SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTICANCER ACTIVITY OF SOME TETRAZOLE DERIVATIVES

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Different tetrazole derivatives containing isoxazole has been synthesized. 5-phenyl tetrazoles **(1)** was cyclized using sodium azide and ammonium chloride and benzonitrile. The 5-phenyl tetrazoles on treatment with acetic anhydride forms 5-phenyl 1-acetyl Tetrazole (2) which on reaction with different aromatic aldehydes forms chalcones (3a-h).The chalcones further undergo cyclisation with hydroxylamine hydrochloride in presence of KOH to form 5-phenyl-1-(5-substituted phenyl isoxazol-3-yl)-1*H*-tetrazole (4a-h).The chemical structures were confirmed by means of FT-IR, ^{1}H-NMR and elemental analysis. Among the synthesized tetrazole derivatives, eight compounds have been selected and evaluated for their anticancer activity at the National Cancer Institute for testing against a panel of approximately 60 different human tumor cell lines derived from nine neoplastic cancer types. Relations between structure and activity are discussed, the most efficient anticancer compound (**4b**) was found to be active with selective influence on ovarian cancer cell lines, especially on SK-OV-3 with a growth % of 34.94.

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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 it is projected to beginning the primary cause of death there within the coming years [1,2]. 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 medicinal chemistry researchers [3]. 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. The second is the acquisition by cancer cells of multiple-drug resistance. 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 [4].

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 mechanisms 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

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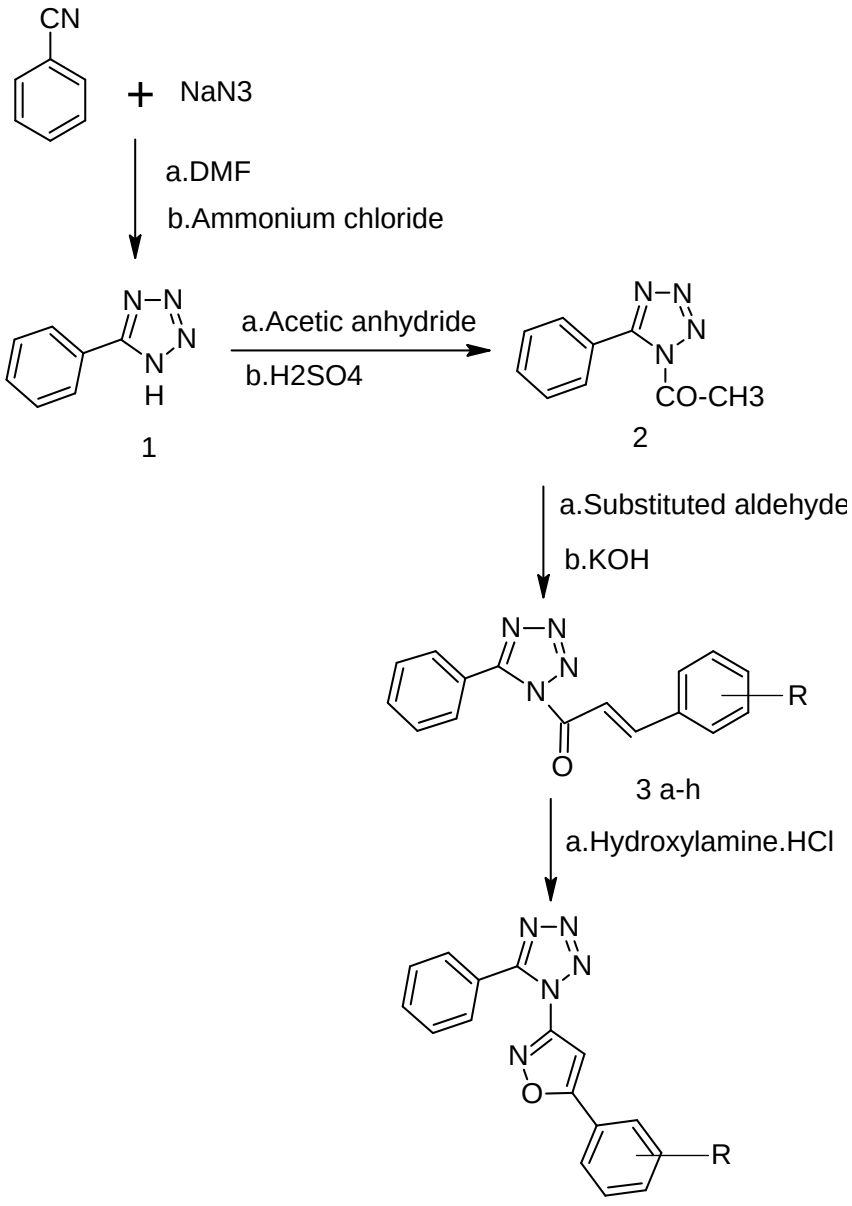
compounds. In this regard, it should be emphasized that National Cancer Institute (NCI) *in vitro* primary anticancer drug screen represents a valuable research tool to facilitate the drug discovery of new structural/ mechanistic types of antitumor agents [5]. The main sources of lead compounds for drug development are natural products, new synthetic compounds and analogues of new agents [6].

