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Research Article

DOCKING INVESTIGATION, SYNTHESIS AND CYTOTOXIC STUDIES OF SUBSTITUTED OXADIAZOLE DERIVATIVES

BIJU.C.R.[1, 3] ILANGO.K[2]

[1]Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala.673634, [2]SRM College of Pharmacy, SRM University, Kattankulathur, Kancheepuram, Tamilnadu.603203, [3]Karpagam University, Echanari, Coimbatore, Tamilnadu, India.
Email: bijucrmpharm@gmail.com

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ABSTRACT

Structural modification involving variations in the nature of groups in different positions of the oxadiazole ring leads to a large number of derivatives that are biologically active. It was planned to suitably incorporate the coumarin ring system into oxadiazole moiety to explore the possibilities of some altered biological action.We report here with the preparation and cytotoxic studies of new series of compounds bearing 1, 3, 4-oxadiazole moiety with substitutions in the 2nd and 5th position.The docking studies were carried out by using GOLD software Most of the compounds shown good binding interaction against various drug target proteins of breast and oral cancer. The compounds which show good docking score were synthesized and characterized by spectral data. The cytotoxic activity of the synthesized compounds was tested by MTT assay and the compounds show good activity on MCF-7 cell lines.

Keywords: 1, 3, 4-oxadiazoles, Coumarin, Molecular docking, Ligand, MTT assay.

INTRODUCTION

1,3,4-oxadiazoles represent an important class of heterocyclic compounds that have wide applications in therapeutic area, having biological activities like anticancer[1], analgesic[2], anti-inflammatory, bactericidal, antifungal, anticonvulsant, anti-tumor, anti-hypertensive, muscle relaxant, psychotropic, plant growth regulating and mono amino oxidase inhibitory activities.[3,4] Coumarins, an old class of compounds, are naturally occurring benzopyrene derivatives. A lot of Coumarins have been identified from natural sources, especially in plant families such as Rutaceae and Umbelliferae. The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution.[7] The lead compound was structurally modified by incorporating various substitutions at the second and fifth position of the 1,3,4-oxadiazole heterocyclic ring system. From the literature review it is clear that 2,5disubstituted 1,3,4-oxadiazole derivatives possess remarkable analgesic, anti-inflammatory and anticancer activity[5,6]

MATERIALS AND METHODS

Target identification andretrieval

Crystallographic structures of the targets of interests were obtained from PBD (Protein Data Bank) and saved in standard 3D coordinate format.

Active Site Identification

All the targets were possessing natural ligand and so active site residue identification was carried out taking advantage of the same. The protein was loaded in SWISS PDB Viewer. Proteins which had many chains were cleaned and a single chain of interest was selected. Using the control panel of this stand-alone software, natural ligand molecules were selected[7, 8].

Preparation of Active site

Explicit Hydrogen atoms missing in the PDB structure were added using Argus Lab, stand-alone docking software. Furthermore, the atom list of the molecules were prepared, which represents numbers of all the atoms of the active site residues involved.

Ligand preparation

The smiles formulas of the drug molecules were obtained from ChemSketch. Molecular Network software packages provide CORINA, which was used for the generation of 3D coordinates from smiles. Again Using Converter of this same server PDB Structures of the Drugs was converted into MDL MOL format which is an acceptable form for any standard docking software. Finally using ARGUS LAB ligand molecules were prepared by the addition of hydrogen atoms[9, 10, 11].

Molecular docking

Docking Studies were carried out using GOLD (Genetic Optimization and Ligand Docking) commercial standalone software developed by CCDC. Entire process was carried out with minimum speed and maximum accuracy. Gold score for each drug molecules against all the targets were saved and analyzed.

Synthesis of 3-carbethoxy coumarin

Salicylaldehyde (0.01 mol, 1.22g) and ethyl malonate (0.01 mol, 1.6g) was dissolved in absolute ethanol and to this mixture was added 5ml of piperidine and 0.5ml of glacial acetic acid. This mixture was heated under reflux for 7 hrs. The hot solution was transferred to a 500ml Erlen Mayer flask. 80ml of hot water (60°C) was added to the solution, the product crystallized out rapidly as the solution cooled, the mixture was stirred from time to time as crystallization proceeded, and stored overnight in a refrigerator. The crystalline product was collected by filtration and washed with ethanol and dried in air[12, 13]. Mol. Wt. 218; Yield :82%; M.p : 245^{0}C; Rf. value: 0.65 (Ethyl acetate: acetone (9;1)) ; IR:(KBr v cm^{-1}); 3090(aromatic C-H str), 1680(C=O,coumarin), 1750(C=O ester), 1131(C-O ester), 800 (aromatic C-H bend).^{1}H NMR ($CDCl_3$) δ ppm^{1}HNMR (DMSO) δ ppm: 2.4(2H, CH_3),; 3.4(2H, OCH_2: 6.2—8.2 (Ar-H, 9H)Mass- M^+ peak (m/z)218, Base peak (m/z)145

