

Synthesis and cytotoxicity evaluation of [(2,4-dichlorophenoxy)methyl]-5-aryl-1,3,4-oxadiazole/4*H*-1,2,4-triazole analogues

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Received: 09.03.2018

Accepted/Published Online: 30.05.2018

Final Version: 11.10.2018

Abstract: We report herein the synthesis, characterization, and cytotoxicity evaluations of some newer oxadiazole and triazole analogues (**5a-j**). The cytotoxicity of all the title compounds were evaluated as per the National Cancer Institute protocol in a one-dose assay (10 μ M) on nine different panels of 59 cancer cell lines. 2- {5-[(2,4-Dichlorophenoxy)methyl]-1,3,4-oxadiazol-2-yl} phenol (**5e**) showed the maximum cytotoxicity among the series of ten compounds. The cytotoxicity of **5e** was comparable to that of the standard anticancer drug, 5-fluorouracil, and better than that of imatinib. The structure activity relationship was also discussed.

Key words: Anticancer activity, cancer cell lines, cytotoxicity, one dose assay, oxadiazole, triazole

1. Introduction

Cancer is uncontrolled growth of abnormal cells that grow outside their usual boundaries and then assault the adjoining parts of the body and spread to other organs. Cancer is the second leading cause of deaths worldwide and accounted for 8.8 million deaths in 2015.¹ In 2018, 1,735,350 new cancer cases and 609,640 cancer deaths are projected to occur in the United States.² There are many types of cancer treatment, and the treatment strategy depends upon cancer type and stage. The most important cancer treatments are chemotherapy, surgery, radiation therapy, immunotherapy, targeted therapy, and hormonal therapy. Stem cell transplant and precision medicine may also help during cancer treatment.³ Chemotherapy is a major part of cancer therapeutics; however, it has its own limitations of limited efficacy, selectivity, high cost, genotoxicity, and drug resistance.⁴ Extensive research and development, especially in the design and discovery anticancer agents, is the need of the present day.

Compounds containing heterocyclic rings are of great importance both in medicine and industry.⁵ Oxadiazole is one among the heterocyclic rings and has fascinating diverse biological potentials. Oxadiazoles have a large impact on multiple drug discovery programs across a variety of therapeutic areas, including tuberculosis,⁶ cancer,⁷ HIV,⁸ diabetes,⁹ obesity,¹⁰ inflammation,¹¹ and infection.¹² The carbonyl compounds of amides, ester, carbamates, and hydroxamic acids have been successfully replaced with oxadiazole rings for improved efficacy.¹³⁻¹⁵ Similarly, triazole analogues are well reported anticancer agents.^{16,17} The literature on oxadiazoles and our previous published work¹⁸ is a source of inspiration to continue research on further exploration of oxadiazole, and in the present investigation we report herein the synthesis and cytotoxicity evaluation of some new oxadiazole analogues. A few triazole analogues were also synthesized and are reported herein.

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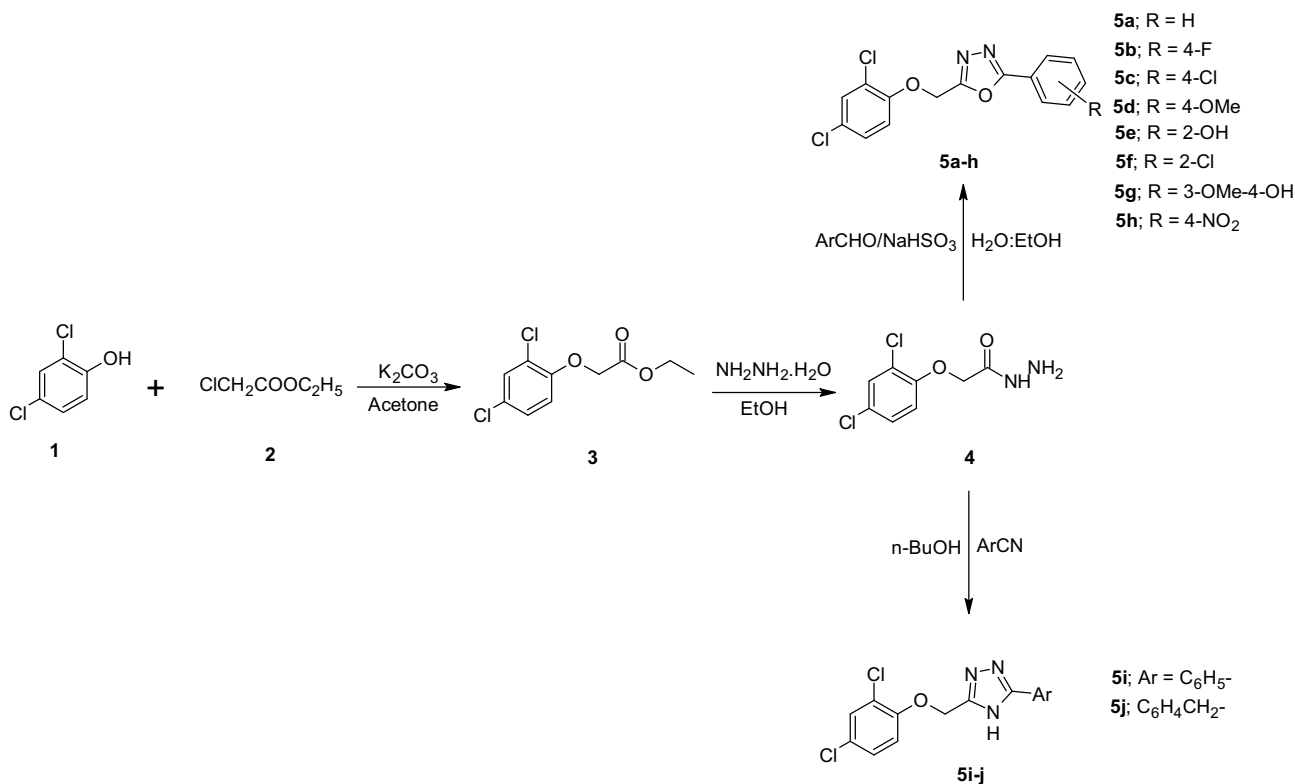
2. Results and discussion