Tetrazole derivatives possess very interesting pharmacological and biological properties and are reported to exhibit variety of biological activities like antibacterial [7,8] antifungal and anticonvulsant [9], analgesic[10] ,anti-inflammatory[11] ,antitubercular activity [12] and anticancer activity[13]. Similarly 1, 5 disubstituted tetrazoles have long been known for their pharmaceutical activity as stimulants or depressants on the central nervous system and are reported to show oral antidiabetic and antithrombotic and antimicrobial properties. Isoxazoles may show interesting medicinal or crop protection properties or have some other industrial utility. Various pharmacologically important isoxazoles with antimicrobial [14] .anti-inflammatory, analgesic [15] ,antitubercular activity [16] , antitumoral and antimycobacterial activity [17] .have already been reported. Isoxazoles are unique in their chemical behavior, not only among heterocyclic compounds in general, but also among related azoles[19-22].

It must be emphasized that combination of 5-phenyl tetrazole with other heterocycles is a well-known approach for drug-like molecules build-up, which allows achieving new pharmacological profile, action strengthening or toxicity lowering. Thus, a single molecule containing more than one pharmacophore, each with different mechanism of action could be beneficial for the treatment of cancer. Taking inspiration from the above, and as a part of our enduring research on the "chemistry-driven" approach of tetrazoles.In present work we have synthesized, characterized the different tetrazole derivatives and evaluated for their anticancer activity at National Cancer Institute(NCI),USA. [18].

1.1.Chemistry

Compounds were prepared as shown in Fig.1. The 5-substituted tetrazoles can be synthesized by number of methods, viz.reaction of hydrazoic acid or its salts with imidoyl chloride or imino ethers or diazo coupling of heterocyclic hydrazines or hydrocyanic acid. Most of these methods have limited use in preparative organic chemistry because the use of hydrazoic acid presents considerable experimental difficulties due its toxicity and tendency to explode. However, the simple route reported by Mohite P.B.et al. was adopted for the preparation of 5-phenyl-1, 2, 3, 4- tetrazoles **(1).** This route replaces the toxic hydrazoic acid by inorganic azide to afford the titled compounds in good yield (59–78%). Compound **1** was cyclized using sodium azide and ammonium chloride and benzonitrile. The 5-phenyl tetrazoles on treatment with acetic anhydride forms 5-Phenyl 1-Acetyl Tetrazole **(2)** which on reaction with different aromatic aldehydes forms chalcones **(3a-h).**The chalcones further undergo cyclisation with hydroxylamine hydrochloride in presence of KOH to form 5-phenyl-1-(5-substituted phenyl lisoxazol-3-yl)-1*H*-tetrazole **(4a-h).**



R=H; 2-Cl,4-Cl; 4-Br; 4-OCH3; 3-NO2;; 4-CH3; 4-N-(CH3)2

Fig.1: Synthetic protocol for synthesis of titled compounds

2. Materials and Methods

2.1 Material s

Melting points were determined in open capillaries and were uncorrected. Reactions were monitored by thin layer chromatography using silica gel-G as adsorbent using benzene: ethyl acetate (9:1) as eluent. IR spectra (KBr pellets) were recorded on Shimadzu FT-IR model 8010 spectrophotometer. ^{1}H NMR spectra (DMSO-d6) were taken a Varian mercury spectrometer

(model YH- 300 FT NMR) using TMS as internal standard and chemical shift are expressed in δ ppm.

2.2 General Procedures for synthesis of 5-Phenyl 1-Acetyl Tetrazole (2)[23-24].

A solution of 5-phenyl tetrazole (12.8g, 0.08 moles) and acetic anhydride (0.08 moles) and 2-3 drops of concentrated sulphuric acid was warmed for 15-20 min.on water bath. Cooled and poured into ice cold water.The product separated was filtered and dried .It was further purified by crystallization from ethanol and was obtained in 75% yield as a white amorphous solid: m.p. 214-215 °C IR: 3445 (NH), 3054(Ar-CH), 1608(C=N), 1575(-N==N-),1569(NHdef.),1164,1072(-CN),^{1}H- NMR(DMSO,d)8.80(s,1H,NH),7.08(6H,Ar H).

