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Design, Synthesis of Newer Substituted of 1,3,4-Oxadiazole Analogues and Study for Anticancer Activity

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Abstract— *A new novel series of 1,3,4-oxadiazole were designed and prepared via the reaction of the Substituent phenyl semicarbazide of appropriate different aldehydes groups (ArCHO) was added synthesized. The 1,3,4-oxadiazole compounds are selected for 3 Compound* ***(4a, 4d, 4f)*** *were evaluated for their anticancer activity in one dose assay and showed moderate activity on various cell lines. The Compound* ***5-(2, 4-dichlorophenyl)-N-(4-chlorophenyl)-1,3,4-oxadiazole-2-amine (4a)*** *showed maximum activity with growth percent (GP) of 59.73 on SR (Leukemia), 72.77 on NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC) and mean growth percent (GP) of 96.31. and Compound* ***5-(2, 4-dichlorophenyl)-N-(2,6-Dimethylphenyl)-1,3,4-oxadiazole-2-amine(4f)*** *showed maximum activity with growth percent (GP) of 66.70 on T-47D (Breast Cancer), 68.96 on NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC), 75.64 on SNB-75 (CNS Cancer) and mean growth percent (GP) of 92.62.*

Keywords— oxadiazole; structure activity relationship, anticancer activity, cytotoxicity.

I. Introduction

Cancer is a serious and life-threatening disease and has been a major health problem for decades. The administration of chemotherapy is still considered an important part of cancer treatment, but is often limited by the severity of the disease and the growth of tumor cell resistance to cytotoxic drugs. Using high doses of immunosuppressive drugs to overcome resistance can lead to serious diseases (11). Therefore, there is an urgent need to develop new vaccines and strategies.

The compounds bearing a five membered heterocyclic ring containing Nitrogen (N) and Oxygen (O) like antibacterial [1], antitubercular [2], anticancer [3], antifungal [4], cytotoxic [5], anti-inflammatory [6], analgesic [7], antimicrobial activities [8]. Also, Imidazoacridone derivatives showed very good cytotoxicity against number of human cancer cell line. It has been found that they bind non-covalently to DNA to induce its cytotoxic effect. Several 9-acridone derivatives with or without an alkyl side chain

attached to the N-position were found to exhibit anticancer activities of novel pyrimidoacridones, pyridophenoxadines and pyrimidocarbazones [6].

II. Experimental

GENERAL METHOD OF SYNTHESIS OF 1,3,4-OXADIAZOLE

Step I: Synthesis of Phenyl urea analogues (2a-i):

Substituent aniline (0.05 mol) was dissolved in 20 ml of glacial acetic acid and 10 ml of hot water, to this sodium cyanide (0.05 mol; 3.25 g) in 80 ml of hot water was added with stirring .it was then allowed to stand for 30 min, then cooled in ice bath and filtered with suction, dried and recrystallized from boiling water to obtain substituent phenyl urea. The purity of the compound was checked by TLC using chloroform + methanol ((9:1) as mobile phase.

Step II: Synthesis of substituent phenyl semicarbazide analogue (3a-i):

Equimolar quantities (0.05mol) of substituent phenyl urea and hydrazine hydrate (0.05mol, 2.5ml) in ethanol were refluxed for 48 hr. with stirring. The two third volume of alcohol was distilled by vacuum distillation and then poured into crushed ice. The precipitate was filtered, washed with water and dried. The solid was recrystallized from 50 ml of 90% ethanol to obtain semicarbazide analogues the purity of the semicarbazide was checked by TLC using chloroform+ methanol (9:1) as mobile phase.

Step III: General method for the synthesis of 5-substituent N-aryl -1,3,4-oxadiazole-2-amine analogues (4a-i):

The Substituent phenyl semicarbazide (0.1mol) and 2,4-dichlorobenzeldihyde (0.001mol,0.175g) was refluxed 12-15 hr. using 20% $NaHSO_3$ and ethanol water solvent. The two third volume of alcohol was distilled by vacuum distillation and then poured into crushed ice. The precipitate was filtered, washed with water and dried. The solid was recrystallized from 50 ml of 90% ethanol to obtain the final product. The purity of the compound was monitored by thin layer chromatography using chloroform+ methanol (9:1) as mobile phase.