2.1. Chemistry

As shown in the Scheme, ethyl(2,4-dichlorophenoxy)acetate (**3**) was synthesized by stirring a mixture of 2,4-dichlorophenol (**1**) and ethylchloroacetate (**2**) suspended in acetone and potassium carbonate for 24 h.^{18,19} Ethyl(2,4-dichlorophenoxy)acetate (**3**) was further refluxed with hydrazine hydrate in ethanol for 6 h to synthesize 2-(2,4-dichlorophenoxy)acetohydrazide (**4**).^{18,19} In the final step, an equimolar quantity of 2-(2,4-dichlorophenoxy)acetohydrazide (**4**) and aromatic aldehyde was refluxed in an ethanol/water system (1:2, v/v) solvent by adding 20 mol% NaHSO₃ for 10 h to obtain 2-[(2,4-dichlorophenoxy)methyl]-5-aryl-1,3,4-oxadiazole (**5a–h**).¹² The progress of the reaction was monitored throughout by thin-layer chromatography (TLC) using *n*-hexane:ethylacetate (1:1) as mobile phase. Base-catalyzed synthesis of 3-[(2,4-dichlorophenoxy)methyl]-5-aryl-4*H*-1,2,4-triazole (**5i,j**) was achieved by refluxing 2-(2,4-dichlorophenoxy)acetohydrazide (**4**) and nitrile in *n*-butanol for 2–3 h in the presence of K₂CO₃.²⁰ The progress of the reactions was monitored throughout by TLC (Silica gel 60 F₂₅₄) using mobile phase, chloroform–methanol (9:1), and benzene–acetone (9:1). The spots were visualized under either iodine vapor or UV light. All the compounds were obtained in satisfactory yield ranging between 52% and 81%. All the chemicals were procured from CDH (New Delhi, India), Merck (Kenilworth, NJ, USA), and SD Fine (Mumbai, India). The title compounds (**5a–j**) were further characterized by infrared (IR), nuclear magnetic resonance (¹H NMR and ¹³C NMR), and mass spectral data. IR, NMR, and mass spectra data were obtained on a Shimadzu 8201 PC (Kyoto, Japan), Bruker AC 400 MHz spectrometer (in DMSO-*d*₆) (Billerica, MA, USA), and Bruker Esquire LCMS using ESI, respectively. The purity of compounds was checked by elemental analyses (PerkinElmer 2400 elemental analyzer, Waltham, MA, USA). In the ¹H NMR, the prototype compound **5e** showed a singlet at δ 5.27 ppm corresponding to the protons of CH₂, a doublet at δ 6.85 ppm corresponding to one aromatic proton, a double doublet at δ 7.11 ppm corresponding to another aromatic proton of 2,4-dichlorophenyl, a multiplet at δ 7.23–7.70 ppm corresponding to the four aromatic protons, a singlet at δ 8.31 ppm corresponding to the one aromatic proton (2,4-dichlorophenyl), and a singlet at δ 10.03 ppm corresponding to the phenolic proton (ArOH). The ¹³C NMR of compound **5e** showed δ ppm: 166.52, 155.61, 152.83, 142.18, 131.49, 130.51, 128.91, 128.20, 128.13, 124.09, 121.99, 117.17, 116.31, 112.22, and 67.0. The mass spectra showed (M+H)⁺ and (M+2)⁺ at 337 and 338, respectively.

