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Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,3,4-OXAZOLE SUBSTITUTED NOVEL COUMARIN DERIVATIVES

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Abstract

Heterocyclic compounds have shown to possess deep impact on biological activities like antitumor, anti-im-inflammatory, Antimicrobial, antiviral etc activities. A number of nitrogen containing heterocyclic groups are present in many of the biologically active compounds. Hence it was a subject of interest to synthesize and study some new heterocyclic derivatives. The nucleus selected for the work is a combination of two hetero aromatic molecules of Coumarin and 1,3,4-oxadiazole.Accordingly,the synthesize were carried out either conventionally as well as Microwave assisted method. There are numerous reports in the literature about the wide spectrum of activities possessed by these versatile lead molecules in pharmaceutical development. So I was synthesized 25 different classes of 1,3,4-oxadiazole derivatives of Coumarin and 10 of them found biologically which were selected for research work. They are *CX1, CX3, CX6, CX8, CX10, CX13, CX15, CX18, CX19*, *CX21and f*ound pharmacologically suitable. The acute toxicity of synthesized oxadiazole derivative were determined by Brine Shrimp Lethality assay. The LD_{50} values of the compounds were found to be in the range of 400-600µg/ml. The compounds *CX1, CX6, CX8* and *CX10* were selected for cytotoxicity studies against breast cancer cell and fibrosarcoma cell line. The compound CX8 and CX10 showed better inhibition than other analogues. As the compounds *CX1, CX8, CX13* and *CX21* showed highest glide scores on docking studies, these analogues were tested for Antitubercular activity. It was found that the analogue *CX8* and *CX1* showed better activity against the organism. The derivatives *CX3* and *CX 18* were selected for antibacterial screening according to *PASS* value. The analogue *CX3* was found better inhibition of growth of both gram positive and gram-negative organisms. The derivatives *CX3* and *CX18* were subjected to antifungal screening with *Candida albicans* and *Aspergillus Niger*. The Analogue *CX3* was found to exhibit better inhibition of proliferation at concentration 400 µg/ml and Anthelmintic activity CX3 was found higher anthelmintic activity.

Keywords: QSAR, Drug discovery, Target, Lead Optimization, Coumarin, oxadiazole etc.

INTRODUCTION

Drug discovery is most portrayed as a linear, consecutive process that starts with target and lead discovery followed by lead optimization and preclinical *in vitro* and *in vivo* studies[1]. After confirmation of the biological activity and chemical feasibility the hit becomes a lead structure, which is improved through lead optimisation until it fulfills the criteria for a drug candidate[2].The lead is a prototype compound that has the desired biological or pharmacological activity, but may have many undesirable characteristics like high toxicity, other biological activity and insolubility or metabolic problems. Such organic compounds once identified, are easy to exploit[3].by High Throughput Screening (HTS),virtual screening, de-novo design, invitro, In-silico ADME,T-screening and structural-based drug design[4]. Computers can be used to stimulate a chemical compound and design including quantitative energy calculations and graphical methods, has been rapidly introduced in the pharmaceutical industry[5].The physicochemical parameters of the proposed molecules are carried out using different software, like ACD Labs, *Chem sketch, Log P, molar volume, molar refractivity, parachor, polarizability, surface tension* etc are determined[6].Drug likeness and violation of the "Lipinski rule 5" are carried out using Molinspiration software and Maestro software[7].In 1986,*Gedye and Giguire* reported for the first time at organic reactions could be conducted very rapidly under microwave irradiation[8]. Since then the reaction time significantly decreased, it also results in lesser evaporations of solvents, preventing pollution[9]. Irene Kostova *et al.* (2005) reported the synthesis of New Zirconium complexes of Coumarin and screened their cytotoxic activity[10]. Xin-Hua-Liu *et al.* (2010) synthesized and performed molecular docking study of novel coumarin derivatives containing 4,5-Dihydropyrazole moiety as potent tumour agent[11] but non of them tried for 1,3,4-oxadiazole derivatives, chemotherapy drugs are sometimes feared because of patient concern about toxic effects. Majority of drugs currently on the market are nonspecific, which leads to many common side effects associated with cancer therapy[12]

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MATERIALS AND METHODS

All the chemicals and reagents used in this research work were of analytical or synthetic grades.

