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Research Article

SYNTHESES AND ANTICANCER EVALUATION OF SOME NOVEL 3-[5-(4-SUBSTITUTED) PHENYL-1,3,4-OXADIAZOLE-2YL]-2- PHENYLQUINAZOLINE-4(3*H*)-ONES

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ABSTRACT

A novel series 7(a-f) 3-[5-(4-substituted) phenyl-1,3,4-oxadiazole-2yl]-2 phenylquinazoline-4(3*H*)-ones have been synthesized and screened for its percent growth inhibition on K_{562} cell lines at different concentrations. IC_{50} values of 7d, 7e and 7f have been reported. Doxorubicin was used as reference drug. Percent growth inhibition on K_{562} cell lines at 1 μM was found to be 50.1, 47.4 and 17.7 for compounds 7d, 7e and 7f. The results indicated that substitution of electronegative atoms showed low anticancer activity

Keywords: Quinazoline-4(3*H*)-one, 1,3,4-oxadiazole, Anticancer activity, K_{562} cell lines

INTRODUCTION

Cancer is basically a disease of cells characterized by a shift in the control mechanism that govern cell proliferation and differentiation. Cell that have undergone neoplastic transformation usually express cell surface antigens that may be of normal fetal stype, and may exhibit qualitative or quantitative chromosomal abnormalities, including various translocations and appearance of amplified gene sequences. Such cell proliferates excessively and form local tumors that can compress or invade adjacent normal structure[1]. The era of cancer chemotherapy began in the1990s with the initial use of antifolate drugs and nitrogen mustards. The clinical use of these cytotoxic (cell killing) chemotherapeutic agents against malignant tumors are successful in many cases, but suffer from major drawbacks: the one being the selection of drug resistance[2]. Recent development in chemotherapy begins with the use of antibiotics, hormonal agents and various other synthetic molecules. Quinazolinone nucleus containing molecules are known to display a wide range of anticancer activity. Raffa *et al*.[3,4] synthesized new series of 3-heterocyclo-substituted 2-styrylquinazolinone as anticancer agent. They observed that substitution of 2-pyrimidinyl at third position and styryl position at second position of 4(3*H*) quinazolinone showed anticancer activity

Experimental Section

Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded for the compounds on Perkin Elmer Spectrophotometer in KBr pellets, H^1-NMR spectra was recorded on Bruker Avance Spectrometer at 400 MHz using $CDCl_3$ as the solvent. Chemical shifts were reported in parts per million (ppm) using trimethylsilane (TMS) as an internal standard. The purity of the compounds was confirmed by thin layer chromatography using silica gel glass plates and a solvent system of chloroform: methanol (8:2). The spots were developed in iodine chamber and visualized under ultra violet lamp.

The synthetic route is outlined in Fig. 1. Substituted aryl semicarbazones **(2)** were prepared according to the method reported in the literature using semicarbazide hydrochloride and substituted aldehydes in alcohol[5]. 2-Amino-5-aryl-1,3,4-oxadiazoles **(3)** were prepared by cyclization of semicarbazones of the corresponding aromatic aldehydes in the presence of bromine in glacial acetic acid[6]. *N*- benzoyl anthranilic acid **(5)** was prepared by reaction of anthranilic acid with benzoyl chloride. The reaction is followed by dehydrative cyclization of *N*-benzoyl anthranilic acid to form 2-phenyl benzoxazinone **(6)**. Title compounds **7(a-f)** were synthesized by refluxing 2-phenyl benzoxazinone (6) with 2-amino-5-aryl-1,3,4-oxadiazole (3) in glacial acetic acid . All the synthesized compounds were characterized by spectral analysis (IR, H^1-NMR). Thin layer chromatography (TLC) was run throughout the reaction to optimize the reaction for purity and completion.

Synthesis of 4-substitutedbenzaldehyde semicarbazone (2a-2f)

A solution of aromatic aldehyde (0.2 mol) in warm alcohol (250 mL) and a solution of semicarbazide (0.2 mol) and sodium acetate (0.4 mol) in 50-60 mL of warm water were mixed slowly with continuous stirring. The product separated immediately on cooling.

