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SYNTHESIS OF *N*-{[5-(2,4-DICHLOROPHENYL)-1, 3, 4-OXADIAZOL-2-YL] METHYL}AMINE DERIVATIVESAS ANTICANCER PRECURSORS

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ABSTRACT

In the present study *N*-{[5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}amine derivatives5(a-h) were synthesized and tested for *invitro* anticancer activity. Cyclisation of 2, 4-dichlorobenzohydrazide in chloroacetic acid and phosphorous oxy chloride gives 2-(chloromethyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole.This on reaction with various primary and secondary aliphatic or aromatic amines gives the title compounds. The anticancer activity of some of the prepared compounds was evaluated using three human tumour cell lines, representing cervic, liver and breast. The compounds tested were, in most of cases, selective towards liver cancer, where the most potent compound showed IC_{50} = 2.46 µg/ mL. The synthesized compounds were purified by column-chromatography and characterized by LCMS, TLC, IR, and ^{1}HNMR spectral data. Three different cell lines are used for the present study namely (Hela, Hep-G2 and MCF7).

Keywords: Hela, Phosphorus oxy chloride, Hep-G, Anticancer, Triethylamine, MCF7.

INTRODUCTION

In recent years 1,3,4-oxadiazoles and its derivatives have received considerable attention owing to their synthetic and effective biological importance [1]. The heterocycles bearing a symmetrical oxadiazole moiety were reported to show a broad spectrum of pharmacological properties viz., anticonvulsant, insecticidal [2], fungicidal [3], antiviral, anti-cancer [4] activities etc. Cancer is a known medically as a malignant neoplasm is a broad group of diseases involving unregulated cell growth that affecting millions of people worldwide. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Cancer is usually treated with chemotherapy, radiation therapy and surgery. In the present study *N*-{[5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-l] methyl}amine derivatives have been found to be more potent molecules in inhibiting cancer cell lines. 1, 3, 4 - oxadiazole ring are known to have potent anticancer properties. This importance of oxadiazole nucleus and continuing demand for new anticancer agents, prompted us to synthesis about eight 1,3,4-oxadiazole derivatives.

EXPERIMENTAL

The chemicals required for the study were obtained from spectrochem and s-d fine chemicals. The melting points of these synthesized compounds were determined in open capillary tube. The IR spectra were recorded by preparing KBr pellets containing 1% compounds using FTIR-8400 spectrometer. Liquid Crystal Mass spectra(LCMS) of the samples were recorded using Agilet and the ^{1}HNMR spectra wasrecordedusing Varian (400MHz) NMR spectrometer.

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EXPERIMENTAL PROCEDURE

Step-1: Synthesis of ethyl 2, 4-dichlorobenzoate

2,4-Dichlorobenzoic acid (0.0523mol, 10g) was refluxed with concentrated H_2SO_4 (2.5ml) in absolute ethanol (100cm^3) for 4 hours. The formation of ester was monitored by TLC and the solvent was removed under reduced pressure. Ice cold water was added and later the aqueous was neutralised with saturated solution of $NaHCO_3$.The product is extracted with ethyl acetate (25ml x 3) and organic layer was washed with brine (10ml), dried over Na_2SO_4 and evaporated under reduced pressure. The product is taken as such for next step. Yield (%) 88, ^{1}HNMR-1.15(t,3H),3.85(q,2H),7.5(m,2H),8.5 (s,1H) TLC ethyl acetate: hexane (2:8).

Step 2: Synthesis of 2,4-dichlorobenzohydrazide

The ethyl 2, 4-dichlorobenzoate (8.8g) was refluxed with excess of hydrazine hydrate in ethanol for 8 hrs. The reaction was monitored by TLC. After the completion of reaction solvent was removed under reduced pressure. The liquid residue was added with few ice pieces and the solid thus obtained was filtered, washed with cold water, and dried under vacuum. The crude solid was recrystallized with ethanol and used for the next step (3). Yield (%) 68. ^{1}HNMR-4.3(bs,2H),7.5(m,2H),7.8(d,1H),9.4 (s,1H) . IR 1350cm^{-1}(C-N stretching), 3458 cm^{-1} (NH- stretching),1570 cm^{-1}(NH bend),TLC Ethyl acetate: hexane (5:5).

