, 2H, Ar-H) 3.68 (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), 7.41-7.29 (m, 5H, Ar-H), 6.97 (s, 1H, isoxazole), 6.91 (d, 1H, Ar-H), 6.80 (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: 7.52 (d, 2H, Ar-H), 7.42-7.26 (m, 5H, Ar-H), 7.11 (d, 2H, Ar-H), 6.98 (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: 7.92 (s, 1H, Ar-H), 7.74 (d, 1H, Ar-H), 7.56-7.27 (m, 5H, Ar-H), 7.13 (d, 1H, Ar-H), 7.05 (s, 1H, isoxazole), 4.92 (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:7.51 (d, 1H, Ar-H), 7.43-7.25 (m, 5H, Ar-H), 7.18 (d, 1H, Ar-H), 7.02 (s, 1H, Ar-H), 6.95 (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: 7.57-7.28 (m, 5H, Ar-H), 7.18(d, 1H, Ar-H), 6.84 (s, 1H, isoxazole), 6.77 (d, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 3.78 (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: 7.42-7.29 (m, 5H, Ar-H), 6.89 (s, 1H, isoxazole), 6.82 (s, 2H, Ar-H), 3.65 (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: 7.73-7.58 (m, 4H, Ar-H), 7.50-7.27 (m, 5H, Ar-H), 7.08 (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:7.48 (s, 1H, Ar-H), 7.42-7.25 (m, 5H, Ar-H), 7.21 (d, 1H, Ar-H), 7.12(s, 1H, isoxazole), 6.91 (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 as per the protocol of National Cancer Institute, USA (Bekircan et al., 2006). The synthesized compounds were evaluated at single concentration of 10^{-5} M towards the panel of 60 cell lines derived from different cancer types such as: 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 was determined by a protein binding dye, sulforhodamine B (SRB). The result of anticancer activity of each compound was reported as the percent growth of treated cell lines when compared to untreated control cells (Zahera et al., 2017, Lokhande et al., 2013).

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

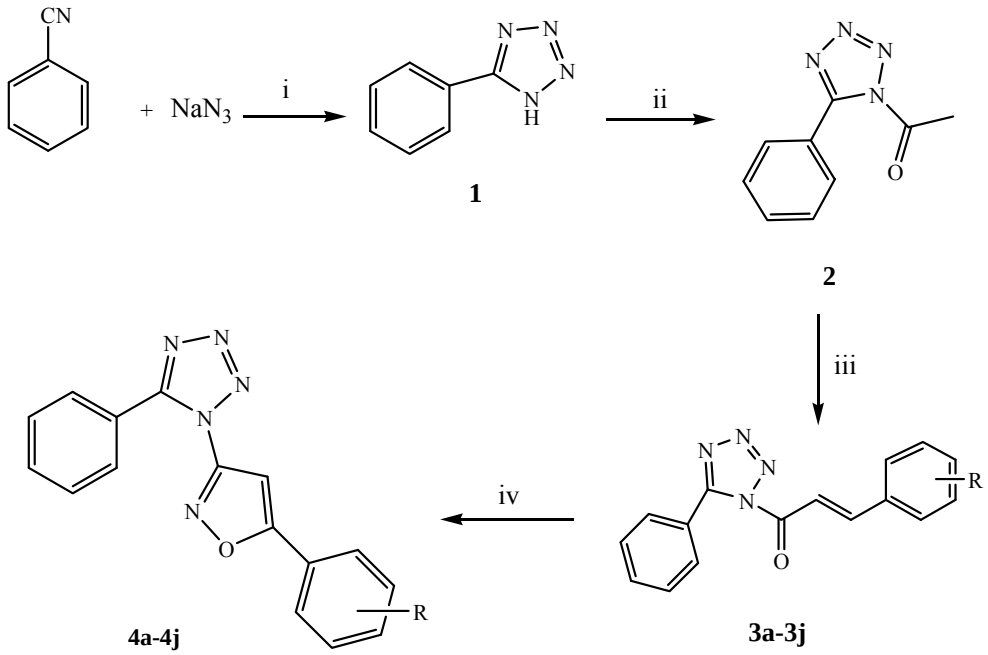
Molecular docking studies were done to check out interaction between the synthesized compounds and the active site of the receptor. The computation was carried out using Schrodinger molecular modeling software package. Docking was performed by using the Glide integrated with Maestro (Schrodinger, LLC, 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 equal stoichiometric amount 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 the synthesized compounds were characterized 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 ^{1}H-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 under the drug discovery program. Only seven compounds were selected according to NCI protocol and 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 activity 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