Synthesis of 2-amino-5-aryl-1,3,4-oxadiazoles (3a-3f)

Substituted semicarbazone (0.1 mol) and sodium acetate (0.2 mol) was dissolved in 300-350 mL of warm glacial acetic acid with continuous stirring. Bromine (7 mL in 50 mL of glacial acetic acid) was added slowly to it. Solution was stirred for an hour and then poured onto crushed ice. The separated solid was dried and recrystallized from 95% hot ethanol.

Synthesis of N-benzoyl anthranilic acid (5a-5f)

Anthranilic acid (4) (0.1 mol) was added in 360 mL of (5%) sodium hydroxide solution in a well-corked conical flask. Benzoyl chloride (36 mL) was added slowly with constant shaking until the odour of benzoyl chloride disappeared. The resulting solid was separated, dried and recrystallised from 95% ethanol.

Synthesis of 2-Phenyl benzoxazinone (6a-6f)

The mixture of N-benzoyl anthranilic acid (5) (0.01mol) and acetic anhydride (0.1 mol) was refluxed under anhydrous condition for 2 h. The excess acetic anhydride was distilled off under reduced pressure. The product so obtained as a solid mass was used up immediately for next step. Physico-chemical data and m.p. of the 5 and 6 was in agreement with the data reported in the literature.

Synthesis of title compounds (7a-7f)

To the mixture of 2-phenyl benzoxazinone (0.1 mol), 2-amino-5-aryl-1,3,4- oxadiazole (0.1 mol) in 100mL of glacial acetic acid was added and refluxed under anhydrous condition for 4 h. After cooling it was poured into crushed ice. The solid was filtered out, dried and recrystallised from hot ethanol (95%). The yields and melting point of synthesized compound are recorded in table 1.

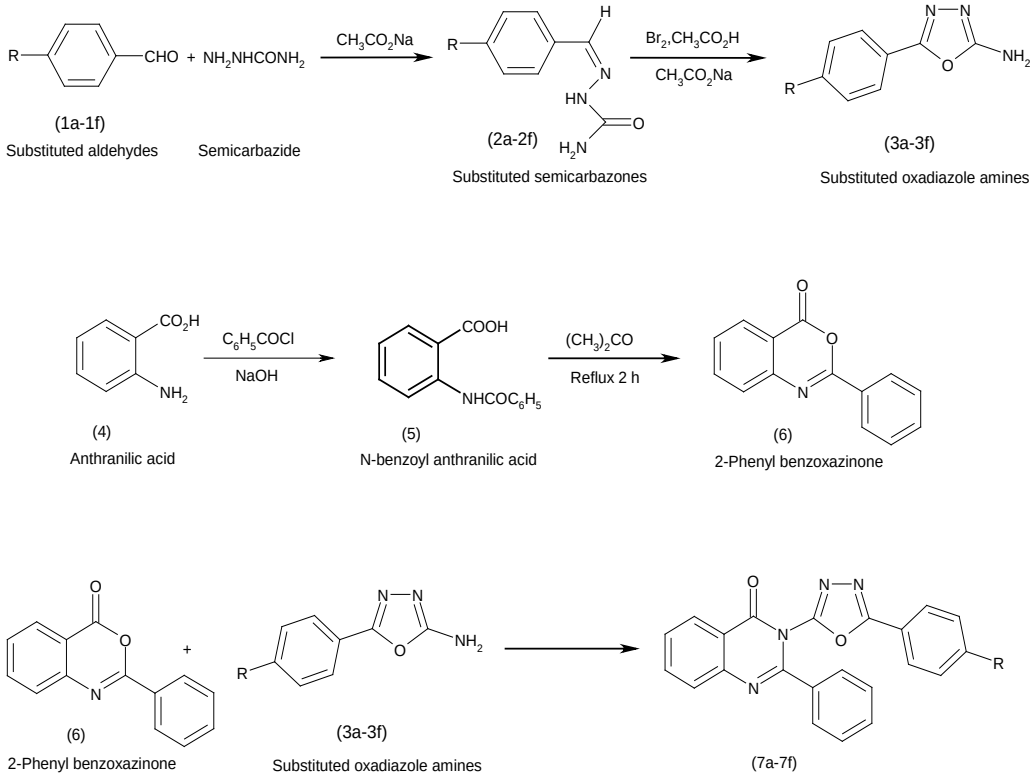


Fig. 1: Scheme for synthesis of target compounds

7a IR (cm^{-1}) 1683.1 (C=O str. in quinazolinone ring), 1608.7 (C=N str.), 1159.3 (C-O-C str. in oxadiazole ring), 849.3 (Ar-CH out of plane bending vibration), ^{1}HNMR (400 MH_z, δ) 7.22-7.9 (m, 14H, 3Ar-H)