Table 1. Chemicals and Reagent used for the synthesize

REAGENT/SOLVENT	MANUFACTURER
Diethylmalonate	SISCO Research Laboratories
Salicylaldehyde	Loba chemie Pvt ltd
Ethanol	Nice chemicals Pvt ltd
Piperidine	Sami Labs
Hydrazine hydrate	Reachem Laboratory Chemicals Pvtltd
Glacial Acetic acid	Bipin Enterprise
P-Hydroxybenzaldehyde	Central Drug House Ltd
N, N-Dimethyl benzaldehyde	Loba Chemie Pvt ltd
P-Chlorobenzaldehyde	Loba Chemie Pvt ltd
4-Methoxybenzaldehyde	Bipin Enterprises
Chloramine T	Central Drug House Ltd
Chloroform	Loba ChemiePvtltd
Benzene	Nice chemicals

Step –I

Synthesis of ethyl 2-oxo-2H-chromene-3-carboxyllate Conventional method

Salicylaldehyde (0.01mol,1,22 gm) and Diethyl malonate (0.01 mol,1.6 g) was dissolved in ethanol(20ml) to give a clear solution. To this mixture piperidine (1.2ml) and 2 drops of Glacial acetic acid was added and refluxed for 5 hours. The hot solution was transferred to a beaker containing hot wat(20ml). The product crystallises out readily as the solution cools; the mixture was stirred from time to time as crystallisation proceeds and was finally stored overnight in refrigerator. The crystalline product was collected by filtration a recrystallized from ethanol to give a white shining crystal.

Percentage yield -85% (1.48 gm), Melting point -92^{o}C, Rf value-0.65, Solvent System-Toluene: Acetic acid (9:1),IR-KBR-1670cm^{-1}(C-O Coumarin),1563cm^{-1}(C=O),1764cm^{-1} (ester).

Microwave Assisted synthesis

A mixture of Salicylaldehyde (0.01 mol,1.22g) and Diethylmalonate (0.01mol,1.6 g) and 4-5 drops of Piperidine was placed in a beaker and irradiated(360W) in an unmodified domestic oven for 3 minutes. At the end of the exposure to microwave, the reaction mixture was cooled to room temperature and the crude product was recrystallized from ethanol.

Percentage yield :92%, Melting point -92^{o}C, Rf value -0.65, Solvent System-Toluene: Acetone

Step-II

Synthesis of 2-oxo-2H-Chromene-3-carbohydrazide

Compound (1) (0.01 mol,2.18 g) and Hydrazine hydrate (99%), (0.01mol,0.5gm) was dissolved in sufficient quantity of ethanol(50ml) to give clear solution and refluxed for 10 hours. The excess solvent was removed by distillation, allowed to cool; the solid mass that separated out on cooling was washed with small amount of ice-cooled ethanol, dried and recrystallized from ethanol.

Percentage yield – 80%(1.87gm), Melting point -212oC, Rf value-0.63, Solvent system– Chloroform: Methanol, IR(KBr)-3384cm^{-1}(NH-stretching),2925(C-H-aromatic stretching), 1671cm^{-1}(C=O Coumarin)1492cm^{-1}(C=O).

Microwave Assisted synthesis

A mixture of compound 1(0.01mol) and Hydrazine (0.01mol) was dissolved in a closed Teflon vessel and was irradiated in domestic microwave oven for 2 minutes at 360W.At the end of the exposure the reaction mixture was cooled and recrystallized from ethanol.

Percentage yield -80%, Melting point-212^{o}C, Rf value-0.63, Solvent System-Chloroform: methanol (7:3).

Step III

General Methods for synthesis of 2-oxo-N'-[(E)-aryl methylidiene]-2-H-chromene-3-carbohydrazide

Conventional Method

Mixture of compound (2) (0.01mol,2.04g) and 0.01mol of substituted aldehyde was dissolved in ethanol(20ml) and added a drop of Glacial Acetic acid to provide acidic medium and refluxed for 2hrs.After completion of reaction, the contents were concentrated to a small volume and allowed to cool to room temperature. A solid mass separated out, which was filtered and washed with water. The dried product was recrystallised from ethanol. A single spot-on TLC plate established the purity of the compound. The solvent system used was Chloroform: Ethanol (9.5:0.5).

