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Anticancer and Molecular Docking Studies of 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazoles

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Authors' contributions

This work was carried out in collaboration among all authors. Author N. Kaushik designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors N. Kumar and AK managed the analyses of the study. Author SK managed the literature searches. Author BKC managed the molecular docking study. All authors read and approved the final manuscript.

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ABSTRACT

Cancer a leading cause of human mortality worldwide is characterised by the unseemly growth of cellular mass and signalled through the enlargement of stress. Management of cancer treatment is still buried and has been recently alerting the need to discover a drug molecule with lesser side effects. The objective of the 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

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evaluated for *in-vitro* anticancer activity under the drug discovery program of National Cancer Institute (NCI), USA. Only seven compounds were 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 analogues 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 [1,2]. The identification of novel structures that can be potentially useful in the development of new, potent, selective and less toxic anticancer agents is a major challenge to researchers till date [3,4]. 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 the attainment of multiple-drug resistance by cancer cells. Undesired effects of anticancer drugs could be overcome with the agents capable of astute tumour cells from normal proliferative cells, and the resistance is minimised by using combined modality approach with the different complementary mode of action [5].

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 unique 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 emphasised 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 [6].

Tetrazole derivatives are well known compounds with a high level of biological activity [7] and screened for various biological activities such as antibacterial, antifungal [8], anticancer [9], analgesic [10], anti-inflammatory [11], anti-diabetic, anti-hyperlipidemic [12] and anti-tubercular agents [13]. 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 [14].

They are important ligands for many useful transformations and also precursors for a variety of nitrogen-containing heterocycles [15]. It was also noticed that the toxic properties of a drug could decrease through the introduction of a tetrazole ring into the molecule [16]. The tetrazole moiety is also generally accepted to exhibit stronger resistance to *in vivo* metabolisation than the carboxylate group, thus conferring to the corresponding drug with longer bioavailability [17]. The isoxazole moiety act as lead molecule in the drug development and has a broad range of biological activities like antimicrobial [18], anti-inflammatory, analgesic , [19], antitubercular [20], anticancer [21], anti-hyperglycemic, lipid-lowering activity [22] and antihypertensive activity [23]. 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 the 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 the

open capillary method and is uncorrected. The purity of synthesised compounds, commercial reagents used and monitoring of chemical reaction was done by thin layer chromatography. The spots were observed under iodine vapours and UV light. IR spectra of synthesised 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 ionisation (ESI).

2.2 General Procedure for the Synthesis of 5-phenyl Tetrazole, 1

The synthetic procedure and spectral characterisation of synthesised compounds described in earlier reports by author [24,25]. The equal quantity of sodium azide, ammonium chloride and benzonitrile were refluxed with dimethylformamide at 125 °C for 7-8 hours, the 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 recrystallised by using ethanol to obtain the 5-phenyl tetrazole (1).

White solid, Yield: 82%; m.p.(210-212°C).IR (KBr disk, cm^{-1}):v3348 (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 were added to the solution and warmed this solution at 60-70 °C for 15-20 minutes on a water bath. The reaction mixture was cooled to room temperature and poured into cold water. The white precipitate was filtered, washed, dried and recrystallised 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}):v3060 (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}):v3583 (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}):v3054 (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}):v3054 (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}):v3064 (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}):v3074 (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}):v3034 (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}):v 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}):v3051 (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}):v3093 (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}):v3063 (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}):v3577 (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}):v3095 (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}):v3056 (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}):v3055 (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}):v3086 (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}):v3066 (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 [26]. 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 [4,27].

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 the interaction between the synthesised compounds and the active site of the receptor. The computation was carried out using the Schrodinger molecular modelling 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 synthesised 1-(5-substituted phenyl) isoxazol-3-yl)-5-phenyl-1H-tetrazole derivatives was built on Maestro build panel and minimised in Schrodinger and optimised using the OPLS molecular mechanics force field using default settings. For Glide (Schrodinger) calculations, the protein Tubulin-Colchicine: Stathmin-Like Domain Complex [PDB: 1SA0] was optimised with the ‘‘protein preparation wizard" workflow by subjecting a cycle of constrained minimisation 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.