7b IR (cm^{-1}) 1684.3 (C=O str. in quinazolinone ring), 1608.9 (C=N str.), 1180.6 (C-O-C str. in oxadiazole ring), 849.3 (Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ) 7.29-7.9 (m, 13H, 3Ar-H)

7c IR (cm^{-1}) 1684.4 (C=O str. in quinazolinone ring), 1609.4 (C=N str.), 1180.5 (C-O-C str. in oxadiazole ring),1231.5 (Asym. C-O-C str. of aryl alkyl ether), 1025.0 (sym. C-O-C str. of aryl alkyl ether), 2983.4 (Alkane C-H str.) 848.3 (Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ) 6.83-7.9 (m, 13 H, 3Ar-H), 3.73 (s, 3H, OCH_3)

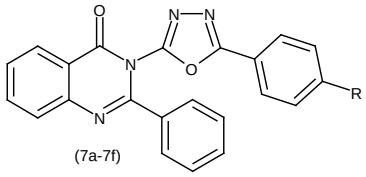
7d IR (cm^{-1}) 1660.9 (C=O str. in quinazolinone ring), 1583.8 (C=N str.), 1181.8 (C-O-C str. in oxadiazole ring), 2853.6 (Alkane C-H str.), 829.1 (Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ) 7.12-7.9 (m, 13H, 3Ar-H), 2.35 (s, 3H, CH_3)

7e IR (cm^{-1}) 1685.0 (C=O str. in quinazolinone ring), 1643.4 (C=N str.), 1158.3 (C-O-C str. in oxadiazole ring), 1538.5 (Asym. NO_2 str.), 1314.9 (Asym. NO_2 str.) 849.6 (Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ) 7.29-8.25 (m, 13H, 3Ar-H)

7f IR (cm^{-1}) 1684.8 (C=O str. in quinazolinone ring), 1645.6 (C=N str), 1157.9 (C-O-C str. in oxadiazole ring), 1039.2 (Aromatic C-F str.) 843.1(Ar-CH out of plane bending vibration) ^{1}HNMR (400 MH_z, δ), 7.03-7.9 (m, 13H, 3Ar-H)

Table 1: Physical data of 3-[5-(4-substituted)phenyl-1,3,4-oxadiazole-2yl]-2-phenylquinazoline-4(3*H*)-ones

S. No.	Code	Molecular Formula	-R	M.P. (°c)	% Yield
1	7a	$C_{22}H_{14}N_4O_2$	-H	123-125	37.20
2	7b	$C_{22}H_{13}ClN_4O_2$	-Cl	113-116	42.56
3	7c	$C_{23}H_{16}N_4O_3$	-OCH_3	132-135	29.32
4	7d	$C_{23}H_{16}N_4O_2$	-CH_3	162-165	46.82
5	7e	$C_{22}H_{13}N_5O_4$	-NO_2	155-158	25.80
6	7f	$C_{22}H_{13}FN_4O_2$	-F	149-152	26.89



(7a-7f)

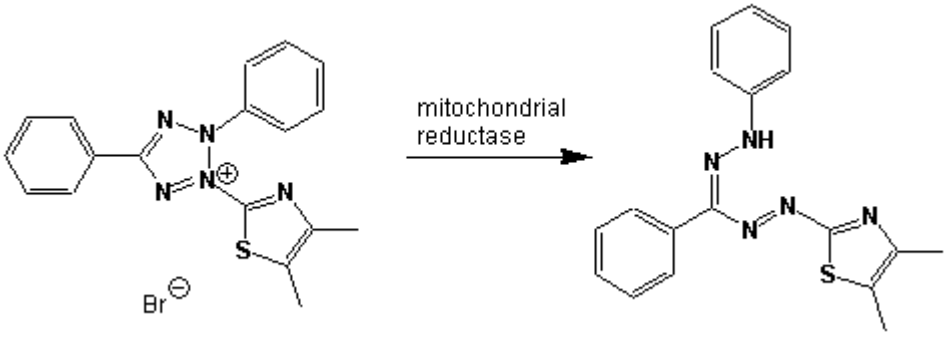
Cell Line Study

The synthesized compounds were tested for their *In vitro* anticancer activity on human myelogenous leukemia K_{562} cells by MTT (3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazoliumbromide) assay. This cell lines (K_{562} cells) were grown at 37°C in a humidified atmosphere containing 5% CO_2, in RPMI-1640 medium (Biochrom KG) supplemented with 10% fetal calf serum and antibiotics