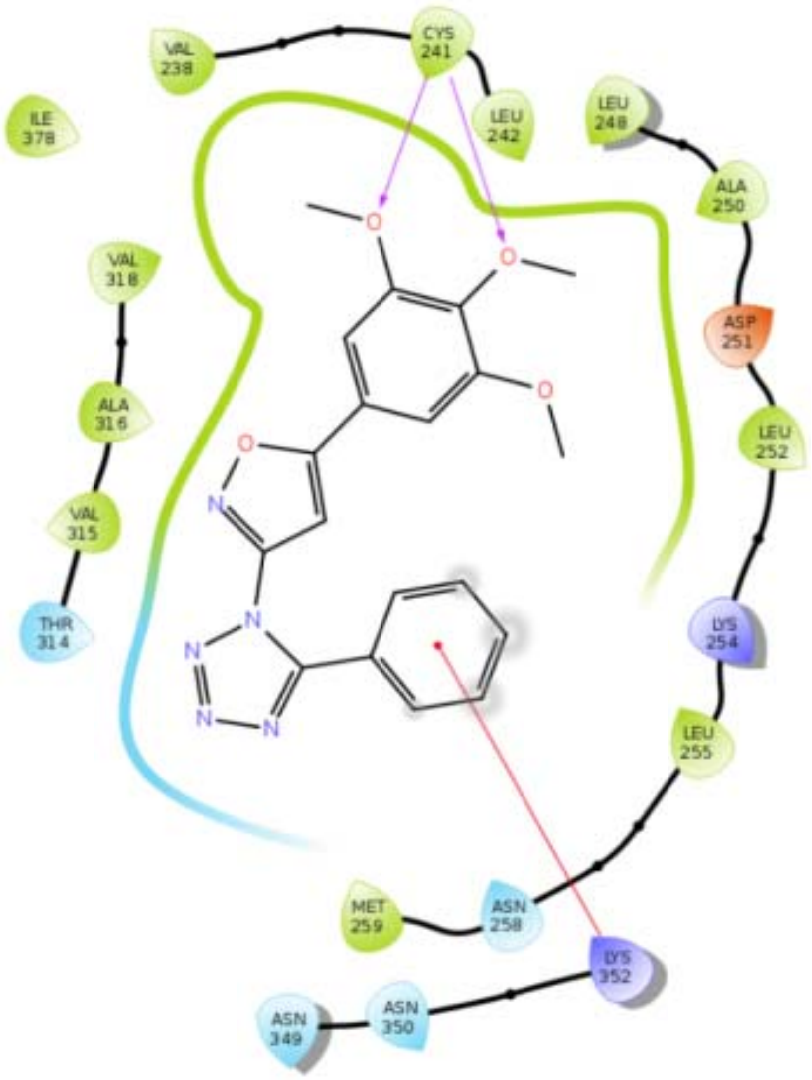
3.3. Molecular Docking studies

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 at the site LYS 352. The ligand receptor interactions of compound **4b** and **4i** are shown in Fig 1.

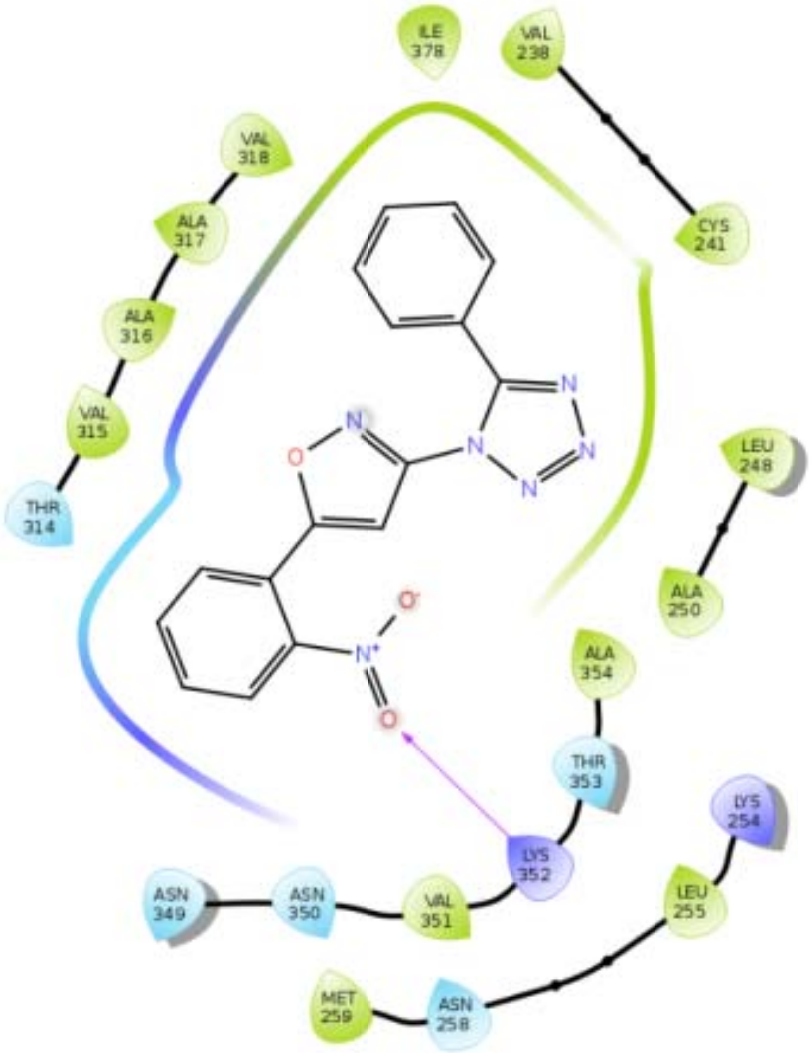
Table 2 Docking results of title compound with tubulin-colchicine: stathmin-like domain complex [PDB: 1SA0]

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


CYS 241
VAL 238
ILE 378
LEU 242
LEU 248
ALA 250
VAL 318
ASP 251
ALA 316
LEU 252
VAL 315
THR 314
LYS 254
LEU 255
MET 259
ASN 258
LYS 352
ASN 349
ASN 350
O
O
O
O
N
N
N
N
N

354

355 **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 the present work, 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazole derivatives have been synthesized from sodium azide and benzonitrile as reported by author earlier (Kaushik et al., 2015). All the synthesized compounds were characterized by different spectral technique. The synthesized compounds were assayed for their in vitro anticancer activity by the National Cancer Institute (NCI), USA under the drug discovery program. 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) cancer 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.

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