3. Cytotoxicity

All the title compounds (**5a–j**) were evaluated for their cytotoxicity at 10 μ M drug concentrations as per the National Cancer Institute (NCI) protocol on nine different panels of 59 human cancer cell lines.^{21–24} The results of cytotoxicity study are given in Table 1. All the tested compounds showed moderate or weaker cytotoxicity except for **5e**, which showed promising cytotoxicity among the series. The compounds **5a**, **5b**, **5f**, and **5g** showed higher sensitivity towards the UACC 257 (melanoma) [percent growth inhibitions (%GIs) = 43.11, 28.58, 45.05, and 40.58, respectively], NCI-H522 (non-small cell lung cancer) (%GIs = 39.29, 39.28, 36.14, and 40.83, respectively) and A549/ATCC (non-small cell lung cancer) (%GIs = 29.97, 23.44, 20.45, and 28.05, respectively). Compounds **5i** and **5j** showed higher sensitivity towards the UO-31 (renal cancer) with %GIs of 22.89 and 23.43, respectively. Similarly, compounds **5e** and **5h** showed higher sensitivity towards the NCI-H522 (non-small cell lung cancer) with %GIs of 98.03 and 36.89. Overall, excellent growth control (%GI of 98.03) regarding NCI-H522 (non-small cell lung cancer) was observed in compound **5e**. Compounds with GIs of $\geq 68\%$



Scheme. Protocol for the synthesis of 2-[(2,4-dichlorophenoxy)methyl]-5-aryl-1,3,4-oxadiazoles (**5a-h**) and 3-[(2,4-dichlorophenoxy)methyl]-5-aryl-4H-1,2,4-triazoles (**5i,j**).

were considered to be active towards that particular cell line.²⁵ Compound **5e** showed promising cytotoxicity (%GIs of $\geq 68\%$) against 15 different cancer cell lines (Figure 1). Figure 1 shows the %GIs of **5e** on 59 human cancer cell lines at 10 μ M drug concentration. The cytotoxicity of **5e** [mean growth percent (MGP) = 46.12] and 5-fluorouracil (5-FU) (MGP = 42.21) was comparable. Compound **5e** showed far better %GIs on nearly 26 cancer cell lines having 50 cancer cell lines in common (Figure 2). Figure 2 shows the %GIs of **5e** and 5-FU on 50 human cancer cell lines in common at 10 μ M drug concentrations. The average %GIs against the nine panels was also calculated for comparative study and **5e** showed higher sensitivity towards leukemia, non-small cell lung cancer, CNS cancer, and ovarian cancer (Table 2). The cytotoxicity data of imatinib and 5-FU were obtained from the NCI data warehouse index.

The structure activity relationship was established with cytotoxicity studies, and the 2-hydroxy substitution showed the maximum cytotoxicity. The 2-chloro substitution showed higher cytotoxicity than the 4-hydroxy-3-methoxy and nitro substitutions. The order of cytotoxicity in the present investigation was 2-OH > 2-Cl > 4-OH-3-OCH₃ > 4-NO₂, while the overall activity was 3,4-(OCH₃)₂ > 2-OH > 2-Cl > 4-OH-3-OCH₃ > 4-NO₂ > 4-Cl > 4-OCH₃.¹⁸

3.1. Conclusion

All the compounds were synthesized in satisfactory yield and evaluated for cytotoxicity on nine different panels of nearly 60 human cancer cell lines. Compound **5e** showed promising cytotoxicity among the series of compounds.

Table 1. The cytotoxicity evaluation of 2-[(2,4-dichlorophenoxy)methyl]-5-aryl-1,3,4-oxadiazoles (**5a-h**) and 3-[(2,4-dichlorophenoxy)methyl]-5-aryl-4*H*-1,2,4-triazoles (**5i,j**).

