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Research Article

Synthesis and Biological Activities of Some 1, 3, 4-Oxadiazole Based Schiff's Bases

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Abstract

In the present study a series of 1, 3, 4-oxadiazole based Schiff's bases (4a-4i) were synthesized and characterized by IR and NMR spectroscopy. The synthesized compounds were evaluated for their preliminary *in-vitro* anti-cancer activities. Compound 4h showed better anticancer activity (GI_{50} value of 2.7×10^{-5}M), which was closer to test standards doxorubicin (GI_{50} value 1.3×10^{-6} M).

1. INTRODUCTION

The literature is flooded with numerous reports on biological, pharmacological and other important uses of various heterocycles comprising of azoles[1] like oxadiazole[2], Triazoles, indole3, benzotriazole, oxazoles, imidazoles[4], pyridine and their derivatives. Among them 1,3,4-oxadiazoles have been associated with varities of activities ranging from herbicidal, diuretic, monoamine oxidase inhibition, anti ulcer[5], anti-inflammatory[6] to anti cancer[7,8], anti-tubercular and anti-human Immunodeficiency virus etc. Moreover schiffs bases of various heterocyclic scaffolds exhibits broad spectrum of biological activities like anti human immunodeficienty virus[9,10], anti cancer , anti bacterical[11], fungicidal and anti inflammatory[12]. The importance of benzotriazole as chemotherapeutic agent is also well known[13, 14, 15, 16]. With this background and our continuous interest[17,18,19] in 1,3,4-oxadiaszole chemistry, we herein report the synthesis of few 1,3,4 oxadiazole derivatives comprising 1,2,3-benzotriazole ar one end and schiff's base structure at other end with biological activities of interest.

2. MATERIALS AND METHODS

The synthetic pathways adopted for the preparation of the parent compounds **3, 4a-4i** are outlined in Scheme. (**Fig. 1**)

2.1 Synthesis of Ethyl-2-(1H-benzo[d] [1,2,3] triazole-1-yl) acetate (1)

To a solution of 1,2,3-(1H)-benzotriazole, (10g, 0.1 mole) in absolute ethanol (20 mL), ethyl bromoacetate (10 mL, 0.1 mole) and anhydrous potassium carbonate (5 g) were added and the reaction mixture was refluxed for 20-22 h. It was filtered off and the excess of ethanol was distilled under vacuum to yield a light yellow color thick liquid **(1)**[20,21].

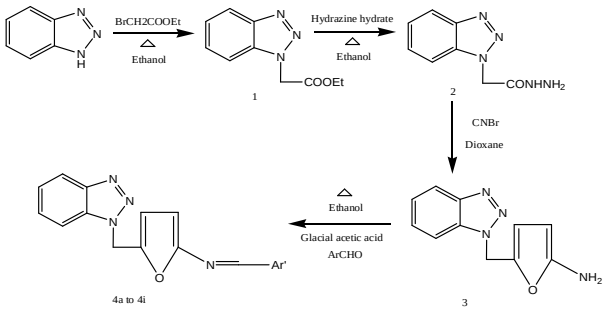

Figure 1. Scheme for synthesis of oxadiazole derivatives.

2.2 Synthesis of 2-(1H-benzo[d] [1,2,3] triazole-1-yl) acetohydrazide (2)

To a solution of **1** (9 g, 0.1 mole) in ethanol (10 mL), hydrazine hydrate (2.2 mL, 0.15 mole) was added and the reaction mixture was refluxed for 9-10 h. The excess of ethanol was distilled under vacuum and diluted with ice cold water. The white precipitate thus obtained was filtered, washed with ice cold water, dried and recrystallized from ethanol to yield **2.** [20, 21, 22]

2.3 Synthesis of 5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4-oxadiazole-2-amine (3)

A solution of **2** (5 g, 0.1 mole), cynogen bromide (3.05 g, 0.1 mole,) and sodium bicarbonate (2.41g, 0.1 mole) in 15 mL dioxane was stirred for 7-8h at room temperature. Excess of solvent was distilled and residue thus obtained was poured into ice cold water. The brownprecipitate was filtered, washed with ice cold water and dried. [23]