All the Compounds prepared were listed below

Table 2. List of Synthesis of Analogues

Sl. No	Name of derivatives	Aromatic group	%age yield	Melting Point
a)	Phenyl	Benzaldehyde	68%	210oC
b)	4-Chlorophenyl	P-chlorobenzaldehyde	80%	218oC
c)	4-Hydroxy phenyl	p-Hydroxybenzaldehyde	80%	218oC
d)	2-HydroxyPhenyl	Salicylaldehyde	86%	215oC
e)	4-Hydroxy-3-methoxy	4-Hydroxyl-3-methoxyBenzaldehyde	68%	210oC
f)	3-NitroPhenyl	3-Nitrobenzaldehyde	68%	210oC
g)	P-Amino phenyl	P-Amino benzaldehyde	68%	210oC
h)	4-(Dimethylamine) Phenyl	N, N'-Dimethylamine benzaldehyde	75%	210oC
i)	4-MethoxyPhenyl	4-Methoxybenzaldehyde	68%	210oC
j)	2-Chlorophenyl	2-Chlorobenzaldehyde	68%	210oC

Step IV

General Procedure of Synthesis of 3-[5-(substituted)-1,3,4-oxadiazol-2-yl]-2H-Chromen-2-one

Hydrazide (0.01mol) was dissolved in ethanol and Chloramine-T(0.05mol) was added and refluxed for 4-6hours.Excess of ethanol was completely removed by boiling on a water bath leaving behind a solid mass, which is recrystallised from ethanol.

Microwave method

Hydrazide(0.01mol) was dissolved in ethanol(15ml) and Chloramine -T was added(0.05mol). It was subjected to microwave irradiation at 300Wintermittently at 30 seconds intervals for a specified time. On completion of reaction was monitored by TLC, the reaction mixture was cooled and treated with cold water. Solid product obtained was filtered and recrystallised from methanol.

Table 3. The same procedure was used synthesis for the following compounds

Sl.No.	Compounds	Aromatic aldehydes	Molecular weight
1	CX1	Benzaldehyde	290.278
2	CX3	P-Chlorobenzaldehyde	324.723
3	CX6	P-Hydroxybenzaldehyde	306.277
4	CX8	Salicylaldehyde	306.277
5	CX10	Vanillin	336.303
6	CX13	m-nitro benzaldehyde	335.275
7	CX15	p-Amino benzaldehyde	305.293
8	CX18	N, N'-Dimethyl amino benzaldehyde	333.347
9	CX19	Anisaldehyde	320.304
10	CX21	2-Chloro benzaldehyde	324.723

BIOLOGICAL EVALUATION

Brine shrimp lethality bioassay

Dried crystal (1gm crystal per litre) was hatched in a hatcher at 28-30^{o}C with strong aeration under continuous light regime.

Approximately 24 hours after hatching the phototropic nauplii were collected with a pipette from lighted shade and concentrated in a small vial. Ten brine shrimps were transferred to each well using adequate pipette. Each test consists of exposing groups of 10 Artemia nauplii aged 24 hr to various concentrations of the oxadiazole analogues. The toxicity was determined after 24 hr and 48 hr of exposure. The numbers of survivors were counted and percentage of deaths was calculated.

Cytotoxicity studies

The cell lines were purchased from NCCS, Pune and was maintained in Dulbecco's modified eagles' media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5%CO2 (NBS, EPPENDORE, GERMANY) in a humidified atmosphere in a CO_2 incubator. The cells were trypsin zed (500µl of 0.025% of trypsinized in PBS/0.5molEDTA solution (HI media) for 2minutes and passed in to T-flasks to complete aseptic condition. The effect of compounds on the proliferative capacity of the breast cancer cells (MCF) and L929 fibrosarcoma T-cells was determined using MTT[3-(4,5-dimethylimidazole-2-yl)2,5-diphenyltetrazolium bromide] assay. Cells were seeded (5000cells/well) in 96-well, flat bottom titre plates along with different concentrations of the compounds, CX1, CX6, CX8 and CX10(1,10,100,250,500µl/ml) and incubated for 48 hours at 37oC in 5%CO2 atmosphere. After incubation the medium was removed and wells were washed with phosphate buffered saline (PBS),100µl of the working *MTT* lysis in Dulbecco's Modified Eagle media (DMEM) was added and incubated for 2 hrs. *MTT* lysis buffer(100µl) was added and incubation was continued for 4 hrs. The absorbance was measured at 570nm and the proliferation rate (PR) was calculated using the formula

$$PR = \frac{\text{Absorbance of test x100}}{\text{Absorbance of Control}}$$

Cytotoxicity of the compoundsCX1, CX6, CX8 and CX10 on the cells was calculated as cell growth inhibition rate (IR)
IR =100-PR