Compound	Cancer cell lines assay in single dose assay 10 μ M concentration				
	Mean GP	GP range	The most sensitive cell lines	GP	% GI
5a	98.36	56.89 to 116.71	UACC 257 (Melanoma) NCI-H522 (Non-small cell lung cancer) A549/ATCC (Non-small cell lung cancer) HT29 (Colon cancer) PC-3 (Prostate cancer)	56.89 60.71 70.03 80.15 80.90	43.11 39.29 29.97 19.85 19.10
5b	98.78	60.72 to 117.88	NCI-H522 (Non-small cell lung cancer) UACC 257 (Melanoma) HT29 (Colon cancer) A549/ATCC (Non-small cell lung cancer) HL-60(TB) (Leukemia)	60.72 71.42 72.64 76.56 87.29	39.28 28.58 27.36 23.44 12.71
5c	97.75	72.06 to 126.71	HCT116 (Colon cancer) T-47D (Breast cancer) A549/ATCC (Non-small cell lung cancer) PC-3 (Prostate cancer)	72.06 77.06 77.21 77.25	27.94 22.94 22.79 22.75
5d	99.88	77.31 to 140.84	A549/ATCC (Non-small cell lung cancer) SNB-75 (CNS cancer) UACC 257 (Melanoma) HL-60(TB) (Leukemia)	77.31 81.46 81.55 85.24	22.69 18.54 18.45 14.76
5e	46.12	1.97 to 89.91	NCI-H522 (Non-small cell lung cancer) HL-60 (TB) (Leukemia) HCT-116 (Colon cancer) CCRF-CEM (Leukemia) HT29 (Colon cancer)	1.97 3.89 8.59 15.45 18.16	98.03 96.11 91.41 84.55 81.84
5f	93.33	54.95 to 116.65	UACC 257 (Melanoma) NCI-H522 (Non-small cell lung cancer) MOLT-4 (Leukemia) CCRF-CEM (Leukemia) A549/ATCC (Non-small cell lung cancer)	54.95 63.86 65.29 71.41 71.55	45.05 36.14 34.71 20.59 20.45
5g	94.97	59.17 to 115.61	NCI-H522 (Non-small cell lung cancer) UACC 257 (Melanoma) HL-60 (TB) (Leukemia) A549/ATCC (Non-small cell lung cancer) UO-31 (Renal cancer)	59.17 59.42 61.28 71.95 75.10	40.83 40.58 38.72 28.05 24.90
5h	96.73	63.11 to 120.45	NCI-H522 (Non-small cell lung cancer) HL-60 (TB) (Leukemia) PC-3 (Prostate cancer) CCRF-CEM (Leukemia) UO-31 (Renal cancer)	63.11 70.44 75.54 77.09 78.27	36.89 29.56 24.46 22.91 21.73
5i	98.01	77.11 to 121.90	UO-31 (Renal cancer) SK-OV-3 (Ovarian cancer) CCRF-CEM (Leukemia) PC-3 (Prostate cancer) HOP-62 (Non-small cell lung cancer)	77.11 80.91 85.89 87.34 86.76	22.89 19.09 14.11 13.66 13.24

Table 1. Continued.

Compound	Cancer cell lines assay in single dose assay 10 μ M concentration				
	Mean GP	GP range	The most sensitive cell lines	GP	% GI
5j	97.14	76.57 to 112.18	UO-31 (Renal cancer) HL-60(TB) (Leukemia) MOLT-4 (Leukemia) SNB-75 (CNS cancer) T-47D (Breast cancer)	76.57 82.56 83.27 86.37 86.79	23.43 17.44 16.73 13.63 13.21
5-FU	42.21	-19.6 to 95.5	SF-539 (CNS cancer) HCC-2998 (Colon cancer) A498 (Renal cancer) HS 578T (Breast cancer) MCF7 (Breast cancer)	-19.6 -17.8 -16.3 -10.8 11.5	119.6 117.8 116.3 110.8 88.5

GP = growth percent; %GI = percent growth inhibition

The data of one-dose assay for 5-fluorouracil (5-FU) were taken from the NCI database compound ID NSC 19893 (<https://dtp.cancer.gov/dtpstandard/servlet/MeanGraphSummary>).

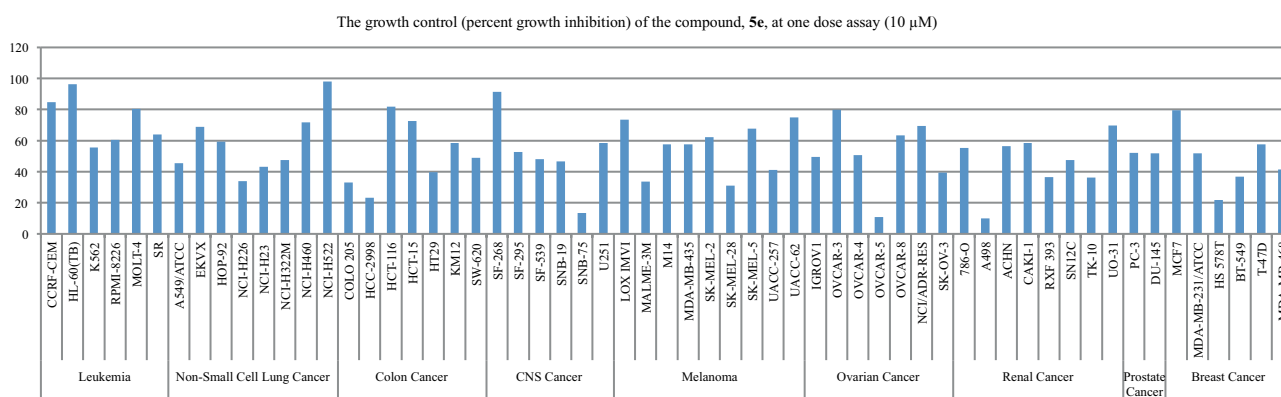


Figure 1. The percent growth inhibitions (%GIs) of the compound, **5e**, on 59 human cancer cell lines at 10 μ M drug concentration.