Step 3: Synthesis of 2-(chloromethyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole

The 2,4-dichlorobenzohydrazide(5.9g,0.042mol) was added with phosphorousoxychloride (10ml) and chloroacetic acid(8.116g,3eq,0.0863mol).The reaction mixture was irradiated using microwave for about 5 minutes(30sec/interval).The reaction mixture was neutralised with saturated solution of $NaHCO_3$.The solid thus obtained was filtered, washed with water and dried under vacuum. Yield (%) 45, NMR-7.97(d,1H),7.60(d,1H),7.42(dd,1H),4.56(s,2H), IR1400cm^{-1}(C-N stretching), 3490cm^{-1} (NH- stretching), 1580 cm^{-1}(NH bend), TLC Ethyl acetate: hexane (2:8).

Scheme-2. General procedure for the synthesis of N-{[5-(2,4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl]methyl}alkyl or arylamine derivatives

The2-(chloromethyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole (250mg,1equ.) was dissolved in 1,4-dioxan(10ml) containing triethylamine (100µl).To this reaction mixture various substituted aliphatic or aromatic amines(1.2eq) was added while stirring. The reaction mixture was heated to 80^oC for 2-8hrs.Reaction was monitored by TLC. After the completion of the reaction, reaction mixture was concentrated under reduced pressure, ice cold water was added and the product was extracted using ethyl acetate (10ml x 2).The organic layer was washed with brine(10ml),dried over sodium sulphate and evaporated to dryness.(synthetic scheme-2)

PURIFICATION

All the final compounds were purified by column chromatography using silica gel 100-200 mesh, 100% n-hexane has been usedas eluent for column and later using ethyl acetate it was decreased to 75%. Yield (%) 68.TLC ethyl acetate: hexane (5:5). Table -02 summarizes the physical and analytical data of compounds.

ANTICANCER ACTIVITY

Eight compounds (**5a**, **5b**, **5c**, **5d**, **5e**, **5f**, **5g** and **5h)** have been selected for the screening: Four different concentrations 1, 2.5, 5 and 10 mg/ mL of each compound were employed. Three human cell lines were used in this experiment namely: a) human cervic carcinoma cell lines b) human liver carcinoma cell line (HepG2) and c) human breast carcinoma cell line (MCF7). Stock cultures were grown in T-75 flasks containing 50 mL of RPMi-1640 medium with glutamine bicarbonate and 5% f*et al.,* calf serum. Medium was changed at 48 hr intervals. Cells were dissociated with 0.25% trypsin. Experimental cultures were plated in micro- titre plates , containing 0.2 mL of growth medium per well at a densities of 1,000-200,000 cells per well.

Cell fixation

Cells attached to the plastic substratum were fixed by gently layering 50 mL of cold 50% TCA (4^OC) on the top of the growth medium in each well to produce a final TCA concentration of 10%. The cultures were incubated at 4^OC for one hour and then washed five times with tap water to remove TCA, growth medium and low-molecular weight metabolites, and serum protein. Plates were air dried and then stored until its further use. Background optical densities were measured in wells incubated with growth medium without cells. The anionic dye sulforhodamine B (SRB, Sigma Chemical Co.) was dissolved in 1% acetic acid for cell staining and extracted from cells with 10 mM buffered trisbase [tris (hydroxymethyl) amino methane].

SRB Assay

TCA-fixed cells were stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. At the end of the staining period, SRB was removed and cultures were quickly rinsed for four times with 1% acetic acid to remove unbound dye. The acetic acid was poured directly into the culture wells from a beaker. This procedure permitted rinsing to be performed so quickly such that desorption of protein-bound dye does not occur. Residual solution was removed by sharply flicking plates over a sink, which ensured the complete removal of rinsing solution. Because of the strong capillary action in 96-well plates, draining by gravity alone often failed to remove the

rinsing solution by simple inverting technique. After being rinsed, the cultures were air dried until no standing moisture was visible. Bound dye was solubilized with 10 mM unbuffered tris base (pH 10.5) for 5 min on gyratory shaker. OD (optical density) was read on a UVmax microtitre plate reader at 564 nm for maximum sensitivity.