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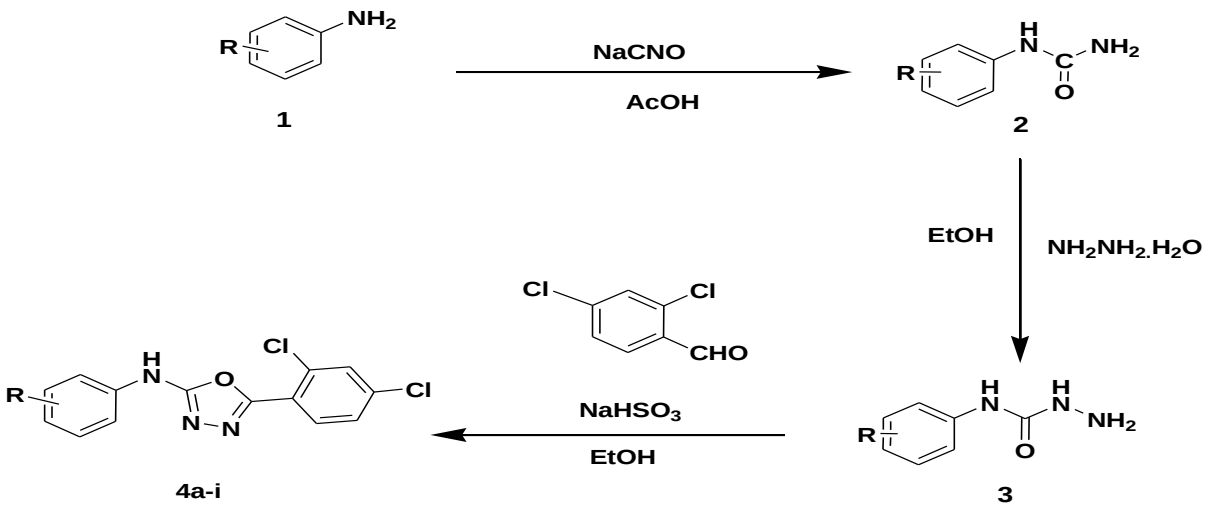

R=4-CH_3; 2-CL; 2-OCH_3; 2-CH_3; 2,4-CH_3; 2,6-CH_3; 4-F; 4CL; 4-OCH_3

SCHEME: - Protocol for the synthesis of Oxadiazole moiety:

III. Chemistry of Thiazole

5-(2, 4-dichlorophenyl)-N-(4-chlorophenyl)-1,3,4-oxadiazole-2-amine (4a) Compound:-

Spectral data ^{1}H-NMR (400 MHz, CD Cl_3) δ: 2.5 (s, 3H, CH_3), 7.27 (d, 2H, *J*= 8 Hz, C_3-H, C_5-H phenyl), 7.9 (d, 2H, *J*= 8 Hz, C_2-H C_6-H phenyl), 8.14 (s, 1H, C4-H oxadiazole). IR (KBr, cm^{-1}): 3278 (OH, Stretch), 3078 (CH, Stretch, Aromatic), 2919 (CH, Stretch, Asymmetric, Aliphatic), 2858 (CH, Stretch, Symmetric, Aliphatic), 1721 (C=O, Stretch), 1603 (C=C, Stretch, Aromatic), 1521, 1454 (C=C, Stretch, Aromatic), 1403, 1342, 1244 (C-O, Stretch), 809. MS (*m/z*): 215 (100, M^+), 175 (22), 134 (30), 119 (95), 115(75), 91 (40), 56 (38).

5-(2, 4-dichlorophenyl)-N-(4-methoxyphenyl)-1,3,4-oxadiazole-2-amine(4b) Compound:-

Spectral data.^{1}H (d, *J* = 7.9 Hz, 2H), 6.38 (d, *J* =1.5 Hz, 1H), 2.38 (s, 3H); ^{13}C NMR (100 MHz,): *δ* 169.6 (C), 150.8 (CH), 140.5 (C), 129.7 (CH), 125.8 (CH), 124.6 (C), 98.0 (CH), 21.4 (CH_3).

5-(2, 4-dichlorophenyl)-N-(4-methylphenyl)-1,3,4-oxadiazole-2-amine(4c) Compound:-

Spectral data ^{1}H NMR (400 MHz): *δ* 8.28 (s, 1H), 7.79(s, 1H), 7.41 (m, 2H), 6.3 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 165.8 (C), 150.8 (CH), 128.7 (C), 127.0 (CH), 125.4 (CH), 124.3 (CH), 98.4 (CH).

5-(2, 4-dichlorophenyl)-N-(4-fluorophenyl)-1,3,4-oxadiazole-2-amine (4d) Compound:-

Spectral data ^{1}H NMR (400 MHz): *δ* 7.28 (s, 1H), 7.79(s, 1H), 7.58 (m, 2H), 6.8 (s, 1H); ^{13}C NMR (100 MHz): *δ* 165.8 (C), 150.8 (CH), 127.7 (C), 127.0 (CH), 128.4 (CH), 125.3 (CH), 88.4 (CH).