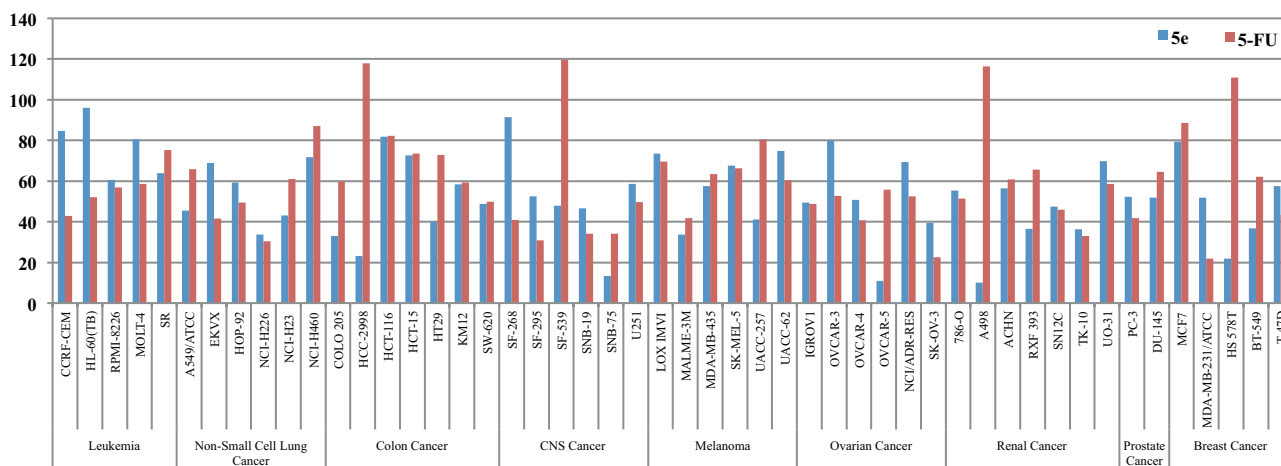


Figure 2. The percent growth inhibitions (%GIs) of the compound, **5e**, and 5-fluorouracil (5-FU) on 50 human cancer cell lines in common at 10 μ M drug concentrations.

Table 2. The average percent growth inhibitions (GIs) of 2-[(2,4-dichlorophenoxy)methyl]-5-aryl-1,3,4-oxadiazoles, 3-[(2,4-dichlorophenoxy)methyl]-5-aryl-4*H*-1,2,4-triazoles, imatinib, and 5-FU.

Compound	Leukemia	Non-small cell lung cancer	Colon cancer	CNS cancer	Melanoma	Ovarian cancer	Renal cancer	Prostate cancer	Breast cancer
5a	5.81	8.69	0.43	-1.07	3.26	-5.82	-0.67	3.83	0.91
5b	2.86	6.88	-0.59	-0.75	3.50	-5.79	0.79	4.99	0.19
5c	108.88	91.81	95.58	99.56	95.72	101.36	100.53	91.4	95.28
5d	112.31	94.41	99.67	97.64	97.92	99.38	102.30	106.86	98.15
5e	73.57	58.45	51.09	51.72	52.96	51.84	46.31	52.02	48.07
5f	23.47	11.64	2.17	1.00	6.65	0.15	2.73	11.27	5.52
5g	15.27	11.97	0.46	2.61	4.01	-1.06	4.27	-0.79	4.91
5h	19.55	9.99	1.64	0.77	1.41	-8.59	0.47	7.31	3.49
5i	3.11	5.69	1.54	2.98	-1.05	2.56	-1.90	0.74	3.58
5j	8.65	3.61	-3.71	5.68	2.50	4.36	0.75	3.39	1.87
Imatinib	9	15.68	5.34	5.80	2.02	-7.15	3.86	12.50	12.15
5-FU	57.23	51.23	73.63	51.57	55.2	45.44	61.69	53.15	65.34

GP = growth percent; %GI = percent growth inhibition. Bold figure shows higher activity.