Synthesis of 2 – oxo – 2H – chromene -3 – carbohydrazide

Compound 1(0.014mole, 3.05g) and hydrazine hydrate (99%) (0.016mole, 0.8ml) were dissolved in sufficient quantity of ethanol to give a clear solution and refluxed for 10 hrs. The contents were concentrated to a small volume. On cooling, the crystals of **2'** separated out which were filtered and crystallized from ethanol to give TLC pure colorless needles.Mol.wt 204; Yield : 45% ; M.p.210-214°C ; Rf: 0.63 (chloroform: methanol, 7:3); IR (KBr) : 3304(NH str), 1667(C=O str), 3100(C-H aromatic str),Mass- M^+ peak (m/z)201, Base peak (m/z)132

General procedure for synthesis of 2-(coumarin-3-yl)-5-aryl-1,3,4-0xadiazoles (3a - e)

In a 100 ml round bottom flask was taken a solution of compound 2 (0.01) in phosphorous oxychloride (5ml) and different aromatic acids were added. The reaction mixture was refluxed for 5 hours,

cooled to room temperature and poured on to crushed ice. On neutralization with 20% $NaHCO_3$ solution a solid mass separated out which were filtered washed and crystallized from methanol to give compounds [14]**3'a – e**, which were TLC pure.[1]H NMR($CDCl_3$) δ ppm[1]HNMR (DMSO) δ ppm: 2.4(2H, CH_3),; 3.4(2H, OCH_2: 6.2—8.2 (Ar-H, 9H)Mass- M^+ peak (m/z)204, Base peak (m/z)181

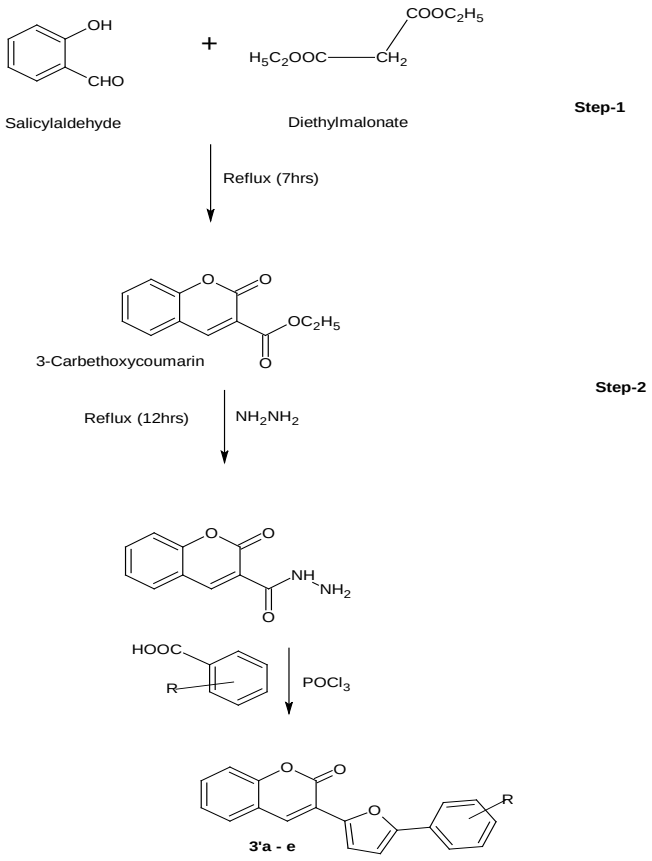

Fig. 1: Synthetic scheme

RESULT AND DISCUSSION

Docking studies were carried out against different breast cancer and oral cancer targets like VEGFR, EGFR, Caspase, Cyclin dependent kinases (CDKs), Akt1, Integrins, inhibitors of apoptosis (IAP) etc.(Fig-1 to 4) Majority of the synthesized chemical compounds showed good fit with the active site of all the docked targets. Compounds 4a, 4b & 4c which showed a maximum GOLD score were taken out for wet laboratory validations of its anti-cancer activities. (Table-1)

Table 1: Docking results of synthesized compounds with selected targets.