Antituberculr screening

Alamar- Blue Assay Method (Resazurin Microlitre Assay, REMA)

The test was performed in black, clear bottomed, 96 well microplates (Packard instrument company, (Meriden) in order to minimize background fluorescence. Initial drug dilutions were prepared in Dimethyl sulfoxide and subsequent in two-fold dilutions were performed in 0.1ml of 7H9Gc media in the micropipettes.100ml of 2000 CFU/ml of Mycobacterium tuberculosis H37Rv in 7H9GC were added to each well of 96 well microplates containing test compounds. Three control well plates containing drug and medium, bacteria and medium and medium only were prepared and microplates were incubated at 37°C.At a day 7 of incubation Almar blue dye solution (20µlAlmar blue solution and 12.5 ml of 20% tween-80) added to all the wells and plates were re-incubated at 37°C for 24 hrs. Reading were taken at 365nm.

Antimicrobial screening

The test solutions were prepared in Dimethyl sulfoxide. The concentrations 0f used for antibacterial screening were25,50, and 100µl/ml. Standard drug solution of gentamycin (100µl/ml) was prepared in distilled water. Filter paper discs, impregnated with compounds to be tested, are then placed on the surface of the media. The test solutions, standard solution and the vehicle control(ethanol) were placed in each cup of each petri dish and incubated at 375°C for 24 hrs. The presence of a definite z with that of one of inhibition of any size was observed and compared with that of standard drug solution,

Antifungal activity

The minimum inhibitory activity was determined by agar well diffusion. Sabouraud dextrose agar plates were prepared and overnight grown different species of fungus such as *Candida albicans* and *Aspergillus Niger* were swabbed. Wells of a after overnight and approximately 10 mm was bored using cutter and samples of different concentrations was added. The zone of inhibition was measured and compared with that of standard drug Clotrimazole.

Anthelmentic activity

Albendazole was diluted with normal saline to obtain100,200,300,400 µg/ml as standards and poured in to petri dishes. The 2,5-Disubstituted Oxadiazole analogues (CX3, CX6, CX18) were prepared in minimum quantity of Dimethyl sulphoxide and diluted to 15ml with normal saline to obtain 100,200,300,400µg/ml and taken in to poetry dishes. Normal saline serves as control for standard. Each earth worm (Eudrillus eugenia obtained from University of Agriculture, Vallayani, Kerala) nearly equal size were placed in each petri dish at room temperature. The time taken for complete paralysis and death were recorded. The mean paralysis time and mean lethal time for each sample were recorded.

RESULTS AND DISCUSSION

Drug design is an integrated developing discipline which involves the study of effects of biologically active compounds on the basis of molecular interaction in terms of molecular structure or their physicochemical properties. The newly synthesized3-[(5-substituted)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-ones become useful as drugs if it possesses anticipated Pharmacological activity and is free from toxicity. Keeping thus view in mind, the new analogues were designed, predicted the drug likeness, synthesized and evaluated for anticancer, Antitubercular, Antibacterial, Antifungal and Anthelmintics studies.

ADME profile

Acute prediction of ADME properties prior to expensive experimental procedures can eliminate unnecessary testing on compounds that will ultimately fail. The ADME can also be used to focus lead optimization efforts to enhance the desired properties of a given compound. All proposed Analogues were efficiently evaluated for pharmaceutically relevant properties within limited time fractions, making an indispensable lead generation and lead optimization tool.

Synthetic methodology

The synthesis of analogues CX1-CX21 were performed by conventional method as well as microwave Assisted methods. On comparison, it was observed that the microwave technique increased the yield and also showed a decreasing the reaction time than that of the conventional method.