The cytotoxicity of **5e** was higher than that of imatinib and comparable to that of 5-FU. The cytotoxicity studies reported herein may provide an insight into the design of other anticancer agents with improved antiproliferative activity.

4. Experimental

4.1. Method for the synthesis of ethyl(2,4-dichlorophenoxy)acetate (**3**)

Equimolar amounts of 2,4-dichlorophenol (0.05 mol; 5.91 mL) and ethyl chloroacetate (0.05 mol; 5.35 mL) in 50–60 mL acetone, and 5 g anhydrous potassium carbonate were refluxed for 24 h with continuous stirring to obtain ethyl(2,4-dichlorophenoxy)acetate (**3**).¹⁹

4.2. Method for the synthesis of 2-(substitutedphenoxy)acetohydrazide (**4**)

A solution of substituted ethyl(2,4-dichlorophenoxy)acetate (0.03 mol; 7.01 mL) (**3**) and hydrazine hydrate (0.045 mol; 2.18 mL) was refluxed in ethanol for 5–6 h to obtain 2-(2,4-dichlorophenoxy)acetohydrazide (**4**). The solid thus obtained was recrystallized with an absolute ethanol.¹⁹

4.3. General procedure for the synthesis of 2-[(2,4-dichlorophenoxy)methyl]-5-aryl-1,3,4-oxadiazole (**5a–h**)

2-(2,4-Dichlorophenoxy)acetohydrazide (**4**) (0.001 mol; 0.235 g) and aromatic aldehydes (0.001 mol) were refluxed in an ethanol–water (1:2, v/v) solvent system for 10–12 h, using 20 mol% solution of NaHSO₃ to obtain the title compounds [(2,4-dichlorophenoxy)methyl]-5-aryl-1,3,4-oxadiazole (**5a–h**).

4.3.1. 2-[(2,4-Dichlorophenoxy)methyl]-5-phenyl-1,3,4-oxadiazole (5a)

Creamy solid; mp 112–114 °C; ^1H NMR (DMSO d_6 , 400 MHz) δ ppm: 5.30 (2H, s, CH_2), 7.07 (1H, dd, $J = 8.8, 8.9$ Hz, ArH), 7.31 (1H, d, $J = 7.4$ Hz, ArH), 7.41–7.96 (5H, m, ArH), 8.02 (1H, s, ArH); ^{13}C NMR (DMSO d_6 , 100 MHz) δ ppm: 168.74, 163.98, 153.30, 134.36, 129.69, 129.20, 128.98, 128.27, 127.62, 127.42, 124.98, 115.53, 66.17; Mass (m/z) 321 (M+H) $^+$, 322 (M+2) $^+$; Calcd/Anal. [C (56.10) 56.06, H (3.14) 3.16, N (8.72) 8.75].

4.3.2. 2-[(2,4-Dichlorophenoxy)methyl]-5-(4-fluorophenyl)-1,3,4-oxadiazole (5b)

Creamy solid; mp 118–120 °C; ^1H NMR (DMSO d_6 , 400 MHz) δ ppm: 5.32 (2H, s, CH_2), 7.11 (1H, d, $J = 8.7$ Hz, ArH), 7.15–7.57 (4H, m, ArH), 7.71 (1H, d, $J = 7.3$ Hz, ArH), 7.98 (1H, s, ArH); ^{13}C NMR (DMSO d_6 , 100 MHz) δ ppm: 166.40, 152.98, 142.27, 132.24, 129.80, 129.73, 128.41, 126.09, 125.57, 122.96, 115.83, 115.32, 67.00; Mass (m/z) 339 (M+H) $^+$, 340 (M+2) $^+$; Calcd/Anal. [C (53.12) 53.10, H (2.67) 2.69, N (8.26) 8.24].

4.3.3. 2-[(2,4-Dichlorophenoxy)methyl]-5-(4-chlorophenyl)-1,3,4-oxadiazole (5c)

White solid; mp 122–124 °C; ^1H NMR (DMSO d_6 , 400 MHz) δ ppm: 5.31 (2H, s, CH_2), 6.99 (1H, dd, $J = 8.0, 7.9$ Hz, ArH), 7.12 (1H, d, $J = 8.0$ Hz, ArH), 7.27 (1H, s, ArH), 7.43 (2H, d, $J = 7.3$ Hz, ArH), 7.78 (2H, d, $J = 7.3$ Hz, ArH); ^{13}C NMR (DMSO d_6 , 100 MHz) δ ppm: 166.52, 152.91, 142.44, 134.27, 131.41, 129.40, 128.93, 128.11, 128.09, 124.57, 124.31, 117.13, 67.11; Mass (m/z) 354 (M+H) $^+$, 355 (M+2) $^+$; Calcd/Anal. [C (50.66) 50.63, H (2.55) 2.59, N (7.88) 7.85].

