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Anticancer Activity of Novel 2, 5- Disubstituted 1, 3, 4- Oxadiazole Derivatives

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ABSTRACT:

1, 3, 4-oxadiazole molecule and its derivatives has become an important construction moiety for the development of novel drugs. A series of 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives were synthesised, by the cyclization reaction of salicylic acid hydrazide in presence of carbon disulfide and ethanol. Further it under go sulfonamide formation reaction and esterification reaction to form the derivatives. The structure was confirmed by IR, NMR spectroscopy and MASS spectrometry. Derivatives were then evaluated for their anticancer. In this OXD 5 showed good activity and OXD1, OXD2, OXD3, OXD4 showed moderate activity.

KEYWORD: 1. 1,3,4-oxadiazole, 2. Derivatives, 3. Synthesis, 4. Charecterisation, 5. Anticancer activity

INTRODUCTION:

Drug discovery and development is a deep and extensive inter-disciplinary endeavor. In recent times, an inclination towards the use of in-silico chemistry and molecular modeling for computer-aided drug design has gained significant momentum. The major advantage of the in-silico drug design is cost effectiveness in research and development of drugs. [1]

New drug development can be forwarded with various pathways for different compounds. At the same time, discovery and development of new drugs is very protracted and costly practice, but a development theory, that has extensively been adequate well as a general model. [2]

Molecular modelling is an art and science of learning molecular structure and function using model structure and computation. The model structure can be as simple as plastic templates or metal rods or as complex as interactive, animated colour stereographic and laser made wooden model. The computation technique includes quantum mechanics, molecular mechanics, molecular dynamics, Monte Carlo, free energy and solvation methods, SAR, chemical/ biochemical informations and databases and many other conventional procedures. [3]

Oxadiazole is an important heterocyclic compound in pharmaceutical chemistry. It is a heterocyclic compound bearing one oxygen and two nitrogen atoms in a five membered ring. It is obtained from furan by the replacement of two methane (-CH=) group by two pyridine type nitrogen (-

N=), which may classified into four isomers based on the position of N- atom in the heterocyclic ring : 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazole. Among heterocyclic compounds, 1, 3, 4-oxadiazole scaffold and its derivatives has become an important construction moiety for the development of novel drugs. [4]

The generally used synthetic route for 1,3,4-oxadiazoles includes the cyclization of diacylhydrazines using a variety of dehydrating agents such as phosphorousoxychloride, thionyl chloride, phosphorous pentaoxide, triflic anhydride, polyphosphoric acid, and reactions of acid hydrazides (or hydrazine) with acid chlorides/carboxylic acids. [3] It has been reported that compounds having 1, 3, 4-oxadiazole ring possess significant biological properties such as anticancer, anti-inflammatory, hypoglycaemic, antifungal, antibacterial, anti-tubercular, analgesic, Antiviral Activity.