Table 4. Prediction of ADME profile of selected oxadiazole analogues

Compd	Horal abs	% Horal Abs	QP logKhsa	QP PCaco	QP logBB	QP logKp	QPlog HERG	#metab	QPP MDCK
CX1	High	93	-0.079	958	-0.436	-1.973	-6.103	1	472
CX3	High	100	-0.044	998	-0.262	-2.109	-5.998	1	1219
CX6	High	84	-0.009	302	-1.000	-3.002	-5.941	2	135
CX8	High	89	-0.008	533	-0.729	-2.464	-5.875	2	250
CX10	High	87	-0.154	357	-0.972	-3.017	-5.935	3	162
CX13	High	74	-0.177	144	-1.475	-3.878	-6.069	2	47
CX15	High	81	-0.005	249	-1.098	-3.177	-5.988	2	110
CX18	High	100	-0.046	963	-0.557	-2.150	-6.139	2	475
CX19	High	96	-0.103	997	-0.502	-2.041	-5.990	2	493
CX21	High	100	-0.154	1046	-0.265	-2.033	-6.000	1	1094

Table 5. Physical Characterisation of Data of synthesized Oxadiazole derivatives

Compounds	Molecular formula	Molecular weight	Melting Point(°o.)	Yield (%)	Rf values Toluene: Acetone 9:1
CX1	$C_{17}H_{10}N2O_3$	290.278	210	65	0.63
CX3	$C_{17}H_9ClN_2O_3$	324.723	220	69	0.62
CX6	$C_{17}H_{10}N_2O_4$	306.277	227	55	0.64
CX8	$C_{17}H_{10}N_2O_4$	306.277	225	56	0.67
CX10	$C_{17}H_{10}N_2O_5$	336.303	232	45	0.68
CX13	$C_{17}H_{11}N_3O_5$	335.275	228	63	0.63
CX15	$C_{17}H_{11}N_3O_3$	305.293	237	53	0.65
CX18	$C_{17}H_{15}N_3O_3$	333.347	230	52	0.62
CX19	$C_{18}H_{12}N_2O_4$	320.304	227	45	0.61
CX21	$C_{17}H_9ClN_2O_3$	324.723	215	63	0.66

Table 6. Characteristic IR peaks obtained for synthesized Oxadiazole Analogues

Compounds	IR Peaks(cm^{-1})
CX1	3304(AR-NH-str.),1681(C-O-Coumarin),1591(C=O),1353(C-N ring-str), 1081(symmetric-O-C ring str)
CX3	3442(Ar-NH),1591(C=C Ar.str),1425(C-N ring str.)1156(Assymetric-C-O-C- ring str.),1092(symmetric ring str),762(CCl-str)
CX6	3774(O-H str),3415(Ar-NHstr),1366(C-N ring str),1179(asymmetric-C-O-C(ring str.),1042(symmetric-C-O-C-ring str.)
CX8	3653(AR-NH str),3423(Ar-NH str),1428(C-N ring str.)1152(asymmetric-C-O-C- ring str)1092(Symmetric-C-O-C- ing str)
CX10	3213(Ar-NHstr),1680(C-O coumarin) ,1645(C=O),1264(C-N ring str), 1085 (symmetric-O-C ring str).
CX13	3100(Ar-NH str),1680(C-o coumarin),1634(C=O),1264(C-N str),1303(N=O) str.,1069(SymmetricC-O-Cring str)
CX15	3196(AR-NH str),1571(C=C Ar.str),1619(C=O),1701(C-O coumarin) ,1271 (C-N ring str),1073(symmetric C-O-C- ring str)
CX18	3194(Ar-C-H str),1680(C-O coumarin),1607(C=O),1264(C-N ring str), 1051 (symmetric C-O-C-ring str)
CX19	2918(Ar-C-H str),1701(C-O ring str.),1619(C=O),1271(C-N ring str.), 1080 (symmetric C-O-C-str)
CX21	3449(Ar-NH str),1487(C-N ring str),1609(C=O),1172(Asymmetric -C-O-C- ring str),761(C-C str)

Characterization

I.R Spectra Data

Infra-red (IR) spectra of the synthesized on Oxadiazole analogues were recorded using KBr pellets in the range of 4000-500cm^{-1}on Jasco FTIR Model 4100nType A spectrophotometer. The presence of characteristic peak of functional groups in the IR spectra of the synthesized Oxadiazole analogues substantiates the formation of the designed compounds.