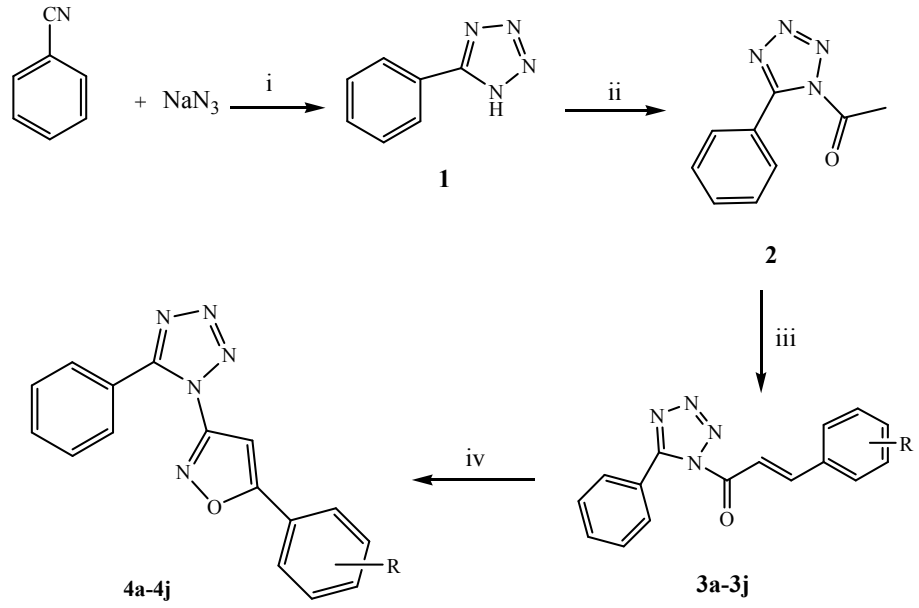
3. RESULTS AND DISCUSSION

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 a 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 methane 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 a 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 a singlet in the range of δ 6.84-7.12 ppm. Protons of methoxy group showed a 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.

3.2 Anticancer Activity

All the synthesised 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 synthesised compounds on NCI cancer cell lines were presented in Table 1. The synthesised compounds displayed moderate to low activity in the *in vitro* screening in all tested



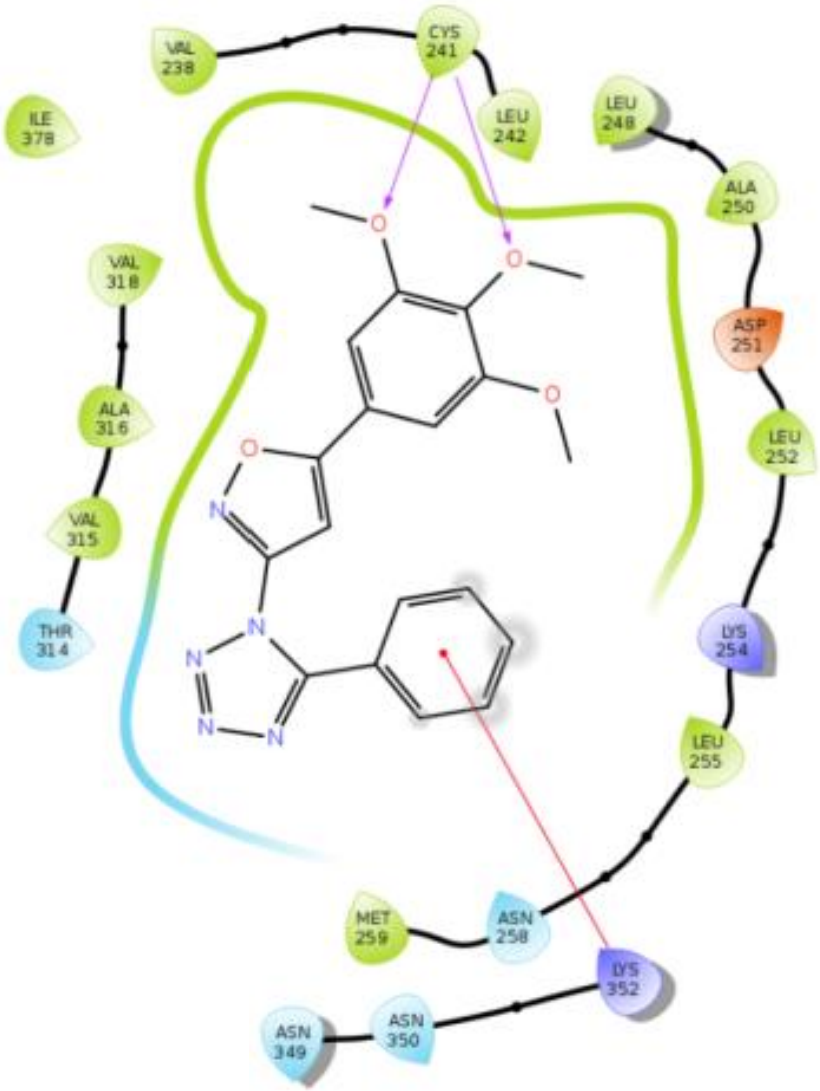
Scheme 1 Synthetic routes to titled compounds (4a-4j).
Reagents and conditions:(i) NH_4Cl, DMF, 125 °C, 7-8 h; (ii) $(CH_3CO)_2O$, H_2SO_4; 60-70 °C, 15-20 min; (iii) R-CHO/NaOH, C_2H_5OH; (iv) $NH_2OH.HCl$, KOH, C_2H_5OH, 130 °C, 4-5h.