Cytotoxic MTT assay

MTS assay is laboratory test for measuring the activity of enzymes that reduce MTT or MTS + PMS to formazan, giving a purple color. A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by spectrophotometer.

500-10,000 cells in 200µl media per well in a 96 well plate was taken and 8 wells were leaved empty for blank controls. These were incubated (37°C, 5% CO_2) overnight, to allow the cells to get attach to the wells. 2 µl of drug solution in DMSO was added to each well. Well plate was shaked at 150 rpm for 5 minutes, to thoroughly mixed the samples into the media. The plates were further Incubated (37°C, 5% CO_2) for 1-5 days. MTT solution was prepared in PBS (5mg/ml). Added 20 µl MTT solution to each well. Well plate was shaked at 150 rpm for 5 minutes, to thoroughly mix the MTT into the media and then Incubated (37°C, 5% CO_2) for 1-5 h. MTT metabolic product formazan was resuspended in 200 µl DMSO and shaked at 150 rpm for 5 minutes. Note the optical density at 560 nm and subtract background at 670 nm. Optical density was directly correlated with cell quantity.



RESULTS AND DISCUSSION

Percent growth inhibition on K_{562} cell lines at 1 µM was 50.1, 47.4 and 17.7 for compounds 7d, 7e and 7f. The percentage inhibition was least for 7f. Below 1 µM concentration all the compounds were inactive. Table 2 summarizes the growth inhibition observed when K_{562} cells were treated with compound and Doxorubicin (fig.2). Inhibitory effect was observed with compound 7d and 7e. IC_{50} value for compound 7d and 7e was 1.0 and 1.5 µM respectively. Between these two compound 7d was more active than 7e. IC_{50} value of 7f (fig.3) was insignificant (table 3). In the prepared series compound 7d and 7e showed different degree of activity against K_{562} cell lines. The most active compound (7d, fig.4) contained *p*-methyl phenyl group in 1,3, 4 – oxadiazol. Whereas compound (7e, fig.5) has *p*-nitro phenyl group in 1, 3, 4-oxadiazole. The result indicated that electronegative constituent showed low anticancer activity.

Table 2: Percent growth inhibition on K_{562} cell lines at different concentration

Code No.	R	% Inhibition Concentration				
		0.01 µM	**0.1 µM**	**1 µM**	**10 µM**	**100 µM**
7d	$-CH_3$	0.0	0.0	50.1	46.4	54.7
7e	$-NO_2$	0.0	0.0	47.4	54.3	68.6
7f	-F	0.0	0.0	17.7	10.6	17.4
Doxorubicin 0.5 µM				54.3		