MATERIALS AND METHODS

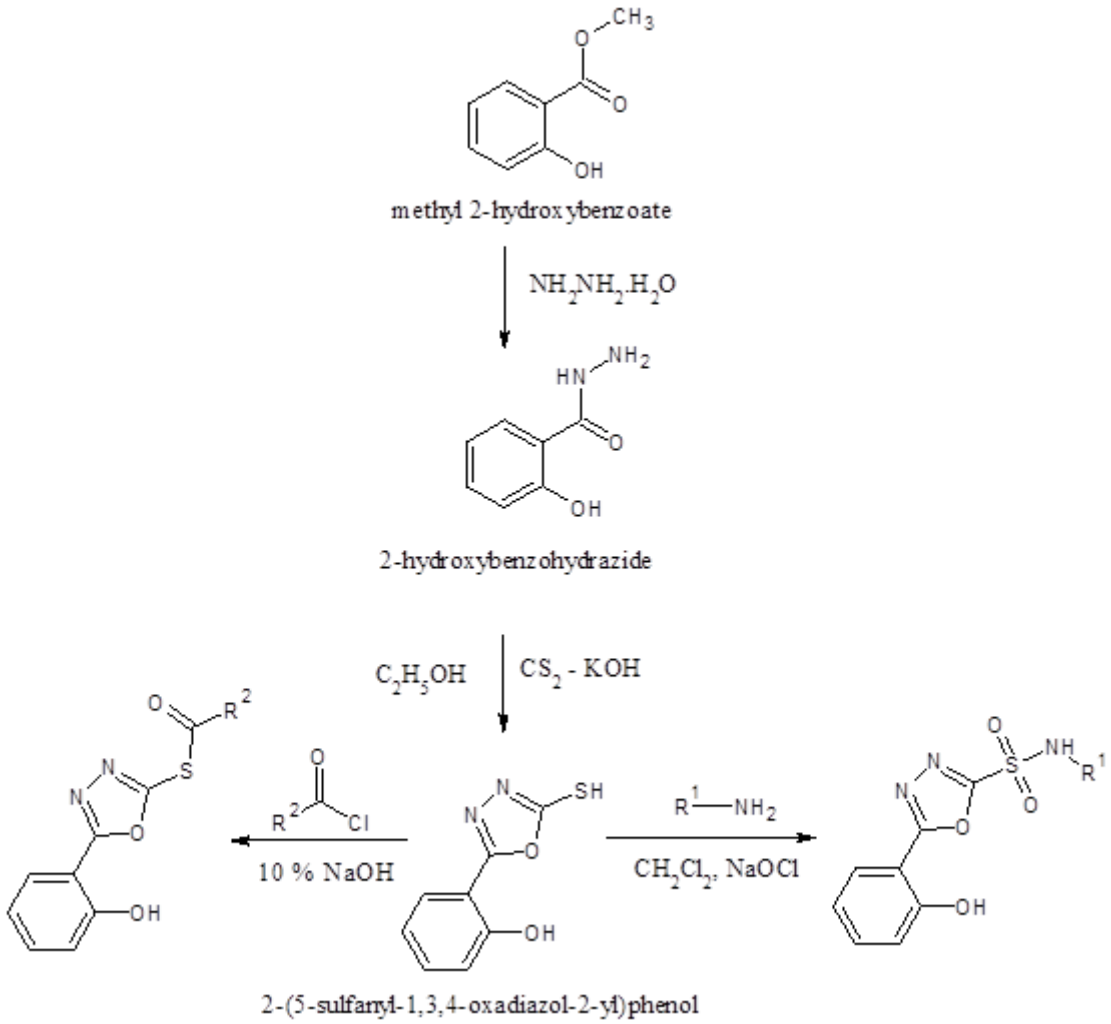


Figure 1: Synthetic scheme of 2, 5-disubstituted 1, 3, 4- oxadiazole

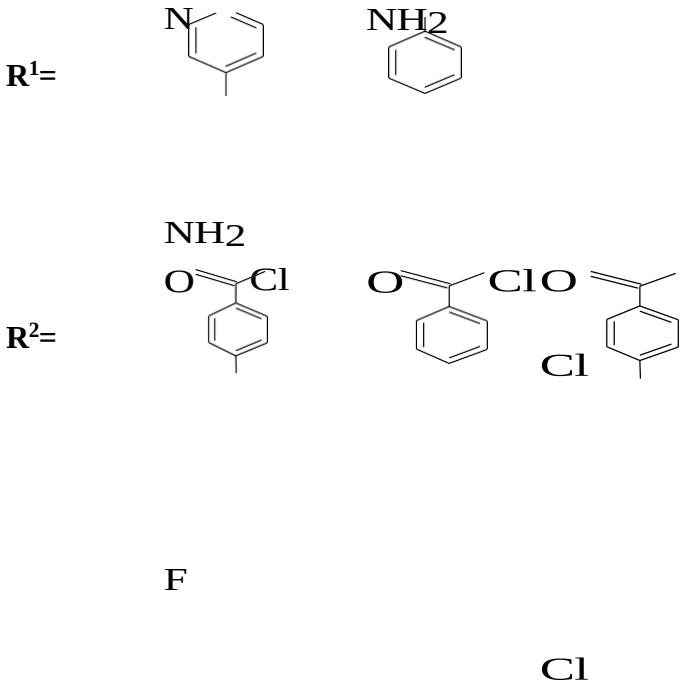


Figure 2: Derivatives

Synthetic Procedure:

Step 1: Synthesis of Methyl salicylic acid hydrazide

Methyl salicylate (0.001 M), Hydrazine hydrate (0.001 M) and Ethanol (10 ml) was exposed in microwave at 5 sec. intervals. The specific reaction time was 1min. It was then cooled in ice water with constant stirring. A powder is formed, which is filtered and recrystalised from ethanol. [6]

Step 2: synthesis of 2-(5-sulfanyl-1, 3, 4-oxadiazol-2-yl)-phenol

Methyl salicylic acid hydrazide (0.001 M), KOH (0.001 M), CS_2 5 ml in 10ml ethanol was exposed to microwave at 5 sec interval for 1 min. It is then cooled and added dil. HCl. the product then filtered and washed with water and recrystalised from ethanol. [6]

Step 3: synthesis of 2-(5-sulfanyl-1, 3, 4-oxadiazol-2-yl)-phenol derivatives

2-(5-sulfanyl-1, 3, 4 - oxadiazole-2-yl) phenol (0.561 g, 5 mmol) were stirred in a mixture of 25 ml of CH_2Cl_2 and 25 ml of 1 M HCl in a 125mL Erlenmeyer flask for 10 min at -10 to -5 ^{0}C (internal temperature). Cold (5 ^{0}C) sodium hypochlorite (6% solution, 0.68 M, 26 ml, 18 mmol, 3.3 equiv) was added drop wise with very rapid stirring, maintaining the internal temperature at -10 to -5 ^{0}C. The mixture was stirred for 15 min at -10 to -5 ^{0}C (internal temperature) after the addition was completed. The reaction mixture was then transferred to a separating funnel that pre-cooled with ice water. The separated CH_2Cl_2 layer was then collected in a clean 125-mL Erlenmeyer flask and cooled in a dry ice-acetone bath. Different amines (1.4 ml, 12.5 mmol) were added with stirring, whereupon the CH_2Cl_2 layer became a white suspension. The flask was removed to an ice-water bath and the suspension was stirred for 30 min at 0 ^{0}C. The suspension was then washed with 1 M phosphoric acid (all solids

dissolved at once), then with water and brine. Drying (Na_2 SO_4). [7]

Step 4: synthesis of 2-(5-sulfanyl-1, 3, 4-oxadiazol-2-yl)-phenol derivatives

2-(5-sulfanyl-1, 3, 4 - oxadiazole-2-yl) phenol (0.001 M) was dissolved in10% NaOH solution (15ml) in a flat bottom flask which was stirred using a magnetic stirrer. benzooyl chloride (0.011M) was added drop wise and stirring was continued for 1 hour. After completion of reaction, precipitated compound was filtered and washed with cold water, dried and recrystallised from rectified spirit. [8]

Characterisation

The structural characterisation of the synthesised derivatives was done by IR, NMR and MASS spectroscopy.

Biological evaluation

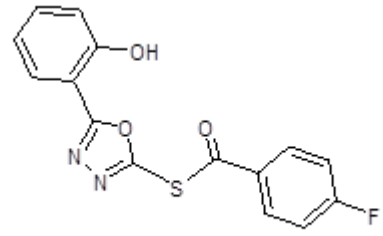
In-vitro anticancer activity

Trypan blue exclusion method: the tumor cells taken from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Viable cell suspension (1x 10^6 cells in 0.1 ml) was then combined with tubes containing various concentrations of test compounds and the volume was made up to1ml using phosphate buffered saline (PBS). Set control tube with cell suspension. The assay mixture was then incubated for 3 hrs at 37^0C. Further it was mixed with 0.1ml of 1% trypan blue and kept for 2-3 min and loaded on haemocytometer. Dead cells taken up the blue colour of trypan blue but live cells not take. The number of stained and unstained cells was counted separately. [10]

$$\% \text{ cytotoxicity} = \frac{\text{No. of dead cell}}{\text{No. of dead cell+ No. of live cell}} \times 100$$

RESULT:

OXD1



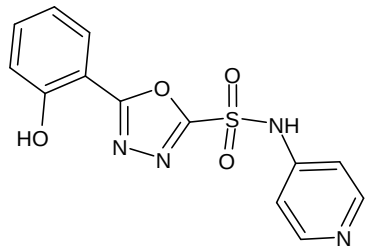
S-[5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl] 4-fluorobenzene-1-carbothioate

Figure 3: derivative OXD 1

White colour solid (yield: 88%); melting point: 243^0C; IR Vmax/ cm^{-1}(KBr); 3411cm^{-1} (O-H str), 3090 cm^{-1} (C-H str), 1285 cm^{-1} (C-O str), 1595 cm^{-1} (C=C str), 1674 cm^{-1} (S-C=O, thioester), 1317 cm^{-1} (C=N str), 1057 cm^{-1} (C-O-C str), 761 cm^{-1} (C-S str), 1151 cm^{-1} (C-F str). ^{1}H NMR ppm (400 MHz in

DMSO-d6) Mass: molecular ion peak at *m/z*: 317. Molecular formula: $C_{15}H_9FN_2O_3S$; Molecular weight (g/mol): 316.3

OXD2

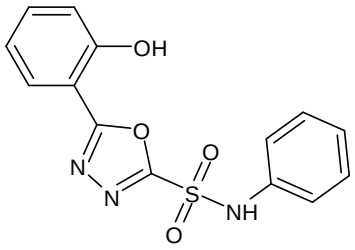


5-(2-hydroxyphenyl)-*N*-(pyridin-2-yl)-1,3,4-oxadiazole-2-sulfonamide

Figure 4: derivative OXD 2

Light brown colour solid (yield: 60 %); melting point: 273^{0}C; IR Vmax/ cm^{-1}(KBr); 3413 cm^{-1} (O-H str), 3226 cm^{-1} (C-H str), 1596 cm^{-1} (C=C str), 1502 cm^{-1} (C=N), 1347 cm^{-1} (C-N str in secondary amine), 3337 (N-H str), 1282 cm^{-1} (C-O str), 1168 cm^{-1} (S=O str), 765 cm^{-1} (C-S str), 1200 cm^{-1} (C-O-H str). ^{1}H NMR ppm (400 MHz in DMSO-d6) Mass: molecular ion peak at *m/z*:319. Molecular formula: $C_{14}H_{10}N_4O_4S$; Molecular weight (g/mol): 318.3

OXD3

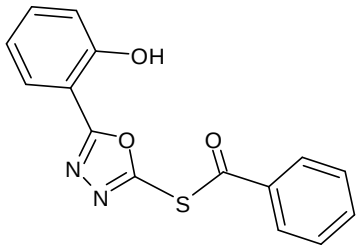


5-(2-hydroxyphenyl)-*N*-phenyl-1,3,4-oxadiazole-2-sulfonamide

Figure 5: derivative OXD 3

Dark green colour solid (yield: 53 %); melting point: 265^{0}C; IR Vmax/ cm^{-1}(KBr); 3412 cm^{-1} (O-H str), 3005 cm^{-1} (C-H str), 1595 cm^{-1} (C=C str), 1351 cm^{-1} (C=N str), 1098 cm^{-1} (C-O-C ring), 1222 cm^{-1} (C-O str), 1151 cm^{-1} (S=O str), 3346 cm^{-1}(N-Hstr), 686 cm^{-1} (C-S str). ^{1} H NMR ppm (400 MHz in DMSO-d6) Mass: molecular ion peak at *m/z*: 318. Molecular formula: $C_{13}H_{11}N_2O_3S$; Molecular weight (g/mol): 317.3