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.

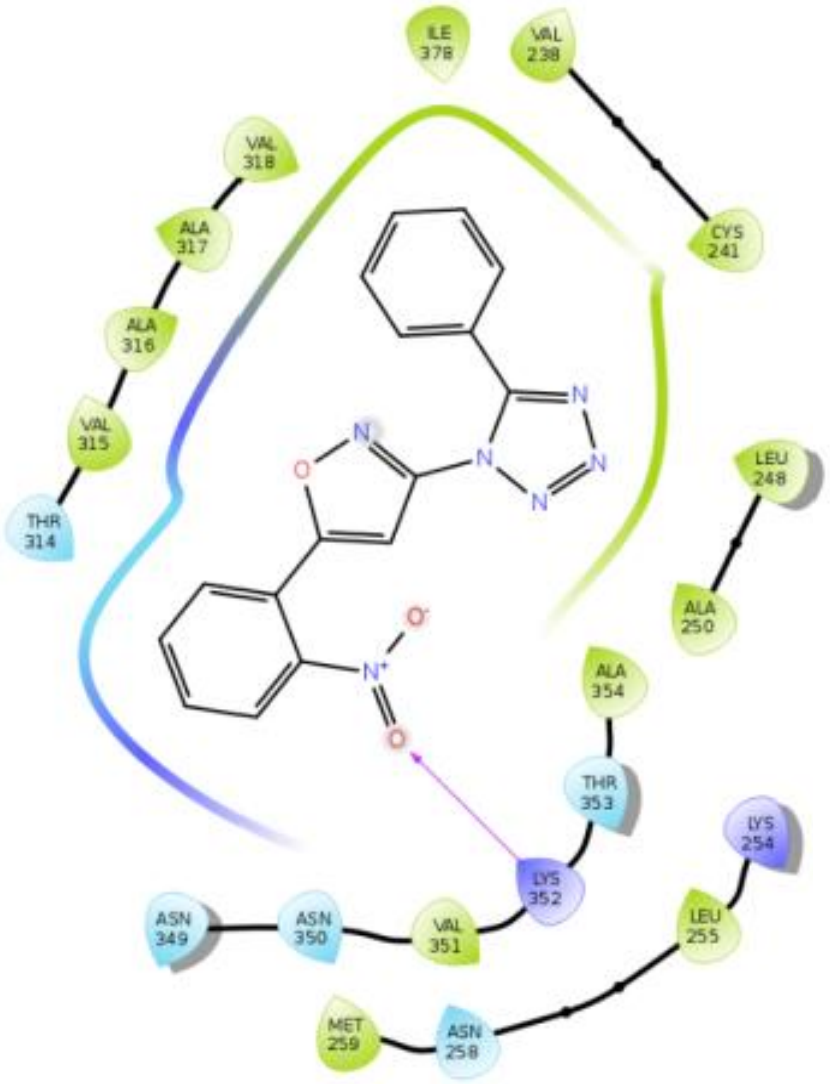
The possible mechanism of action of synthesised compounds would be inhibition of non-covalent polymerisation of tubulin into microtubules. Tubulin, the major structural component of microtubules, is a target for the development of anticancer agents. Microtubules are a key component of the cytoskeleton, 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.

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-



4b



4i

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

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 the 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 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) SR (Leukemia)	65.5 69.34
4c	778577	100.12	75.41 - 116.41	A498 (Renal cancer) HOP-92 (Non small cell lung cancer)	75.41 81.22
4d	761444	95.48	73.81 - 131.39	HL-60 (Leukemia) UO-31 (Renal cancer)	73.81 78.96
4f	778579	99.11	68.97-117.02	UO-31 (Renal cancer) BT-459 (Breast cancer)	68.97 69.64
4g	778578	100.95	80.52-121.84	UO-31 (Renal cancer) A498 (Renal cancer)	80.52 84.9
4h	778580	101.81	79.84-118.88	A498 (Renal Cancer) BT-459 (Breast cancer)	79.84 80.62
4i	761445	93.18	58.95 - 119.00	CCRF-CEM (Leukemia) SR (Leukemia)	58.95 74.13

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

Comp.	Glide core/docking score	Glide ligand efficiency	Glide rotatable 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

These compounds show an increased anticancer activity, and hence these are 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 indicate the need for additional investigations concerning 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 synthesised from sodium azide and benzonitrile as reported by author earlier (Kaushik et al., 2015). All the synthesised compounds were characterised by different spectral technique. The synthesised compounds were assayed for their in vitro anticancer activity by the National Cancer Institute (NCI), the USA under the drug discovery program. The compound **4b** and **4i** were found to be the 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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