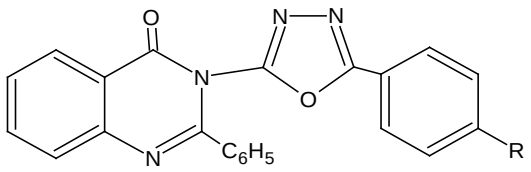


Table 3: IC_{50} values of 7d, 7e and 7f

Compound code	IC_{50} values (μM)
7d	1.0
7e	1.5
7f	n.s*

n.s*: not significant

Fig. 2: Graph between % inhibition of compound and doxorubicin

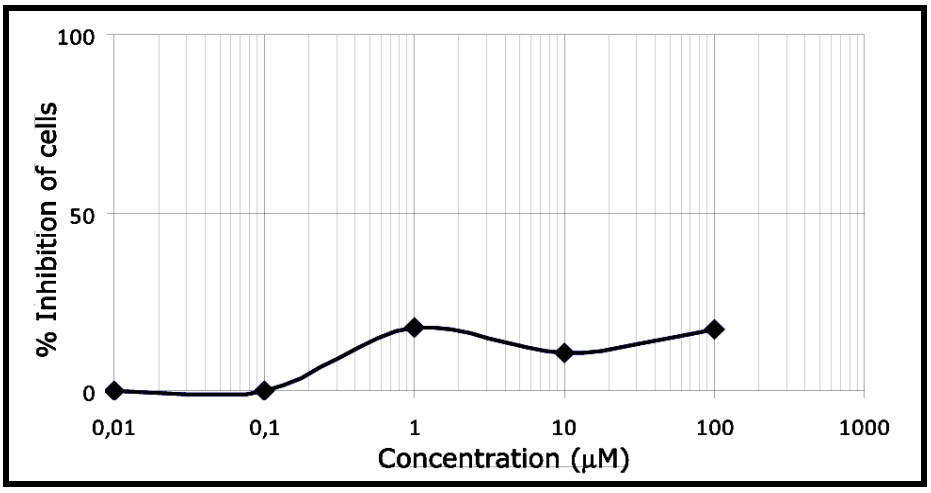


Fig. 3: Graph: % inhibition of cells Vs concentration of compound 7f

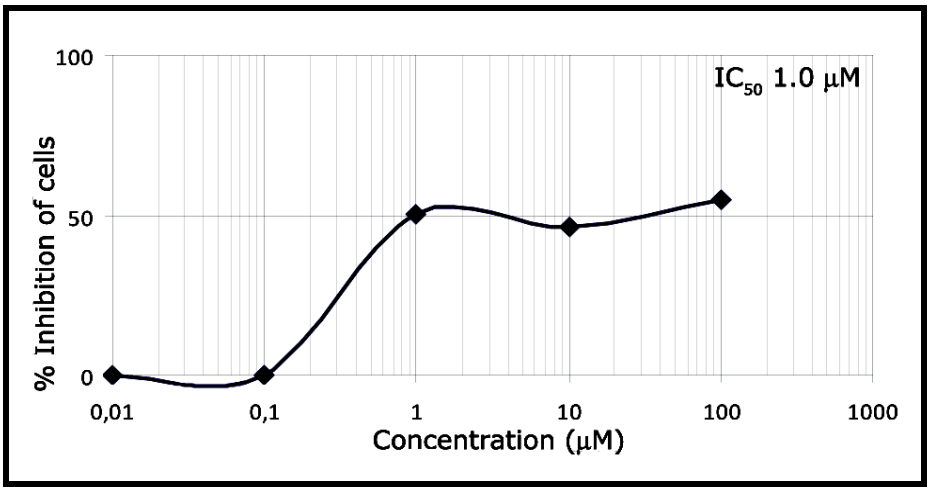


Fig. 4: Graph: % inhibition of cells vs concentration of compound 7d

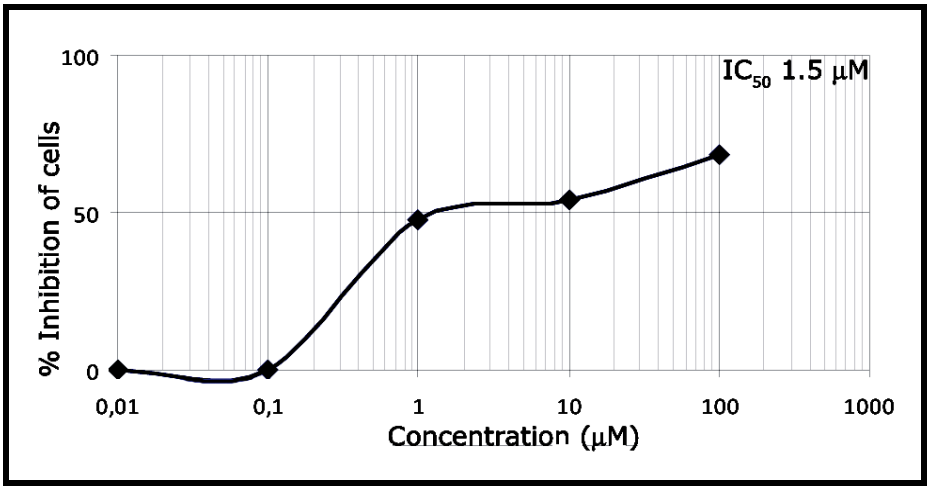


Fig. 5: Graph: % inhibition of cells Vs concentration of compound 7e

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