RESULTS AND DISCUSSION

Starting from 2,4-dichloro ethyl benzoate [5], which are prepared according to the method of kaushik and darpan [6], reaction with hydrazine hydrate with ethyl 2,4-dichloro benzoate yields the appropriate 2,4-dichlorobenzohydrazide [7]. Cyclisation of the hydrazine in the presence of excess chloroacetic acid and phosphorous oxychloridegavethecorresponding2-(chloromethyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole [9].The2-(chloromethyl)- 5-(2,4-dichlorophenyl)-1,3,4-oxadiazole which is a key intermediate for the synthesis of N-{[5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]methyl} alkyl or aryl amine [12]. Different aliphatic and aromatic amines were coupled in the presence of1,4-dioxan and triethylamine base with the intermediate No.4 to get the final molecules. Eight different molecules have been synthesised. In order to know the potency of these molecules these have been tested with leukemic cell lines. The AR grade amines were purchased from the commercial sources for the above synthesis.

On the other hand, according to the known chemotherapeutic activities of 1,3,4-oxadiazoles as antiviral, antifungal [14] and anticancer agents [13], it was of interest to incorporate such moiety into the 2,4-dichloro phenyl derivatives. In this connection author has synthesised methylamine derivatives of the various 1,3,4-oxadiazoles into the parent 2, 4-dichloro phenyl backbone to obtain more active and less toxic anticancer agents. So, fusion of the various aliphatic side chain as well as aromatic side chain amines (a-h) with 2-(chloromethyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole [10] afforded the desired N-{[5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}alkyl or aryl amine 5(a-h) respectively.

The presence of methyl amine side chain might overcome the water insolubility problem of 1,3,4-oxadiazole compounds, thus increasing their Bio availability (Scheme 2). On the other hand, since many 2, 4-disubstituted phenyl 1,3,4-oxadiazole derivatives possess anticancer activity [13] it prompted us synthesize some novel 2,4-disubstituted phenyl derivatives.

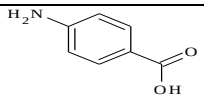
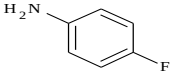
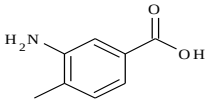
ANTICANCER SCREENING

Above eight compounds were screened for anti-cancer activity at Centre for Cellular and Molecular Platforms (C-CAMP),Tata institute of Fundamental Research (TIFR), Bangalore, India. Three cell lines were used for the evaluation (Human cervic carcinoma cell line, human liver carcinoma cell line and human breast carcinoma cell line).The results are expressed in the form of the concentration of compound that causes 50% inhibition of cells growth. The *invitro* evaluation revealed that the activity of the tested compounds was higher towards both the liver cancer and cervic cancer than the breast cancer. Two compounds (5c, 5d,) were showed the activity towards all the 3-cell lines while compounds **5b,** 5c, 5d, 5g, 5e and 5f were selective towards the liver cancer. Compound 5h was the only compound not selective towards any cell line. The cytotoxicity of the compound 5h is very high towards all the 3 cell lines. The results of the anticancer screening of the tested compounds are illustrated in Table no 3.

Structure-activity relationship (SAR)

From the data it is found that the compounds 5(a-h) were the most effective against the Cervical and Liver carcinoma cell line (HepG2) having IC_{50}value 3.54µg/ml and 2.46µg/mL respectively. Whereas compounds 5b, 5f, 5g, and 5h, were nontoxic against the breast cell line. Compound 5f is the only one compound selective towards liver cancer cell lines showing the IC_{50} of 6.08 µg/mL. The activity of the tested compounds could be correlated with structure variation and modification. The 5a, 5b, 5g, three compounds exhibited good result on Cervic cancer cell line showing the IC_{50} of 6.08µg/ml,4.08µg/ml and 9.04µg/ml respectively.