NMR Spectra

Nuclear magnetic resonance spectra were recorded on Bruker Ultra shield Model -400 MHz spectrometer using Deuterated chloroform as solvent and Tetramethyl Silane (TMS) as internal standard. The characteristic peak of protons in δ ppm found in 1H-NMR spectra of selected oxadiazole analogues substantiates the formation of the designed compounds

Table 7. Characteristic δ ppm in 1 H-NMR spectra for selected oxadiazole analogues

Compound	1HNMR (400MHz) δ ppm
	8.538-7.707(1H, Aromatic proton in coumarin ring)
CX3	7.63-7.542(1H, Aromatic protons in benzene ring)

Mass spectral data

Mass spectrum of the compound was recorded by FAB+ ionization mode on JEOOL JMS 600 instrument.

Table 8. Mass spectra of the selected Oxadiazole analogue

Compound	M+ peak(m/z)	Base peak
CX3	306	256

Anticancer activity

All the proposed derivatives were subjected to Anticancer activity and the results were tabulated as follows.

Heatshock protein HSP90-alpha-iuyk

Table 9. Glide Scores of compounds with Anticancer activity

Compound	Glide score
CX8	9.51
CX10	9.23
CX6	8.37
CX1	8.37
CX18	8.07
CX21	8.05
CX15	7.79
CX19	7.5
CX3	6.92
CX13	6.46

Antitubercular activity

Anoyl-[acyl-carrier-protein] reductase[nadh]-2h7p

Table 10. Glide score of compounds with antitubercular activity

Compounds	Glide score
CX8	8.25
CX1	7.78
CX21	7.65
CX13	7.31
CX3	7.22
CX15	7.12
CX10	6.95
CX19	6.85
CX6	6.81
CX18	6.32

HSV serine protease-iat3

Table 11. Glide scores of compounds with activity against Herpes Simplex Virus

Compounds	Glide score
CX21	7.86
CX19	7.34
CX10	7.19
CX8	6.98
CX6	6.91
CX1	6.74
CX3	6.63
CX18	6.54
CX15	6.21
CX13	5.88

Biological evaluation

Brine shrimp toxicity studies

The acute toxicity studies of the synthesized oxadiazole derivatives were determined by Brine shrimp lethality Bioassay.

Table 12. Results of Brine shrimp lethality Bioassay

Sample	Conc (µg/ml)	Log conc	Average Number of deaths (n=10)	% Mortality	Corrected % Mortality	P-value
control	0	0	0	0	0	0
CX1	1000	3	9	90	90	6.28
CX3	1000	3	9	90	90	6.28
CX6	1000	3	8	80	80	5.84
CX8	1000	3	10	100	100	6.96
CX10	1000	3	10	100	100	6.96
CX13	1000	3	9	90	90	6.28
CX15	1000	3	9	90	90	6.28
CX18	1000	3	9	90	90	6.28
CX19	1000	3	9	90	90	6.28
CX21	1000	3	9	90	90	6.28

Table 13. LD50 values of synthesised oxadiazole analogues

Compound code	LD50
CX1	2.56
CX3	2.6
CX6	2.68
CX8	2.6
CX10	2.6
CX13	2.65
CX15	2.67
CX18	2.79
CX19	2.69
CX21	2.6

The acute toxicity studies of the synthesized oxadiazole derivatives were determined by Brine Shrimp Lethality Bioassay. The LD_{50} values of compounds were found to be in the range of 400-600µg/ml.

Cytotoxicity study

Table 14. Cytotoxicity of the compounds against breast cancer cell lines

Conc. in µg/ml	Percentage of Inhibition				
	CX1	CX6	CX8	CX10	Doxorubicin
1	18.91	13.54	32.14	31.84	57.17
10	24.66	19.36	42.39	36.67	62.16
100	33.42	29.56	45.42	43.28	63.7
250	42.54	38.24	52.34	49.64	65.37
500	45.68	40.81	54.66	51.41	69.50

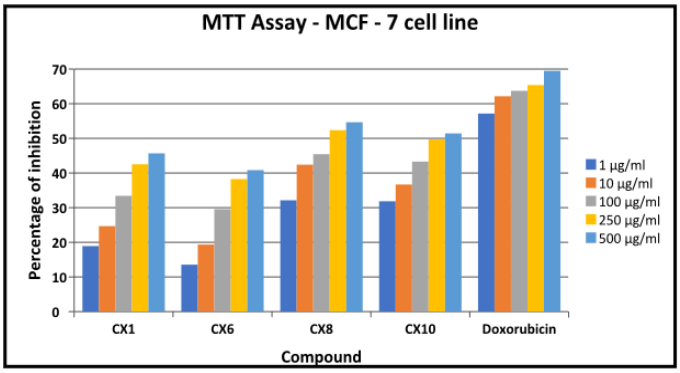

Figure 1. Showing the comparison of MTT Assay-7 cell line method

ANTITUBERCULAR ACTIVITY

For antitubercular activity Mycobacterium tuberculosis H37RV was used as the test organism for antimycobacterial screening studies