5-(2, 4-dichlorophenyl)-N-(2,4-Dimethylphenyl)-1,3,4-oxadiazole-2-amine(4e) Compound :-

Spectral data ^{1}H NMR (400 MHz): δ 5.36 (s, 1H), 6.36(s, 1H), 7.23 (m, 2H), 6.2 (s, 1H); ^{13}C NMR (100 MHz): δ 176.32(C), 150.8 (CH), 122.3 (C), 128.0 (CH), 121.4 (CH), 127.3 (CH), 81.4 (CH_3).

5-(2, 4-dichlorophenyl)-N-(2,6-Dimethylphenyl)-1,3,4-oxadiazole-2-amine(4f) Compound:-

Spectral data ^{1}H NMR (400 MHz): δ 5.36 (s, 1H), 6.36(s, 1H), 7.23 (m, 2H), 6.2 (s, 1H); ^{13}C NMR (100 MHz): δ 178.32(C), 150.8 (CH), 122.3 (C), 128.0 (CH), 121.4 (CH), 127.3 (CH), 81.4 (CH_3).

5-(2, 4-dichlorophenyl)-N-(2-Chlorophenyl)-1,3,4-oxadiazole-2-amine(4g) Compound:-

Spectral data ^{1}H NMR (400 MHz): δ 4.36 (s, 1H), 6.34(s, 1H), 7.21 (m, 2H), 6.24 (s, 1H); ^{13}C NMR (100 MHz): δ 178.44(C), 144.8 (CH), 117.3 (C), 141.0 (CH), 121.4 (CH), 127.7 (CH), 85.4 (CH_3).

5-(2, 4-dichlorophenyl)-N-(2-methoxyphenyl)-1,3,4-oxadiazole-2-amine(4h) Compound:-

Spectral data ^{1}H NMR (400 MHz): δ 5.63 (s, 1H), 6.47(s, 1H), 6.32 (m, 2H), 6.27 (s, 1H); ^{13}C NMR (100 MHz): δ 174.5(C), 147.8 (CH), 117.3 (C), 141.0 (CH), 121.4 (CH), 127.7 (CH), 87.4 (CH_3).

5-(2, 4-dichlorophenyl)-N-(2-methylphenyl)-1,3,4-oxadiazole-2-amine(4i) Compound:-

Spectral data ^{1}H-NMR (400 MHz) δ: 2.7 (s, 3H, CH_3), 7.17 (d, 2H, *J*= 6 Hz, C_3-H, C_5-H phenyl), 7.4 (d, 2H, *J*= 7 Hz, C_2-H C_6-H phenyl), 8.45(s, 1H, C_4-H oxadiazole). IR (KBr, cm^{-1}): 3278 (OH, Stretch), 3078 (CH, Stretch, Aromatic), 2919 (CH, Stretch, Asymmetric, Aliphatic), 2818 (CH, Stretch, Symmetric, Aliphatic), 1741 (C=O, Stretch), 1603 (C=C, Stretch, Aromatic), 1541, 1454 (C=C, Stretch, Aromatic), 1413, 1342, 1274 (C-O, Stretch), 819. MS (*m/z*): 205 (100, M^+), 185 (22), 124 (30), 129 (95), 125(75), 91 (40), 58 (38).

Table 1 Physical Data of Synthesized compound (4a-4i):-

Compounds	R	% Yield	Mp(°C)	R_f Value
4a	4-Chloro	68.23	201-204	0.724
4b	4-Methoxy	71.09	140-142	0.724
4c	4-Methyl	61.87	120-123	0.655
4d	4-Fluoro	82.71	160-162	0.678
4e	2,4-Dimethyl	73.30	180-184	0.620
4f	2,6-Dimethyl	65.56	160-163	0.586

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4g	2-Chloro	60.29	180-182	0.666
4h	2-Methoxy	63.09	180-183	0.642
4i	2-Methyl	70.62	180-184	0.703

IV. Results & Discussions

Molecular docking:-

Oxadiazole derivatives are a class of anti-proliferative agents, privileged scaffold and have been reported as newer tubulin inhibitors [5]. In the present studies, the oxadiazole analogues were designed, based on the core skeleton of anti-tubulin agent, IMC038525, hence we selected the tubulin as a potential target of the oxadiazole analogues (**4a-i**). Tubulin active site consists of large hydrophobic cavity which can accommodate a range of smaller to larger scaffolds. 1,3,4-Oxadiazole derivatives comprising of different substitutions like phenyl on one side ring comprising of chloro, hydroxyl, methoxy, dimethoxy, trimethoxy and nitro on the other side were tested for anticancer studies. The binding mode analyses of these compounds were studied using Glide. According to the docking simulation frame work, compounds **4a-i** were well accommodated in the colchicine binding site, while remaining compounds **4a-i** were differed in their binding modes with respect to the compounds **4a-i**. Binding site of colchicine in tubulin enzyme consists of hydrophic cavity, lined with a few active residues like Lys254, Cys241, Lys352, THR179, Ala250 and Ala317. For the compound **4f**, the presence 3,4,5-trimethoxy seems to be unfavorable for its potency despite of exhibiting H-bond (Cys241) binding with O-atom in phenyl ring; H-bond (Thr179) binding with NH group; and π-Cationic (Lys254) binding with naphthalene ring. In case of the compounds **4d** they exhibited the H-bond with crucial residue Ala250 binding to N-atom oxadiazole ring, The compounds **4a, 4b** and **4c** are similar binding modes for π-Cationic (Lys352)

binding with naphthalene ring, in case the compound **4g** they exhibited of with crucial residue π-Cationic (Lys254) binding with phenyl ring.