2.3 General Procedures for synthesis chalcones (3a-h):

A solution of 5-phenyl 1-acetyl tetrazole (2g, 0.010 moles) and aromatic aldehydes (0.010 mole) in ethanol (12 ml) was cooled to 5 to 10.^{o}C in an ice bath. The cooled solution was treated with drop wise addition of aqueous potassium hydroxide (2.5 ml, 50%). The reaction mixture was magnetically stirred for 30 min and then left over night. The resulting dark solution was diluted with ice cold water and carefully acidified using diluted hydrochloric acid. The tetrazole analogues of chalcone which crystallized, were collected by filtration by washing with sodium bicarbonate and water. It was further purified by crystallization from ethanol.

2.4 General Procedures for synthesis of isoxazole (4a-h):

A mixture of Chalcone, (0.01 mol), hydroxylamine hydrochloride (0.01mol, 0.695 g) in ethanol and 40% KOH solution were refluxed for 10 h. Then the reaction mixture was cooled and poured into crushed ice and the product separated out was filtered, washed with water, dried and recrystallised from alcohol to give isoxazole**.**

FT-IR., 1H NMR of newly synthesized compounds

3-phenyl -1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one (3a)

Yield 72% as a white solid: m.p. 197-198 °C ,FT-IR:,1285(N-N=N-),1108 and 1138(Tetrazole ring) ,1735(C=O), 1630(C=C), 3054(Ar-CH).^{1}H NMR:6.61(1H,d,-CO-CH=),7.05(1H,d,=CH-Ar), 7.14-7.80 (10H, m, Ar-H). Anal. For $C_{16}H_{12}N_40$.

3-(2-chlorophenyl)-1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one (3b)

Yield 78% as a white solid: m.p. 161-162 °C ,FT-IR: 1285(N-N=N-),1108 and 1138(Tetrazole ring) ,1733(C=O), 1627(C=C), 3052(Ar-CH), 785(C-Cl).^{1}H NMR: 6.62(1H,d,-CO-CH=),7.06(1H,d,=CH-Ar), 7.14-7.75 (9H, m, Ar-H). Anal. For $C_{16}H_{11}ClN_40$

3-(4-chlorophenyl) -1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one (3c)

Yield 66% as a white solid: m.p. 223-224 °C ,FT-IR: 1284(N-N=N-),1108 and 1138(Tetrazole ring) ,1734(C=O), 1632(C=C), 3050(Ar-CH), 785(C-Cl).^{1}H NMR: 6.63(1H,d,-CO-CH=),7.06(1H,d,=CH-Ar), 7.14-7.80 (9H, m, Ar-H). Anal. For $C_{16}H_{11}ClN_40$

3-(4-bromophenyl) -1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one (3d)

Yield 72% as a white solid: m.p. 248-249 °C ,FT-IR: 1283(N-N=N-),1108 and 1138(Tetrazole ring) ,1735(C=O), 1630(C=C), 3055(Ar-CH), 652(C-Br),^{1}H NMR: 6.5(1H,d,-CO-CH=),7.03(1H,d,=CH-Ar), 7.14-7.81 (9H, m, Ar-H). Anal. For $C_{16}H_{11}BrN_40$

3-(4-methoxyphenyl) -1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one (3e)

Yield 64% as a white solid: m.p. 234-235 °C ,FT-IR: 1285(N-N=N-),1108 and 1138(Tetrazole ring) ,1736(C=O), 1635(C=C), 3058(Ar-CH),1251(-OCH3)^{1}H NMR: 6.58(1H,d,-CO-CH=),7.02(1H,d,=CH-Ar), 7.14-7.75 (9H, m, Ar-H). Anal. For $C_{17}H_{14}N_40_2$

3-(2-nitrophenyl) -1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one(3f)

Yield 59% as a white solid: m.p. 251-252 °C ,FT-IR: 1285(N-N=N-),1108 and 1138(Tetrazole ring) ,1734(C=O), 1630(C=C), 3055(Ar-CH), 1578(-NO2).^{1}H NMR: 6.60(1H,d,-CO-CH=),7.01(1H,d,=CH-Ar), 7.14-7.68 (9H, m, Ar-H). Anal. For $C_{16}H_{11}N_50_3$

3-(4-methylphenyl) -1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one(3g)