4.3.4. 2-[(2,4-Dichlorophenoxy)methyl]-5-(4-methoxyphenyl)-1,3,4-oxadiazole (5d)

Light yellow solid; mp 102–104 °C; ^1H NMR (DMSO d_6 , 400 MHz) δ ppm: 3.81 (3H, s, OCH_3), 5.32 (2H, s, CH_2), 6.97 (1H, d, $J = 8.1$ Hz, ArH), 7.02 (2H, d, $J = 8.0$ Hz, ArH), 7.12 (1H, dd, $J = 8.1, 8.0$ Hz, ArH), 7.19 (1H, s, ArH), 7.39 (2H, d, $J = 8.0$ Hz, ArH); ^{13}C NMR (DMSO d_6 , 100 MHz) δ ppm: 166.52, 160.67, 152.81, 142.48, 131.47, 128.51, 128.40, 128.13, 124.01, 118.99, 117.57, 114.31, 67.11, 55.62; Mass (m/z) 351 (M+H) $^+$, 352 (M+2) $^+$; Calcd/Anal. [C (54.72) 54.69, H (3.44) 3.47, N (7.98) 7.95].

4.3.5. 2-{5-[(2,4-Dichlorophenoxy)methyl]-1,3,4-oxadiazol-2-yl} phenol (5e)

Light brown solid; mp 152–154 °C; ^1H NMR (DMSO d_6 , 400 MHz) δ ppm: 5.27 (2H, s, CH_2), 6.85 (1H, d, $J = 7.3$ Hz, ArH), 7.11 (1H, dd, $J = 7.1, 7.2$ Hz, ArH), 7.23–7.70 (4H, m, ArH), 8.31 (1H, s, ArH), 10.03 (1H, s, ArOH); ^{13}C NMR (DMSO d_6 , 100 MHz) δ ppm: 166.52, 155.61, 152.83, 142.18, 131.49, 130.51, 128.91, 128.20, 128.13, 124.09, 121.99, 117.17, 116.31, 112.22, 67.01; Mass (m/z) 337 (M+H) $^+$, 338 (M+2) $^+$; Calcd/Anal. [C (53.44) 53.49, H (2.99) 2.97, N (8.31) 8.29].

4.3.6. 2-[(2,4-Dichlorophenoxy)methyl]-5-(2-chlorophenyl)-1,3,4-oxadiazole (5f)

White solid; mp 152–154 °C; ^1H NMR (DMSO d_6 , 400 MHz) δ ppm: 5.32 (2H, s, CH_2), 6.85 (1H, d, $J = 7.9$ Hz, ArH), 7.05 (1H, dd, $J = 7.9, 7.8$ Hz, ArH), 7.19 (1H, s, ArH), 7.23–7.46 (4H, m, ArH); ^{13}C NMR (DMSO d_6 , 100 MHz) δ ppm: 166.51, 155.60, 152.81, 142.18, 139.49, 132.31, 131.91, 130.22, 129.44, 128.90, 128.43,

128.09, 127.21, 124.11, 117.56, 67.12; Mass (m/z) 354 (M+H)⁺, 355 (M+2)⁺; Calcd/Anal. [C (50.66) 50.65, H (2.55) 2.57, N (7.88) 7.86].

4.4. 4-{5-[(2,4-Dichlorophenoxy)methyl]-1,3,4-oxadiazol-2-yl}-2-methoxyphenol (5g)

Creamy solid; mp 160–162 °C; ¹H NMR (DMSO *d*₆, 400 MHz) δ ppm: 3.73 (3H, s, OCH₃), 5.20 (2H, s, CH₂), 7.09 (1H, s, ArH), 7.31–8.15 (4H, m, ArH), 8.39 (1H, s, ArH), 11.86 (1H, s, ArOH); ¹³C NMR (DMSO *d*₆, 100 MHz) δ ppm: 168.93, 164.23, 153.26, 142.16, 140.47, 133.45, 131.86, 130.31, 129.69, 128.29, 127.54, 125.00, 122.65, 115.60, 67.59, 66.20; Mass (m/z) 367 (M+H)⁺, 368 (M+2)⁺; Calcd/Anal. [C (52.34) 52.38, H (3.29) 3.33, N (7.63) 7.65].