Table 15. Antitubercular activity of selected Oxadiazole derivatives

Conc. In µg/ml	Percentage inhibition			
	CX1	CX8	CX13	CX21
0	0	0	0	0
50	42	46	26	45
100	47	57	45	54
150	66	79	63	64
200	72	84	67	70
250	80	85	76	76

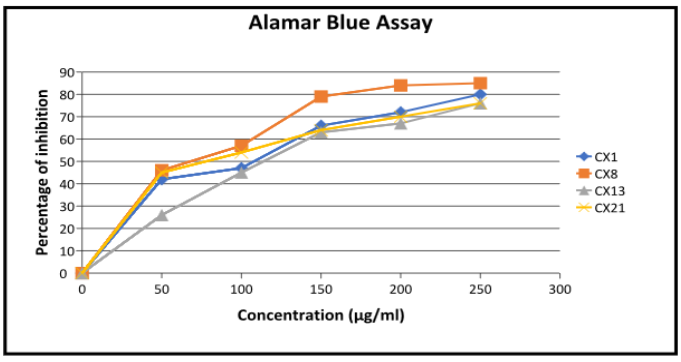

Figure 2. Comparison of Antitubercular activity by Alamar Blue Assay

Antibacteria activity

The Antibacterial activity was performed on selected synthesized oxadiazole analogues. Gentamycin was used as

standard for both gram positive (Staphylococcus aureus) and gram-negative organism (Escherichia coli).

Table 16. Antibacterial activity of selected oxadiazole analogues on gram positive bacteria

Sample	Zone of inhibition					
	Staphylococcus aureus			Streptococcus mutans		
	25µg/ml	50µg/ml	100µg/ml	25µg/ml	50µg/ml	100µg/ml
Control	-	-	-	-	-	-
Gentamycin	-	-	16	-	-	30
CX3	3	8	12	NIL	10	13
CX18	5	9	12	NIL	10	11

Table 17. Antibacterial activity of selected oxadiazole analogues on gram negative bacteria

Sample	Zone of inhibition		
	Pseudomonas aeruginosa		
	25µg/ml	50µg/ml	100µg/ml
Gentamycin	-	-	28
CX3	10	11	12
CX18	7	9	10

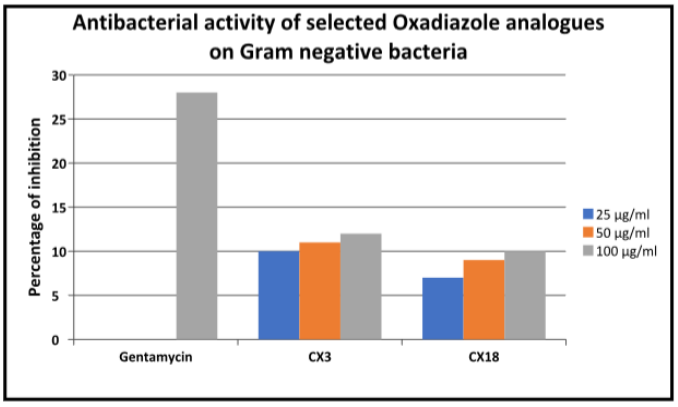

Figure 3. Showing Comparison of Antibacterial activity of Oxadiazole derivatives

Antifungal Activity

Randomly selected oxadiazole analogues were subjected to Antifungal activity using Candida albicans and Aspergillus Niger comparing with the standard Clotrimazole

Table 18. Results of Antifungal Activity

Sample	Zone of Inhibition(mm)			
	Asper Niger		Candida albicans	
Control	50µg/ml	100µg/ml	50µg/ml	100µg/ml
Clotrimazole		20		20
CX3	5	15	NIL	13
CX18	NIL	NIL	NIL	5

Antihelmentic activity

The analogues were studied for their anthelmintic activity using Eudrilus eugenia. Albendazole was used as standard.