OXD4

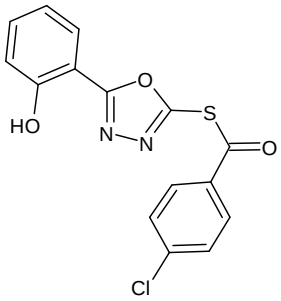


S-[5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl] benzenecarbothioate

Figure 6: derivative OXD 4

White colour solid (yield: 80 %); melting point: 240^{0}C; IR Vmax/ cm^{-1}(KBr); 3401 cm^{-1} (O-H str), 3064 cm^{-1} (C-H str), 2823 cm^{-1}, 2644 cm^{-1} , 2537 cm^{-1} (C-H overtones), 1268 cm^{-1} (C-O str), 1321 cm^{-1} (C=N str),1673 cm^{-1} (S-C=O, thioester), 1575 cm^{-1} (C=C str). ^{1}H NMR ppm (400 MHz in DMSO-d6) Mass: molecular ion peak at *m/z*: 299[Annexure 14]. Molecular formula: $C_{15}H_{10}N_2O_3S$; Molecular weight (g/mol): 298.3

OXD5



S-[5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl] 4-chlorobenzene-1-carbothioate

Figure 7: derivative OXD 5

White colour solid (yield: 82 %); melting point: 242^{0}C; IR Vmax/ cm^{-1}(KBr); 3445 cm^{-1} (O-H str), 3021 cm^{-1} (C-H str), 1584 cm^{-1} (C=C str), 1421 cm^{-1} (C=N str), 1085 cm^{-1} (C-O-C str), 679 cm^{-1} (C-S str), 755 cm^{-1} (C-Cl str). ^{1}H NMR ppm (400 MHz in DMSO-d6) data on Mass: molecular ion peak at *m/z*: 333 Molecular formula:$C_{15}H_9ClN_2O_3S$;Molecular weight (g/mol): 332

Biological activity

In-vitro anticancer activity

	Percentge growth inhibition (%)	
	(concentration in µg/ml)	**IC50**

Derivatives	10	20	50	100	200	
OXD1	**5**	**17**	**32**	**48**	**62**	**137.6**
OXD2	**0**	**0**	**10**	**22**	**32**	**182.8**
OXD3	**0**	**0**	**2**	**7**	**18**	**534.4**
OXD4	**11**	**18**	**35**	**55**	**67**	**120.1**
OXD5	**15**	**21**	**42**	**52**	**70**	**112.4**
5-flurouracil	**20**	**45**	**60**	**71**	**80**	**55.85**

Table 1: anticancer activity

DISCUSSION

The present study involves synthesis of different 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives and evaluation of their anticancer activity.
All the reactions for the synthesis of derivatives are detailed in the scheme. The derivatives were drawn using chemsketch software, and were examined using lipinski rule of five. On the basis of 5 factors derivatives are selected, namely OXD1, OXD2, OXD3, OXD4, OXD5.

For the synthesis of 1, 3, 4-oxadiazole, the first step was the synthesis of 2-hydroxybenzohydrazide. This was done by reacting Methyl- 2 hydroxybenzoate with hydrazine hydrate in presence of ethanol. In the second step this 2- hydroxybenzohydrazide undergo cyclisation reaction with carbon disulfide and KOH in presence of ethanol forms 2-(5-sulfanyl-1, 3, 4-oxadiazol-2-yl)phenol. Different derivatives were synthesised by sulfonamide formation reaction and by esterification reaction. All the derivatives were recrystalised and dried. The structure was confirmed by IR, NMR spectroscopy and MASS spectrometry. Derivatives were then evaluated for their anticancer activity. In this OXD 5 showed good activity and OXD1, OXD2, OXD3, OXD4 showed moderate activity.

CONCLUSION

The present study reports the successful synthesis of various 2,5-disubstituted 1,3,4-oxadiazole derivatives and assessment of their anticancer activity. The presence of an aryl substituent on the oxadiazole nucleus was more favorable in the case of better activity. Presence of a halogen compound in the molecule is an important structural feature for the anticancer activity. These results make these synthesized 1, 3, 4-oxadiazole derivatives an interesting lead molecule for more synthetic and biological evaluation. These compounds certainly hold great promise towards the pursuit to discover novel class of anticancer agent in order to further improve these activities in future.

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“Words have never expressed human sentiments. This is only an attempt to express our deep gratitude

which come our heart". First and foremost, I am highly grateful to the Almighty God for giving me strength, courage, knowledge, and opportunity to complete this thesis work satisfactorily. Without his blessing, this achievement would not be possible.

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