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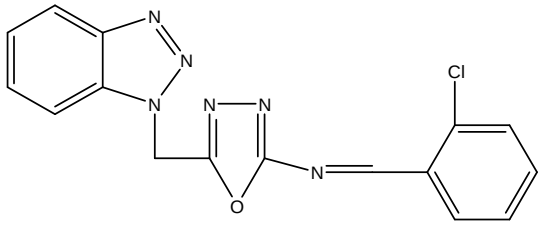
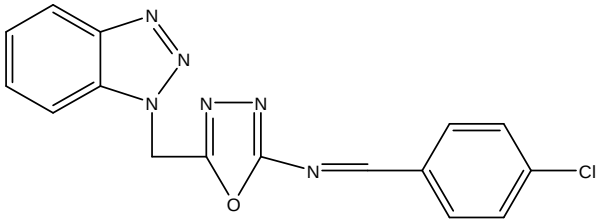
2.4Synthesis of Schiff's bases (4a-4i)

In a mixture of **3** (4 g, 0.1 mole) in 20 mL ethanol, was added an appropriate aldehyde (0.1 mole). The mixture was refluxed for about 12 h in presence of 3 mL of glacial acetic acid. The solvent was distilled off and residue was poured into ice cold water. The precipitate thus obtained was filtered, washed with ice cold water and dried. All these compounds were subjected to purification through column chromatography using Ethyl acetate: Hexane (1:4).[24, 25]



4a



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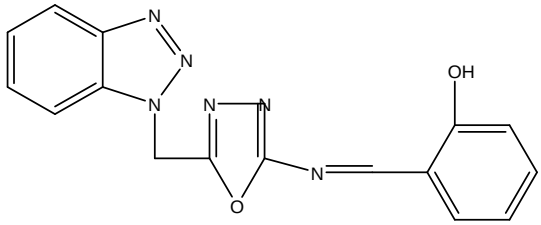

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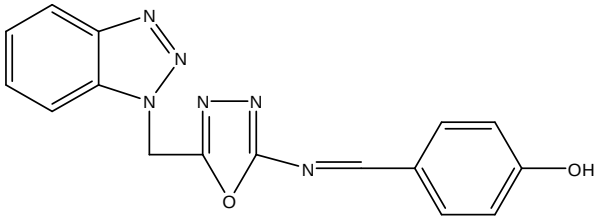

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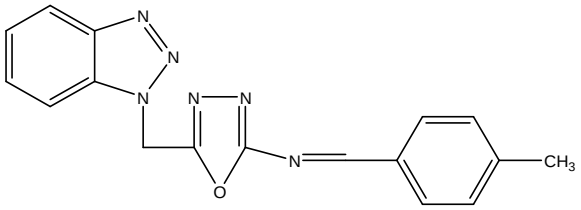

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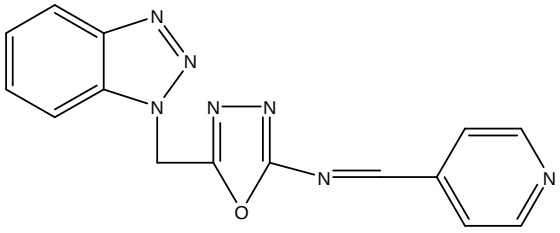

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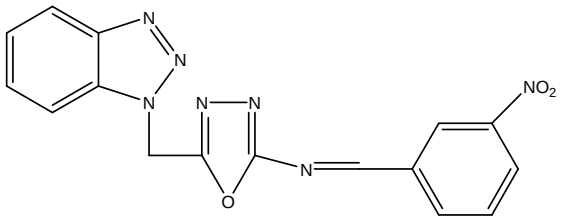
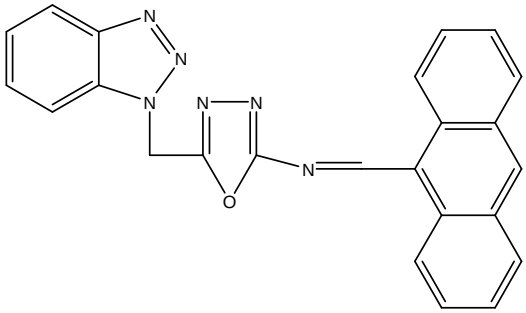
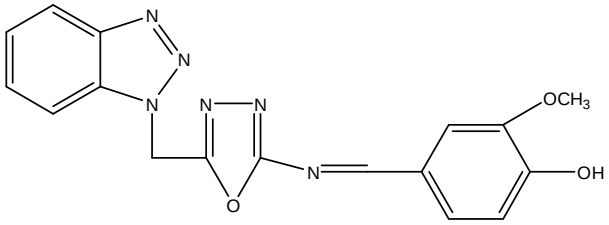