Yield 54% as a white solid: m.p. 217-218 °C ,FT-IR: 1285(N-N=N-),1108 and 1138(Tetrazole ring) ,1733(C=O), 1630(C=C), 3054(Ar-CH),1365(CH3).^{1}H NMR: 3.72(3H,CH3)6.62(1H,d,-CO-CH=),7.02(1H,d,=CH-Ar), 7.14-7.50 (9H, m, Ar-H). Anal. For $C_{17}H_{14}N_40$

3-(4-dimethylaminophenyl) -1-(5-phenyl-1*H*-tetrazol-1-yl) prop-2-en-1-one(3h)

Yield 60% as a white solid: m.p. 230-231 °C ,FT-IR: 1285(N-N=N-),1108 and 1138(Tetrazole ring) ,1735(C=O), 1630(C=C), 3054(Ar-CH),1321(-N(CH3)2.^{1}H NMR: 2.9(6H,d,CH_3)6.63(1H,d,-CO-CH=),7.03(1H,d,=CH-Ar), 7.14-7.50 (9H, m, Ar-H). Anal. For $C_{18}H_{17}N_50$

5-phenyl-1-(5-phenyl isoxazol-3-yl)-1H-tetrazole (4a):

Yield 78% as a white solid: m.p. 161-162 °C.FT-IR:, 3048 (Ar-H).,1285(N-N=N-),1108 and 1138(Tetrazole ring) ,2950 and 1610(C=N ring stretch) , 1580(C=C),1540,1470,1450 (N-O ring stretch) .^{1}H NMR (DMSO) d: 6.90 -7.80 (10H, m, Ar-H) 7.05(1H,d,=CH in isoxazole) .Anal. For $C_{16}H_{11}N_5O$.

1-[5-(2-chloro phenyl) isoxazol-3-yl]-5-phenyl-1*H*-tetrazole (4b):

Yield 72% as a yellow solid: m.p. 155-156 °C.FT-IR:, 3055 (Ar-H).,1285(N-N=N-),1108 and 1138(Tetrazole ring) ,2950 and 1610(C=N ring stretch) , 1580 (C=C),1540,1470,1450 (N-O ring stretch) ,758 (C-Cl).^{1}H NMR (DMSO) d: 6.92-7.80 (9H, m, Ar-H) 7.05(1H,d,=CH in isoxazole).Anal. For $C_{16}H_{10}ClN_5O$.

1-[5-(4-chloro phenyl) isoxazol-3-yl]-5-phenyl-1*H*-tetrazole (4c):

Yield 73% as a yellow solid: m.p. 145-146 °C. FT-IR:, 3055(Ar-H).,1285(N-N=N-),1108 and 1138(Tetrazole ring) ,2950 and 1610(C=N ring stretch) , 1580 (C=C),1540,1470,1450 (N-O ring stretch) ,758 (C-Cl).^{1}H NMR (DMSO) d: 6.92 -7.80 (9H, m, Ar-H) 7.05(1H,d,=CH in isoxazole).Anal. For $C_{16}H_{10}ClN_5O$.

1-[5-(4-bromo phenyl) isoxazol-3-yl]-5-phenyl-1*H*-tetrazole (4d)

Yield 65% as a brown solid: m.p. 150-151°C.FT-IR:,3056 (Ar-H).,1285(N-N=N-),1108 and 1138(Tetrazole ring) ,2950 and 1610(C=N ring stretch) , 1580 (C=C),1540,1470,1450 (N-O ring stretch) ,674 (C-Br).^{1}H NMR (DMSO) d: 6.90-7.80 (9H, m, Ar-H) 7.05(1H,d,=CH in isoxazole),Anal. For $C_{16}H_{10}BrN_5O$.

1-[5-(4-methoxy phenyl) isoxazol-3-yl]-5-phenyl-1*H*-tetrazole(4e):

Yield 82% as a redish brown solid: m.p. 125-126 °C. FT-IR:, 3052(Ar-H).,1285(N-N=N-),1108 and 1138(Tetrazole ring) ,2950 and 1610(C=N ring stretch) , 1580 (C=C),1540,1470,1450 (N-O ring stretch) ,1251(-OCH3), 6.91 -7.80 (9H, m, Ar-H) 7.05(1H,d,=CH in isoxazole), 2..37(-OCH3).Anal. For $C_{17}H_{13}N_5O_2$.

1-[5-(3-nitro phenyl) isoxazol-3-yl]-5-phenyl-1*H*-tetrazole (4f):

Yield 62% as a yellowish amorphous solid: m.p. 167–168 °C. FT-IR:, 3054(Ar-H).,1285(N-N=N-),1108 and 1138(Tetrazole ring) ,2950 and 1610(C=N ring stretch) , 1580 (C=C),1540,1470,1450 (N-O ring stretch) ,1578 (-NO2). ^{1}H NMR: 6.80 -7.80 (9H, m, Ar-H) 7.05(1H,d,=CH in isoxazole) Anal. For $C_{16}H_{10}N_6O_3$.