Table 1. Percentage yield and m/z of the various 1, 3, 4-oxadiazole derivatives

Compounds Name/No	R^2	m/z	Yield(%)
5a	 (a)	364	85
5b		338	76
5c		378	65

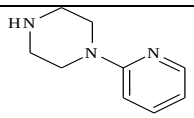
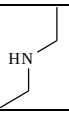
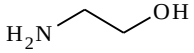
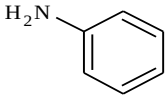
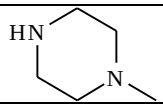
5d		390	38
5e		300	78
5f		288	68
5g		320	55
5h		327	45

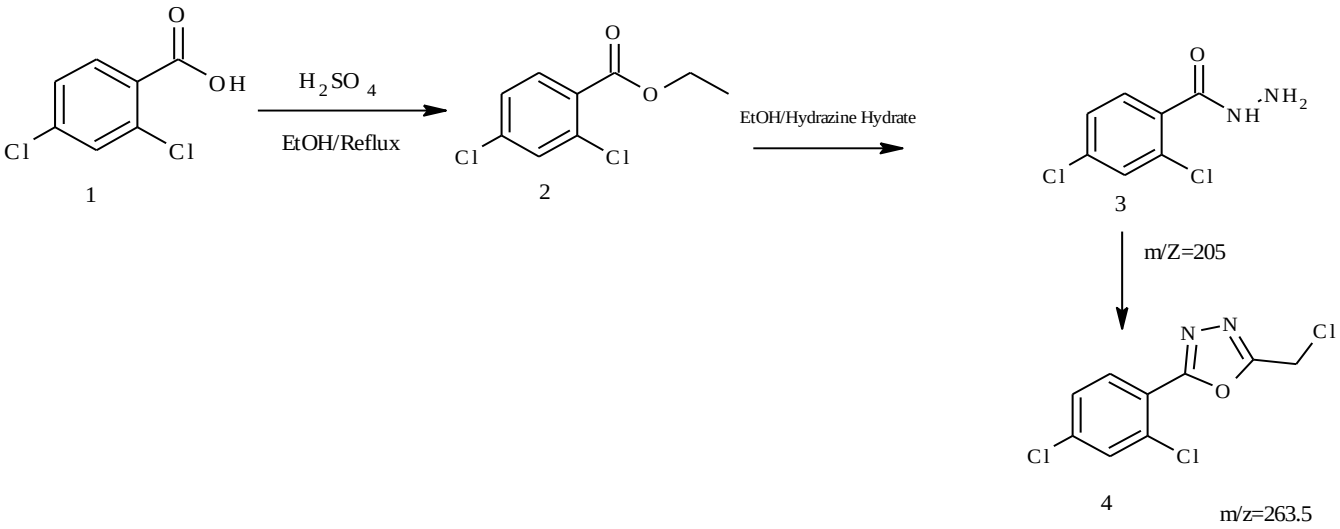
Table 2. Physicochemical characteristics of compounds 5(a-h)

Compounds	Molecular formula	IR cm^{-1}	LCMS (m+1)	M.P (^oC)	^1H NMR (dmso-d_6)
5a	$C_{16}H_{11}Cl_2N_3O_3$	3300(NH-stretch), 1650 (C=O), 3186 (Ar-H)	366	161	3.7(s,2H),7.1(dd,1H),7.6 (m,2H),8.6(m,3H),8.9 (dd,2H)
5b	$C_{15}H_{10}Cl_2FN_3O$	3322(NH-stretch),3146 (Ar-H)	340	143	5.1 (bs,2H),6.85 dd,2H),7.15 (dd,2H)
5c	$C_{17}H_{13}Cl_2N_3O_3$	3414 (NH-stretch), 1690(C=O), 3032 (Ar-H)	380	148	1.5(s,1H),3.7(s,2H),7.5 (m,3H),7.7(m,3H),11.5 (bs,1H)
5d	$C_{18}H_{17}Cl_2N_5O$	3373(NH-stretch), 3142 (Ar-H)	392	155	2.5 (q,2H),2.7(q,2H),3.3 (s,2H),6.9(dd,2H),7.10 (dd,2H),7.7 (m,2H),7.85(m,1H)
5e	$C_{13}H_{15}Cl_2N_3O$	3350(NH-stretch), 3122 (Ar-H)	301	208	0.6 (t,6H),2.3(q,4H),3.6 (s,2H),7.6(dd,1H),7.45 (m,2H)
5f	$C_{11}H_{11}Cl_2N_3O_2$	3120(Ar-H),1280(C-N), 1560 (NH-bend), 3340(NH-stretch), 1529(C-O) bend	289	211	2.6(m,4H),3.6 (s,2H),7.45 (m,2H),7.6(dd,1H),12.35(bs,1H)
5g	$C_{15}H_{11}Cl_2N_3O$	3152 (Ar-H),1300 (C-N), 1580(NH-bend), 3360(NH-stretch),	321	189	5.1(s,1H),3.7 (s,2H),6.9 m,2H),7.7(dd,2H)
5h	$C_{14}H_{16}Cl_2N_4O$	3120(Ar-H), 1200(C-N), 1560(NH-bend),3340(NH-stretch),	328	176	1.85(s,3H),2.6(q,4H),3.7(s,2H),7.6 (dd,,2H),7.45(m,2H)