4g



4h



4i

2.5 Anti-cancer Activity

All the compounds were screened for *in vitro* anti-cancer activity against human Leukemia Cell line (K 562), human breast cancer cell line (MCF 7) and human colon cancer cell line (HCT 15). The cell lines were grown on 96 well micro-titer plates containing RPMI 1640 inoculated, incubated at 37°C, 5% CO_2, 95% air and 100% RH for 24 h prior to addition of experimental drugs. Aliquot of 10 µL of these different dilutions were added to micro-titer plate containing 90 µL of medium, resulting in required final concentration. Doxorubicin was used as a standard. Plates were incubated for 48 h and assay was terminated by addition of cold trichloro acetic acid (TCA). Supernatant was discarded, plates were washed with water. Sulforhodamine B in acetic acid was added to each well and incubated for 20 min at R.T. Unbound dye was removed by washing with 1 % acetic acid. Bound stain was eluted with 10 mM tris base and absorbance was read on Elisa plate reader at 540 nm. SRB protein assay was used to estimate cell viability or growth. This allows detection of both growth inhibition and lethality (Table 1). A value of 100 meant no growth inhibition, a value of 40 meant 60% growth inhibition, a value of 0 meant no net growth over the course of the experiment, value of -40 meant 40% lethality. Dose response parameter was calculated for each test compound. Growth inhibition of 50 % (GI_{50}) was calculated from "[(Ti-Tz)/(C-Tz)] × 100 = 50", which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Tz is the measurement of the cell population for each cell line at the time of drug addition and Ti is the measurement of the cell population for each cell line after interaction with drug for four different concentrations. Compound 4h showed GI_{50} value of 2.7×10^{-5}, which was closer to the doxorubicin a test standard (GI_{50} value of 1.3×10^{-6}). All other compounds showed GI_{50} values more than 10^{-4}.

Table 1: Anti-cancer activity of compound **3, 4a-4i**

Cell lines	Growth percentage of treated cells										
Compd./ Conc.	**3**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**Doxorubicin**
Human Leukemia Cell line K562											
10^{-7}	99.5	101.4	109.5	79.08	99.3	99.7	127.7	79.7	58.4	72.3	45
10^{-6}	88.2	100.5	105.7	78.1	83.73	97.4	114.5	67.9	57.1	64.5	24.25
10^{-5}	78.9	83.28	93.3	64.1	83.27	96.1	106.7	65.8	54.8	62.8	24.29
10^{-4}	73.9	72.45	89.8	60.52	77.55	80.7	99.86	55.4	51.4	60.1	18.92
Human breast cancer cell line MCF 7											
10^{-7}	101.8	98.5	97.1	97.3	91.7	96	89	96.5	84.9	101.9	70.8
10^{-6}	92.8	92.4	88.7	90.1	82.1	84.1	76.6	86.6	80.1	92.5	29.2
10^{-5}	86.6	82.3	82.6	81.2	78.8	79.1	77.8	76.4	69.5	83.7	0.9
10^{-4}	42.2	39.1	36.3	43.3	34.4	28.2	30.6	32	20.3	38.8	-57
Human colon cancer cell line HCT 15											
10^{-7}	98.03	99.37	97.92	100.9	104.0	104.1	100.4	96.68	89.0	96.68	90.6
10^{-6}	85.49	89.7	95.38	89.13	97.23	97.39	95	87.19	71.6	87.19	15.97
10^{-5}	81.39	85.14	89.52	81.27	92.4	91.5	87.84	82.91	59.4	82.91	-31.71
10^{-4}	70.1	70.57	59.88	68.45	50.33	45.36	52.74	66.3	37.0	66.3	-59.96

3. RESULTS AND DISCUSSION

All the Schiff's bases were obtained in quantitative yield and they were characterized by spectroscopic technique like NMR and IR (Table 2) after purification through column chromatography. These compounds were screened against cancer cell lines like K-562, MCF-7 and HCT-15 (Table 1). Compound 4h was found to be the active compound as it showed GI_{50} value of 2.7×10^{-5} M. This value was found to be closer to the GI_{50} value of standard drug doxorubicin (1.3×10^{-6} M).