1-[5-(4-methyl phenyl) isoxazol-3-yl]-5-phenyl-1*H*-tetrazole (4g):

Yield 67% as a colorless solid: m.p. 184-185 °C. FT-IR:, 3054(Ar-H).,1285(N-N=N-),1108 and 1138(Tetrazole ring) ,2950 and 1610(C=N ring stretch) , 1580 (C=C),1540,1470,1450 (N-O ring stretch) ,1365 (CH3). ^{1}H NMR: 6.85-7.80 (9H, m, Ar-H) 7.05(1H,d,=CH in isoxazole), 3.72(3H,CH3).Anal. For $C_{17}H_{13}N_5O$

1-[5-(4-dimethylamino phenyl) isoxazol-3-yl]-5-phenyl-1*H*-tetrazole(4h):

Yield 59% as a yellow crystals: m.p. 152-153 °C. FT-IR:, 3048 (Ar-H).,1285(N-N=N-),1108 and 1138(Tetrazole ring) ,2950 and 1610(C=N ring stretch) , 1580 (C=C),1540,1470,1450 (N-O ring stretch) ,1321(-N(CH_3)$_2$) ^{1}H NMR: 7.14-7.80 (9H, m, Ar-H) 7.05 (1H,d,=CH in isoxazole),2.9(6H,d,CH_3). Anal. For $C_{18}H_{16}N_6O$.

Anticancer activity

The compounds (**3b,3c,3d,4b,4c,4d,4e,4f**) were screened for preliminary anticancer assay by National Cancer Institute (NCI), Bethesda, Maryland, USA in an *in vitro* 60 human tumor cell lines panel, derived from nine neoplastic cancer types.

Criterion for submission and selection of compounds for testing in the NCI screens

The compounds (**1,3b,3d,4b,4c,4d,4e,4f**) were submitted to NCI under the Developmental Therapeutic Program (DTP) which operates a tiered anticancer compound screening course for the benefit of the general research community with the goal of identifying novel chemical leads and biological mechanisms. Structures of the compounds were selected for screening based on their ability to add diversity to the NCI small molecule compound collection. In addition, the submission of compounds with drug-like properties utilizing the concept of privileged scaffold or structures based on computer-aided design were preferred .

Process of NCI−60 DTP Human Tumor Cell Line Screen

The screening of the compounds (**3b,3c,3d,4b,4c,4d,4e,4f**) operated with the *In Vitro* Cell Line Screening Project (IVCLSP) which is a dedicated service, providing direct support to the DTP anticancer drug discovery program. The process utilized 60 different human tumor cancers of the lung, colon, brain, ovary, breast, prostate and kidney which was aimed in showing selective growth inhibition or cell killing of particular tumor cell lines by specific compound. The screening begins with the evaluation of all selected compounds against these 60 cell lines at a single dose of 10^{-5} M.

Methodology of in vitro cancer screen

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (*Tz*). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final test concentration (10-5 M) with complete medium containing 50 µg/ml gentamicin. Aliquots of 100 µl of these drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. Using the seven absorbance measurements [time zero, (*Tz*), control growth, (*C*), and test growth in the presence of drug at the 10-5 M concentration level (*Ti*)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$$[(Ti - Tz)/(C - Tz)] \times 100 \text{ for concentrations for which } Ti \geq Tz,$$
$$[(Ti - Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz$$

Table 1. Anticancer screening data of tested compounds

Comp No.	Number assigned by NCI	60 cell line assay in one dose at 10^{-5} concentration			
		Mean growth %	Range of growth %	Most sensitive cell line	Growth of most sensitive cell line %
3b	751204-K	100.78	-20.59 to 40.60	CNS cancer (SNB-75)	-20.59
3c	751201-H	98.35	-29.69 to 55.77	Renalcancer (UO-31)	-29.69
3d	751202-I	96.81	-28.61 to 47.91	Renal cancer (UO-31)	-28.61
4b	75200-1	84.09	-65.06to 98.06	Ovarian cancer (SK-OV-3)	-65.06
4c	753544-C	104.54	-17.68 to44.00	Renal cancer (UO-31)	-17.68
4d	753545-I	107.18	-21.11 to45.87	CNS cancer (SNB-75)	-21.11
4e	753546-G	106.33	-17.26 to 43.97	Ovarian cancer (OVCAR-5)	-17.26
4f	751203-J	97.01	-23.46 to 39.94	Renal cancer (UO-31)	-23.46