Table -2 : The docking score and E model score of synthesized compounds :-

S.NO.	Compound	Docking Score	E-model Score	Interacting Residue
4a	4-Methyl	-6.048	π-Cationic (Lys352)	-51.090
4c	2-Methoxy	-5.744	H-bond (Ala250); H-bond (Leu248)	-58.042
4g	4-Fluoro	-5.893	π-Cationic (Lys352	-48.682
4h	4-Methoxy	-6.398	Halogen bond (Lys254, Asn249, Ala250)	-60.704
4i	4-Chloro	-5.477	H-bond (Ala250)	-52.824

Anticancer Screening of synthesized compounds

The anticancer screening was carried out as per the NCI US protocol. All compounds submitted to the NCI 60 Cell screen were tested initially at a single high dose (10^{-5} M) on leukemia, Melanoma, Lung, Colon, CNS, Ovarian, renal, prostate, and Breast Cancer cell Lines, nearly 60 in number. Compound ***5-(2, 4-dichlorophenyl)-N-(2,6-Dimethylphenyl)-1,3,4-oxadiazole-2-amine*** **(4f)** showed maximum activity with mean growth percent (GP) of 92.62 followed by **5-*(2, 4-dichlorophenyl)-N-(4-chlorophenyl)-1,3,4-oxadiazole-2-amine*** **(4a)** with mean GP of 96.25 while ***5-(2, 4-dichlorophenyl)-N-(4-methylphenyl)-1,3,4-oxadiazole-2-amine*** **(4c)** showed mean GP of more than 96.31. The compound **4f** was highly active on T-47D (Breast Cancer) [GP=66.70], NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC) [GP=68.96], SNB-75 (CNS Cancer) [GP=75.64]. The compound **4a** showed maximum activity on A498 (Renal Cancer) [GP=78.31], SF-268 (CNS Cancer) [GP=83.14], TK-10 (Renal Cancer) [GP=84.77]. The compound **4c** showed maximum activity on SR (Leukemia) [GP=59.73], NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC) [GP=72.77], LOX IMVI (Melanoma) [GP=76.63], MCF7 (Breast Cancer) [GP=81.32]. The maximum activity was observed with **4c** on SR (Leukemia) with GP=59.73. The anticancer activity of the compounds is given in Table 5.2 in biological screening section.

V. Summary

A Series of newer ***5-(2,4-dichloro phenyl)-N-Aryl-1,3,4-Oxadiazole-2-Amine*** Analogues was subjected to molecular properties prediction by mol soft and Molinspiration software and was

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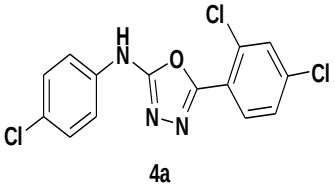
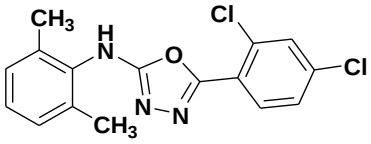
synthesized in satisfactory yields. All the compounds followed the Lipinski "rule of five "which makes them potentially active agents. 3 Compound **(4a, 4d, 4f)** were evaluated for their anticancer activity in one dose assay and showed moderate activity on various cell lines. Compound ***5-(2, 4-dichlorophenyl)-N-(4-chlorophenyl)-1,3,4-oxadiazole-2-amine*** **(4a)** showed maximum activity with growth percent (GP) of 59.73 on SR (Leukemia), 72.77 on NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC) and mean growth percent (GP) of 96.31. and Compound ***5-(2, 4-dichlorophenyl)-N-(2,6-Dimethylphenyl)-1,3,4-oxadiazole-2-amine(4f)*** showed maximum activity with growth percent (GP) of 66.70 on T-47D (Breast Cancer), 68.96 on NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC), 75.64 on SNB-75 (CNS Cancer) and mean growth percent (GP) of 92.62. Compound ***5-(2, 4-dichlorophenyl)-N-(4-fluorophenyl)-1,3,4-oxadiazole-2-amine (4d)*** could be considered as lead further discovery and could be modified to potentiate the anticancer activity.



4a

Mean GP = 96.31.



4f

Mean GP = 92.62

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