Table 2: Physicochemical and spectral data of titled compounds 3, 4a-4i

Compd.	Mol. form. (Mol. wt.)	m.p. (°C) (Yield %)	IR (cm^{-1})	^{1}H NMR (δ PPM)
3	$C_9H_8N_6O$	192-194	3303 (NH str); 3080 (Ar C-H str);1500 (N-H band);	8.15-7.40 (m, 6H, Ar), 7.18 (s, 2H, NH_2),
	(216)	(90)	1575 (C=N);1161, 1103, 1039 (C-O-C oxadiazole), 864, 750 (Ar- CH bend).	6.18 (s, 2H, N-CH_2).
4a	$C_{16}H_{11}N_6OCl$	232-236	3064, 2937 (Ar C-H, str); 1676 (-N=CH);1558 (C=N); 1226,	8.37 (s,1H, N=CH), 8.00-7.20 (m, 8H, Ar),
	(338.5)	(60)	1103 (C-O-C,oxadiazole);813 (Ar C-H, bend); 576 (C-Cl).	5.92 (s, 2H, N-CH_2).
4b	$C_{16}H_{11}N_6OCl$	241-245	3182, 2937 (Ar C-H, str); 1676 (-N=CH);1276,1010	8.37 (s, 1H, N=CH), 8.00-7.20 (m,8H, Ar),
	(338.5)	(60)	(C-O-C , oxadiazole);813,744 (Ar C-H, bend), 549 (C-Cl).	6.00 (s, 2H, N-CH_2).
4c	$C_{16}H_{12}N_6O_2$	224-226	3463 (OH str); 3085, 2833 (Ar C-H, str);1693 (-N=CH);	10.06 (s, 1H, OH), 8.60 (s, 1H, N=CH),
	(320)	(40)	1317 (OH bend); 1234,966 (C-O-C,oxadiazole) 821, 746 (Ar C-H, bend).	8.23-7.42 (m, 8H, Ar), 6.00 (s, 2H, NH_2).
4d	$C_{16}H_{12}N_6O_2$	229-232	3461 (OH str); 3010, 2995 (Ar C-H, str); 1681 (-N=CH);	10.06 (s, 1H, OH), 8.37 (s, 1H, N=CH),
	(320)	(40)	1583 (C-N); 1375(OH bend); 1107,(C-O-C, oxadiazole); 821, 746 (Ar C-H, bend).	8.00-7.2 (m, 6H, Ar); 6.0 (s, 2H, N-CH_2).
4e	$C_{17}H_{14}N_6O$	204-208	3058, 2985 (Ar C-H, str); 1666 (-N=CH); 1211,1022	8.71 (s,1H, N=CH), 7.80-7.00 (m, 8H, Ar),
	(318)	(40)	(C-O-C, oxadiazole); 815, 748 (Ar C-H, bend).	6.00 (s, 2H, N-CH_2), 2.24 (s, 3H, CH_3).
4f	$C_{15}H_{11}N_7O$	198-200	3170, 3070 (Ar C-H, str); 1641 (-N=CH); 1190,1029	8.70 (s,1H, N=CH), 8.15-6.95 (m, 8H, Ar),
	(305)	(60)	(C-O-C, oxadiazole); 810, 754 (Ar C-H, bend).	5.95 (s, 2H, N-CH_2).
4g	$C_{16}H_{11}N_7O_2$	222-225	2972 (Ar C-H, str); 1639 (-N=CH); 1598 (NO_2, str);	8.75(s,1H, N=CH), 8.15-6.85 (m, 8H, Ar),
	(349.3)	(60)	1022(C-O-C,oxadiazole); 846 (Ar C-H, bend).	5.98 (s, 2H, N-CH_2).
4h	$C_{24}H_{16}N_6O$	218-220	3191, 3072 (Ar C-H, str); 1664 (-N=CH); 1139, 1080,	8.68 (s,1H, N=CH), 8.52-7.4 (m, 13H, Ar),
	(404)	(50)	1018 (C-O-C,oxadiazole) ; 954, 729 (Ar C-H, bend).	6.00 (s, 2H, N-CH_2).
4i	$C_{17}H_{14}N_6O_3$	212-215	3492 (OH str); 3147 (Ar C-H, str); 1691 (-N=CH); 1375	10.00 (s,1H, OH), 8.45 (s,1H, N=CH),
	(350.3)	(45)	(OH bend); 1022 (C-O-C, oxadiazole); 817(Ar C-H, bend).	8.00-7.00 (m,7H, Ar), 6.00 (s, 2H, N-CH_2), 3.60 (s, 3H, OCH3).

4. CONCLUSION

All the Schiff's bases were synthesized in good yields and they were characterized by different technique like NMR and IR as given in Table 2. These compounds were screened against cancer cell lines like K-562, MCF-7 and HCT-15. Hence it can be concluded that, the anticancer activity exhibited by Compound 4h was found to be the active compound as it showed GI_{50} value of 2.7×10^{-5} M. This type of synthetic study needs to be thoroughly investigated and analyzed for getting new leads molecules and further it can be investigated by computer aided drug designing.

5. ACKNOWLEDGMENTS

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