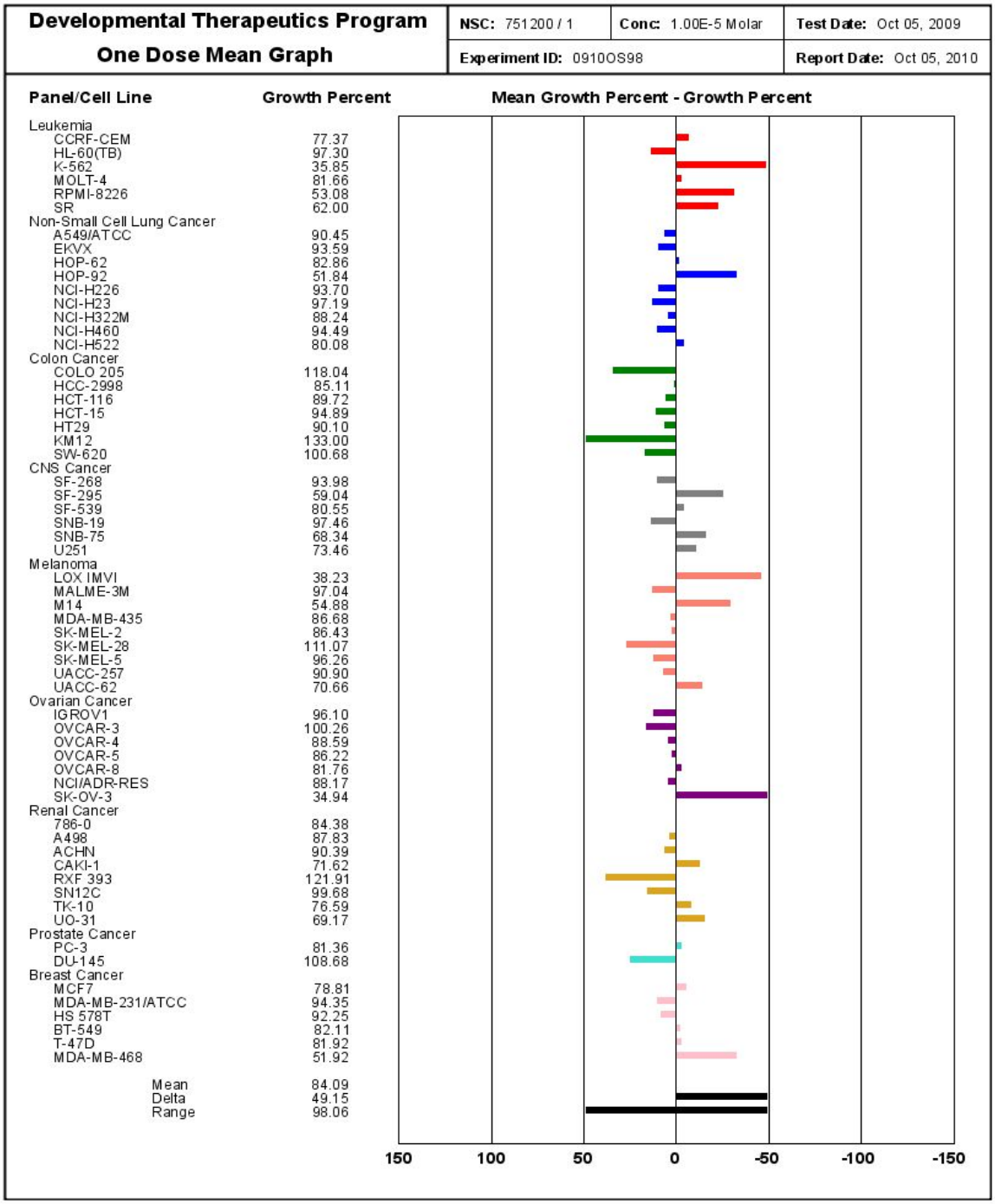


Fig. 1. Selected NCI sixty cell screening data highlighting the potency of compound (1; NSC: D-751200) against renal cancer cell line (RXF 393). Bars to the right of the mean line represent cell lines more sensitive to test compound compared to mean, whereas bars to the left represent less sensitive cell lines.