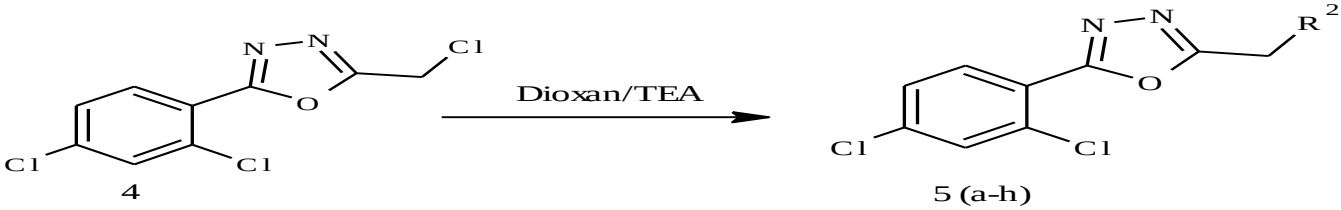
Table 3. Effect of the synthesised compounds on Hela, Liver carcinoma and Breast carcinoma cell lines

Compounds	IC 50(µg/ml)		
	Hela	Hep-G2	MCF7
5a	6.08	-	7.89
5b	4.56	6.78	-
5c	6.78	4.34	5.45
5d	3.54	2.46	8.98
5e	-	3.40	4.56
5f	-	4.10	-
5g	9.04	5.62	-
5h	-	-	-

Reaction Scheme 1. Synthesis of 2-(chloromethyl)-5-(2,4-dichlorophenyl)-1,3, 4-oxadiazole



Reaction Scheme 2. Synthesis of N-{[5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl] methyl} alkyl or aryl amine.



N-{[5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}alkyl or aryl amine

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REFERENCES

1. Kaushik, Darpan *et al.*, *Medicinal Chemistry Research*, 21(11), 2012, 3646-3655.
2. Schmidt, Andreas *et al.*, *Organic Letters*, 9(18), 2007, 3515-3518.
3. Yoshino, Tomonori *et al.*, *Tetrahedron*, 62(6), 2006, 1309-1317.
4. Gaonkar, Santosh L, Nagashima, Izuru, Shimizu, Hiroki. *Organic Chemistry International*, 2011, 751894.
5. Kaushik, Darpan, Kumar, Rajnish, Khan, Suroor A, Chawla, Gita. *Medicinal Chemistry Research*, 21(11), 2012, 3646-3655.
6. Saha, Ajoy, Kumar, Rajesh K, Rajendra, Deva KC. *Indian Journal of Chemistry, Section B: Organic Chemistry Including Medicinal Chemistry*, 49B (4), 2010, 526-531.
7. Lakshman, Bijul A, Gupta R. *Asian Journal of Chemistry*, 21(1), 2009, 86-92.
8. Hols W, Dan W, Jo M, Ondrej K, Stefan. *PCT Int. Appl*, 2010, WO 2010139966 A1 20101209.
9. Zhang, Rong and Qian, Xuhong. *Yingyong Huaxue*, 13(5), 1996, 5-9.
10. Rusay, Ronald J. 1984, US 4488897 A 19841218
11. Singh H *et al.*, *Indian Journal of Pharmaceutical Sciences*, 51(3), 1989, 100-103.
12. Talawar, Mahadev B,Laddi, Umesh V, Bennur, Rajani S, Bennur Shankar C. *Indian Journal of Heterocyclic chemistry*, 4(2), 1996, 111-116.
13. Kamelia MA, Magdi IE, Manal MA, Mohsen MK, Maaly HM. *Acta Poloniae Pharmaceutica and Drug Research*, 66(3), 2009, 279-291.
14. Zhang, Rong, Qian, Xuhong. *YingyongHuaxue*, 13(5), 1996, 5-9.