4.4.1. 2-[(2,4-Dichlorophenoxy)methyl]-5-(4-nitrophenyl)-1,3,4-oxadiazole (5h)

Light yellow solid; mp 220–222 °C; ¹H NMR (DMSO *d*₆, 400 MHz) δ ppm: 5.35 (2H, s, CH₂), 7.10 (1H, d, *J* = 8.75 Hz, ArH), 7.31 (1H, dd, *J* = 7.0, 6.7 Hz, ArH), 7.96 (2H, d, *J* = 8.2 Hz, ArH), 8.09 (1H, s, ArH), 8.25 (2H, d, *J* = 8.5, ArH); ¹³C NMR (DMSO *d*₆, 100 MHz) δ ppm: 169.20, 153.22, 148.37, 142.07, 132.64, 131.40, 128.54, 128.35, 128.27, 124.38, 121.98, 115.79, 67.52; Mass (m/z) 365 (M+H)⁺, 366 (M+2)⁺; Calcd/Anal. [C (49.20) 49.18, H (2.48) 2.50, N (11.48) 11.43].

4.5. General method for the synthesis of 3-[(2,4-dichlorophenoxy)methyl]-5-aryl-4H-1,2,4-triazoles (5i,j).

2-(2,4-Dichlorophenoxy)acetohydrazide (4) (0.001 mol; 0.235 g) and nitriles (0.001 mol) were refluxed in *n*-butanol and K₂CO₃ for 2–3 h to obtain the 3-[(2,4-dichlorophenoxy)methyl]-5-aryl-4H-1,2,4-triazoles (5i,j).²⁰

4.5.1. 3-[(2,4-Dichlorophenoxy)methyl]-5-phenyl-4H-1,2,4-triazole (5i)

Creamy solid; mp 182–184 °C; ¹H NMR (DMSO *d*₆, 400 MHz) δ ppm: 4.55 (1H, s, NH), 4.82 (2H, s, CH₂), 7.01 (1H, d, *J* = 8.3 Hz, ArH), 7.33–7.57 (5H, m, ArH), 7.85 (1H, dd, *J* = 6.9, 7.0 Hz, ArH), 7.93 (1H, s, ArH); ¹³C NMR (DMSO *d*₆, 100 MHz) δ ppm: 157.20, 152.08, 148.37, 131.07, 130.64, 129.31, 128.84, 128.35, 128.27, 127.55, 124.08, 117.79, 71.82; Mass (m/z) 320 (M+H)⁺, 321 (M+2)⁺; Calcd/Anal. [C (56.27) 56.23, H (3.46) 3.48, N (13.12) 13.15].

4.5.2. 3-Benzyl-5-[(2,4-dichlorophenoxy)methyl]-4H-1,2,4-triazole (5j)

Creamy solid; mp 172–174 °C; ¹H NMR (DMSO *d*₆, 400 MHz) δ ppm: 3.33 (2H, s, CH₂), 4.14 (1H, s, NH), 4.82 (2H, s, CH₂), 6.80 (1H, d, *J* = 8.1 Hz, ArH), 7.23 (1H, dd, *J* = 8.1, 8.0 Hz, ArH), 7.43–7.50 (5H, m, ArH), 7.80 (1H, s, ArH); ¹³C NMR (DMSO *d*₆, 100 MHz) δ ppm: 157.25, 152.18, 141.37, 136.37, 131.14, 129.41, 128.81, 128.05, 128.01, 125.85, 124.18, 117.09, 71.82, 36.22; Mass (m/z) 334 (H)⁺, 335 (M+2)⁺; Calcd/Anal. [C (57.50) 57.55, H (3.92) 3.89, N (12.57) 12.54].

4.6. Cytotoxicity

The new synthesized compounds were evaluated for their cytotoxicity at 10 μM drug concentrations as per the NCI protocol on nine different panels of 59 human cancer cell lines.^{21–24} The human tumor cell lines were

grown in RPMI 1640 medium. The cell lines were inoculated into 96-well microtiter having cell densities 5000–40,000 cells/well and further incubated for 24 h at 37 °C (5% CO₂, 95% air, and 100% relative humidity) prior the addition of test compounds. The microtiter plates were incubated for 48 h after addition of test compounds (solution in DMSO) and finally the assay was terminated by addition of trichloroacetic acid (10%). Sulforhodamine B (SRB) was added and excess SRB was removed washing 5 times with 1% acetic acid, and finally absorbance was recorded on an automated plate reader at a wavelength of 515 nm. Using the seven absorbance measurements [time zero (T_i), control growth (C), and test growth in the presence of drug at the five concentration levels (T_f)], the percentage growth was calculated at each of the drug concentrations levels as $\frac{T_f - T_i}{C - T_i} \times 100$ for concentrations for which $T_f \geq T_i$, and $\frac{T_f - T_i}{T_i} \times 100$ for concentrations for which $T_f < T_i$.

Acknowledgments

The author thankfully acknowledges the National Cancer Institute, USA, and Maharishi Arvind College of Pharmacy, India, for in vitro anticancer data and research facility, respectively.

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