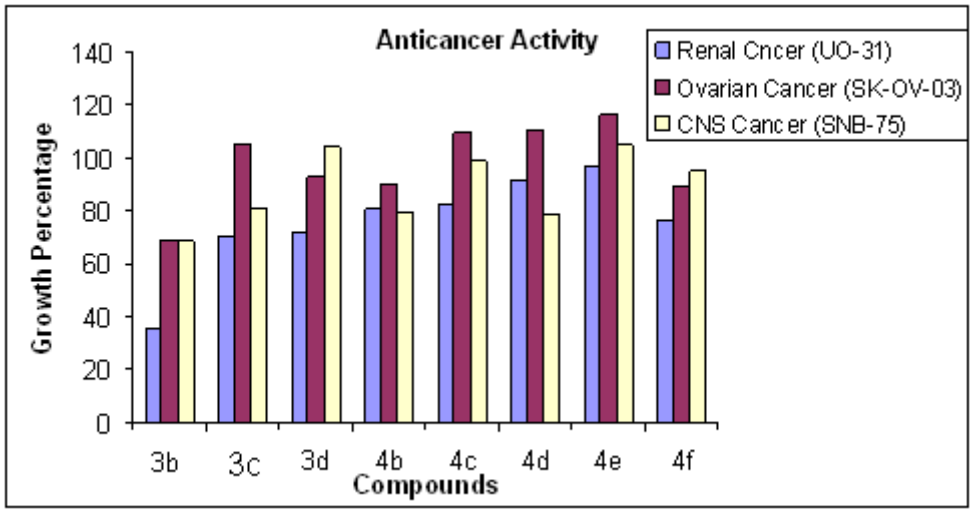


Fig. 2.Anticancer activity of titled compounds against three cell lines.

3. Results and discussion

Tetrazole contains cyclic secondary amino group. All secondary amine undergo acetylation reaction with acetic anhydride and a conc.H_2SO_4. 5-phenyl-1,2,3,4- tetrazoles being a secondary amine was acetylated to compound **2** by acetic anhydride and a conc.H_2SO_4 . The yield of the compound **2** was found to be quantitative and it was readily converted to chalcones by treating them with different aromatic aldehydes and pottasium hydroxide and hence nine different derivatives are synthesized. Infrared spectrum of compound **1** showed absorption bands at 1040, 1108, 1248, 1280 and 1595 cm^{-1} which are attributed to tetrazole ring. An absorption band at 3448 cm^{-1} is attributed to N–H stretching of the tetrazole ring. Characteristic absorption bands were observed for chloro, nitro group, bromo group, dimethylamino group, methyl group, methoxyl group and aromatic region of the synthesized compounds. ^{1}H-NMR spectra of the synthesized compounds showed multiplets in the range of d 6.7–7.80 for aromatic protons. The expected signals with appropriate multiplicities for different types of protons such as methyl, methoxy groups were observed for the derivatives within the range.

The compounds were evaluated at single concentration of 10^{-5} M towards the panel of approximately 60 cancer cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostrate and breast cancers. Preliminary anticancer assay was performed according to the US NCI protocol All the compounds (**3b,3c,3d,4b,4c,4d,4e,4f**) were added to a previously prepared cell culture at a single concentration. The cell culture was incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB).

The mean growth %, range of growth % and growth % relative to most sensitive cell line is depicted in Table 1. The tested compounds showed a broad spectrum of growth inhibitory activity against human tumor cells, as well as some distinctive patterns of selectivity Fig.1. Compound (**4e**) was found to be a highly active growth inhibitor of the ovarian cancer cell line (SK-OV-3)with a growth % of most sensitive cell line to be -17.26, whilst least active over other cell lines. The mean growth % for compound (**4e**) was observed 106.33 % and fall in a range of -17.26 to 43.97 Compounds (**3b**), **(3d)**,(**4c**) and (**4f**) showed selectivity on renal cancer (UO-31) with a growth % of most sensitive cell line to be -29.69, -28.61,-17.68 and -23.46, respectively. These compounds showed varying range of growth % -29.69 to 55.77 for compound (**3b**), -28.61 to 47.91, for compound (**3d**),-17.68 to 44.00 for compound **(4c)** and -23.46 to 39.94 for compound (**4f**). The compound (**4b,4d**) possessed significant activity on CNS cancer cell line(SNB-75) with

growth % of most sensitive cell line as -20.59 and 21.11 respectively. The range of growth % was found to be -20.59 to 40.66 and 21.11 to 45.87 respectively.

The SAR study revealed that anticancer activity of compounds (**3b,3c,3d,4b,4c,4d,4e,4f**)) is sensitive to the nature of substitutents in position-1 of tetrazole.Among the compounds tested, compound (**3b**) with chloro phenyl substitution on isoxazole ring which is present at first position of tetrazoledemonstrates the most marked effect and possessed significant activity (Fig. 2).

Amongst all, the compound (**4f**) with p-methoxyphenyl substituted derivative was found to be least active. The results also states that heterocyclic rings, isoxazole do not support eminently for the anticancer activity. In fact, the chloro substituted tetrazole were found to have encouraging sensitivity to cell lines compared to bromo.