Targets	Compounds	Docking Score	Pose
Integrin beta	*3a*	65.96	9
	3b	53.85	6
	3c	54.96	7
	3d	52.19	6
wee protein	*3d*	64.49	3
	3e	59.09	14
	3a	58.06	2
Ppar alpha	*3a*	73.14	5
	3c	64.05	8
	3d	61.95	10
	3e	59.9	9
Ppar gamma	*3b*	58.93	1
	3d	58.31	6
	3e	55.77	4
IAP	*3a*	53.55	5
	3b	72.02	4
	3c	68.9	5
	3d	67.58	7
VGFR	*3b*	57.49	5
	3d	57.07	3
	3c	56.48	3
	3e	54.46	1
Progestrone	*3a*	57.24	9
	3b	57.17	6
	3c	53.97	1
	3d	51.75	2

Docking Images

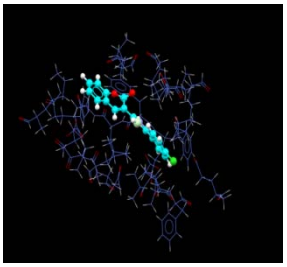

Fig. 1

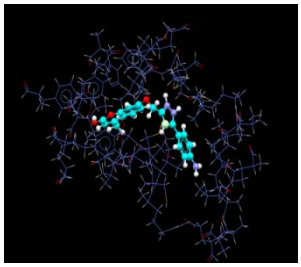

Fig. 2

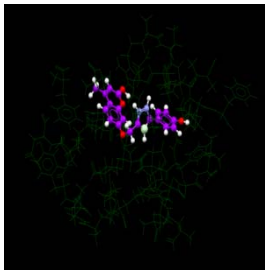

Fig. 3

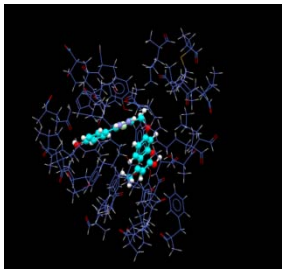

Fig. 4

3c bound to wee protein3d bound to IAP3b bound to IAP 3b bound to VGFR

Table 2: % cell viability – compound 3b

Conc.	% cell viability
.78125µM	102.6901
1.5625µM	94.70706
3.125µM	88.18242
6.25µM	84.40413
12.5µM	77.33023
25µM	53.52863
50µM	46.28265
100µM	41.57505

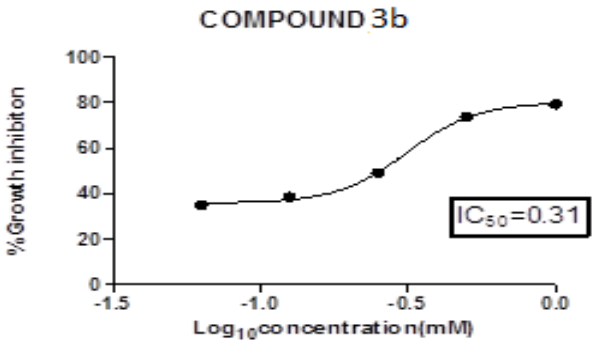

Fig. 5: Graph showing cell viability – compound 3b

Cytotoxicity study

MTT assay was validated best method for screening cytotoxicity. Randomly selected two molecules from the set of synthesized compounds were evaluated for the activity at different concentrations 10-100µg/ml. The drugs are administered as a solution in DMSO and a DMSO control was also used. The drugs showed good cytotoxic behavior and can considered being potent cytotoxic agents.(Table-2),(fig-5)

This research work was focused on the rational approach in design and development of 1,3,4-oxadiazole derivatives as novel anticancer drugs.These analogues also showed good binding with many specific breast cancer molecular targets, which was proved from the docking studies. The high Gold Score for some of the targets is in accordance with the anti cancer mechanism proposed for the oxadiazole moiety in various literature reports.The two derivatives which were having high gold scores have shown good cytotoxic effects on MCF-7 human breast cancer cell lines. Hence we consider these derivatives may be future leads for anti cancer, analgesic and anti-inflammatory drug discovery and further detailed studies on anticancer, analgesic and anti-inflammatory effects are recommended.

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