Table 19. Anthelmintic activity of selected Oxadiazole analogues

Sample	Concentration	Time taken for Paralysis (min)	Time taken for Complete Death (min)
Control	0	No paralysis	No Death
Standard (Albendazole)	400	15.21	35.35
CX3	400	16.88	28.13
CX6	400	20.27	31.88
CX18	400	21.63	33.98

DISCUSSION

The current research work was focused on the rational approach of design and development of 3-(5-substituted)-1,3,4-oxadiazol-2-yl)-2-H-chromen-2-one as anticancer, antitubercular, anti-bacterial, antifungal and anthelminthic agents. The acute toxicity studies of the synthesized oxadiazole derivatives were determined by Brine Shrimp Lethality Bioassay. The LD50 values of the compounds were found to be in the range of 400-600µg/ml, The compounds CX1, CX6, CX8 and CX10 were selected for cytotoxicity studies against breast cancer cell line. The compound CX8 and CX10 showed better inhibition than other analogues. The Cx8 showed better inhibition at all concentrations in both cell lines. Hence further studies can be conducted using this compound by increasing its concentration and also the incubation period. As the compounds CX1, CX8, CX13 and CX21 showed highest glide scores on docking studies these analogues were tested for Antitubercular activity. It was observed that the analogue CX8 and Cx1 showed better activity against the organism. The derivatives CX3 and CX18 were selected for antibacterial screening according to PASS value. The analogue CX3 was found to cause better inhibition of growth of both gram positive and gram negative organisms compared to CX18.Gentamycin was selected as standard. The derivatives CX3 and CX18 were subjected to antifungal screening using Clotrimazole as standard. The fugal strain used were Candida Albicans and Aspergillus Niger. The analogue CX3 was found to exhibit better inhibition of proliferation of the Candida albicans and Aspergillus Niger at concentration of 100µg/ml whereas analogue CX18 didn't show any inhibition against Aspergillus Niger but showed satisfactory inhibition against Candida albicans at concentration of 100µg/ml compare to Clotrimazole. Anthelmintic activity was evaluated using earth worm Eudrillus eugenia because of its anatomical and physiological resemblance with the intestinal round worm parasites in human beings.AnaloguesCx3,Cx6 and CX18 were selected for Antibacterial screening according to PASS value. The analogue CX3 was found to show the highest Anthelmintic activity. The results were found to most satisfactory in comparison to the standard

Conclusion

I was synthesized 25 different classes of 1,3,4-oxadiazole derivatives of Coumarin and 10 of them found biologically which were selected for further work. They are *CX1, CX3, CX6, CX8, CX10, CX13, CX15, CX18, CX19*, *CX21 and f*ound pharmacologically suitable. The acute toxicity of synthesized oxadiazole derivative were determined by Brine Shrimp Lethality assay. The LD_{50} values of the compounds were found to be in the range of 400-600µg/ml. The compounds *CX1, CX6, CX8* and *CX10* were selected for cytotoxicity studies against breast cancer cell and fibrosarcoma cell line. The compound CX8 and CX10 showed better inhibition than other analogues. As the compounds *CX1, CX8, CX13* and *CX21* showed highest glide scores on docking studies, these analogues were tested for Antitubercular activity. It was found that the analogue *CX8* and *CX1* showed better activity against the organism. The derivatives *CX3* and *CX 18* were selected for antibacterial screening according to *PASS* value. The analogue *CX3* was found better inhibition of growth of both gram positive and gram-negative organisms. The derivatives *CX3* and *CX18* were subjected to antifungal screening with *Candida albicans* and *Aspergillus Niger*. The Analogue *CX3*

was found to exhibit better inhibition of proliferation at concentration 400µg/ml and Anthelmintic activity CX3 was found higher anthelmintic activity. *Irene Kostova et al* (2005) reported the synthesis of New Zirconium complexes of Coumarin and screened their cytotoxic activity. *Xin-Hua-Liu et al* (2010) synthesized and performed molecular docking study of novel coumarin derivatives containing 4,5-Dihydropyrazole moiety as potent tumour agent but none of them tried for1,3,4-oxadiazole derivatives. Conventional method as well Microwave method had been tried and found that Microwave method takes little time and more products were obtained.

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