4. Conclusions

In the present paper eight compounds were tested and most of them displayed antitumor activity on renal cancer,CNS cancer cell and ovarian cancer cell llines. The most efficient anticancer compound (**3b**) was found to be active with selective influence on ovarian cancer cell lines, especially on SK-OV-3 with a growth % of 34.94.The obtained results prove the necessity for further investigations to clarify the features underlying the antitumor potential of tested compounds

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References

[1] Gibbs J. B., Science ,287, 1969,(2000).
[2] Unger C., Drug Future,**22**, 1337,(1997).
[3] Heffeter P., Jakupec M. A., W. Orner, S. Wild, N. G. Keyserlingk, L. Elbling, H. Zorbas, A. Korynevska, S. Knasmuller, H. Sutterluty, M. Micksche, B. K. Keppler, W. Berger, Biochem. Pharmacol. **71**, 426(2006) .
[4] Menta E., Palumbo M., Exp. Opin. Ther. Patents ,**7**, 1401,(1997) .
[5] Boyd M.R., Paull K. D., Drug Dev. Res. **34**, 91 (1995).
[6] Shwartsmann G., Winograd B.,Pinedo H.H.',**12**, 301,(1988).
[7] Kemajl, K., Idriz V., Arben ,H., Sevdije,G., Muharrem I. and Sefkija M.,The FASEB Journal. **21**:790-795,(2007).
[8] Mulwad, V. V. ;Pawar, Rupesh ,B. ; Chaskar, Atul ,C. J. Korean Chem.Soc. **52**,3, 249-256,(2008).
[9] Upadhayaya, R. S.; Jain, S.; Sinha, N.; Kishore, N.; Chandra, R.; and Arora, S. K.; Eur. J. Med. Chem. **39**, 579-592, (2004).
[10] Rajasekaran, A. ;Sankar,N.; Murugesh, A.; Kalasalingam, Rajagopal A.; Archives of Pharmacal Research. **29**,7,535-540(2006).
[11] Mohite, P., B., Pandhare, R. B., Khanage, S.G., Bhaskar V.H., *Journal of Pharmacy Research*. **3**,(1),43-46,(2010).
[12] Adamec ,J.;Waisser K.; Kunes J.; Kaustova, J.; ArchPharm.**2005**;338: 385- 389.
[13] De, Souza, AO., Pedrosa, MT., Alderete, JB., Cruz AF., Prado MA., Alves, RB., Silva CL.; Pharmazie.**60**,(5),396-397,(2005).
[14] Dabholkar V.V.,and Faisal ,Y. A. . *J. Serb. Chem. Soc.* ***,74*** (11) 1219–1228,(2009).
[15] Karbasanagouda T.,A.,Vasudeva A..,Girisha M, Indian .J.Chem.**,48B**:430-437, (2009).
[16] Tangallapally R.J.,, Dianqing Sun, R., Nageshwar B., Robin E.B. Lee, Anne J.M. Lenaerts, Bernd M. and Richard E. Lee. *Bioorg Med Chem Lett*. **17**:(23):6638, (2007).
[17] Ray, S.M. ;Lahiri, S.C.; J. Ind. Chem. Soc., **67**, 324-326(1990).

[18] National Cancer Institute, http://dtp.nci.nih.gov.
[19] Furniss, B.S.; Hennaford, A, J; Smith, P.W.G.; Tatchell, A.R. In: Vogel Text Book of Practical organic chemistry, 5th edn, Addision-Wesley, England, pp. 692: 1261-1262, (1998).
[20] Mann, F.G. and Saunder, B.C. Practical Organic Chemistry, 4th edn, Orient Longman Private Limited, New Delhi-110002. pp 118-119, (2003).
[21] Popat ,K.H .,Nirmavat, K.S., Kachhadia ,V.V., Joshi H.S. J Indian Chem Soc.,**80**,707-778, (2003)..
[22] Sherif ,A.F., Rostom, Hayam M.A., Ashour, Heba A., Abd El, Razik, Abd El Fattah H. Abd El Fattah and Nagwa N. El-Din; *Bioorg Med Chem* **. 17**:6: 2410-2422, (2004).
[23] Rajasekaran A. ,Sankaranarayanan ,M., Kalasalingam A. R., *Archives of Pharmacal Research***.29**:7:535-540(2006).
[24] Mohite ,P. B., Pandhare, R. B., Khanage, S. G. , Bhaskar, V. H. *Digest Journal Of Nanomaterials And Biostructures* , **4**: 4:803 -807(2009).