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Molecular Docking Study of Flavanone Derivatives with Human Aromatase Enzyme as target Protein and its Anti-cancer activity against normal Vero cell line and Breast Cancer MCF7 cell Lines

PHARMACOLOGY

KEYWORDS: Flavanone, Anticancer activity, Cytotoxicity, Docking study, glide score, glide energy

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ABSTRACT

The study was aimed to evaluate the invitro cytotoxicity of Flavanone derivatives namely 3-[4-(benzyloxy)-phenyl]-2,3-dihydro-1H-benzo[f]-chromen-1-one– (BDBC) and (3-(4-methoxyphenyl)-2,3-dihydro-1H-naphtho[2,1-b]pyran-1-one – (MDNP) which were synthesized and their cytotoxicity against normal Vero cell and breast cancer MCF7 cell lines were assessed by MTT assay method. Higher IC_{50} values of the compounds with the Vero cell line suggest that they are widely non- toxic. The IC_{50} values of 15.6 and 31. 2 µg/ml, exhibited against the MCF7 cells by the derivatives, BDBC and MDNP suggest the significant anticancer activity of the compounds. Molecular docking studies were performed in order to validate the obtained biological results. Induced fit docking analysis shows that the exemestane (co-crystal) has docked well at the active site of target protein with the glide score of -9.473 and glide energy of -48.055 Kcal/mol. The compounds BDBC and MDNP have been observed to be at the active site of target protein with the glide score of –8.228, -7.952 and glide energy of -55.582, -40.528 Kcal/mol, respectively, which are comparable with the corresponding values of the co-crystal.

INTRODUCTION

Flavanone is an important class of Flavonoids (Hesperitin, Naringenin, Eriodictyol) generally present in all the citrus fruits such as oranges, lemons, grapes etc. The flavanones have attracted considerable attention because they possess antioxidant (Pier-Giorgio Pietta, 2000), anticancer (Harborne, J. B., 1994), cytotoxic (Lie, S., 2010), and anti-inflammatory (Harborne et al., 1994) activities and also an important pharmacophore in numerous pharmacological applications (Yogesh Murti et al., 2014; Bravo, L., 1986). Flavanones have also been used widely in manufacturing varied medicines (Li R et al., 1994; Dinkova-Kostova et al., 1998; Ballesteros et al., 1995; Dimmock et al., 1998).

Breast cancer is the second leading cause of cancer-related death in women. The reduction in mortality and morbidity is being achieved among cancer patients through the use of current chemotherapeutic agents. However, the application of many anticancer drugs is associated with high toxicity due to their mechanisms of action and non-specific targeting. Current anti-estrogen medicine tamoxifen is widely used in the prevention and treatment of estrogen receptor positive breast cancer (Lazarus, et al., 2009) causes severe side effects. Thus, it is imperative to search for new alternatives to breast cancer prevention agents. The 'therapeutic index' is an important parameter to select samples for developing drugs is the ratio of the concentration of the extract at which 50% of the survival of the normal cell in normal cell line to that of the extract at which 50% of cancer cell death occurred in cancer cell lines and a drug with therapeutic index value of 16 or greater is generally used for further testing.

Considering the need for newer anticancer drugs and in an endeavour to search for less toxic and potent new anti-breast cancer drugs,following derivatives of Flavanones have been synthesized and studied for their anticancer activity and the results are communicated in this paper,

I *3-[4-(benzyloxy)-phenyl]-2,3-dihydro-1H-benzo[f]-chromen-1-one (*BDBC)

II *(3-(4-methoxyphenyl)-2,3-dihydro-1H-naphtho[2,1-b]pyran-1-one* (MDNP)

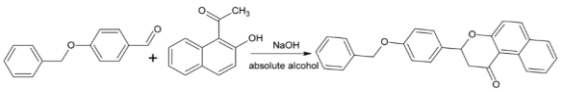
EXPERIMENTAL

(i) SYNTHESIS OF THE COMPOUNDS

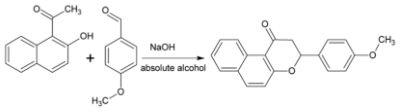
Synthesis of 3-[4-(benzyloxy)-phenyl]-2,3-dihydro-1H-benzo[f]-chromen-1-one (**BDBC**)

In a 250ml round-bottomed flask 2-hydroxy-1-acetonaphthone (0.06mol) and 4-benzyloxybenzaldehyde (0.06mol) were taken to which about 125ml of absolute alcohol was added and stirred at room temperature for a time frame of 10 minutes to ensure complete dissolution to which 10ml of 40% sodium hydroxide solution was added and the mixture was stirred for 24 hours. The precipitate formed on adding ice cold was filtered, washed with distilled water and dried. The crude product was recrystallized twice from chloroform. The compound (3-(4-methoxyphenyl)-2, 3-dihydro-1H-naphtho [2, 1-b] pyran-1-one (**MDNP**) was also synthesized following the same procedure but by including 4-methoxyoxybenzaldehyde (0.05mol) as one of the reactants.

The reaction schemes are given below



SCHEME 1. REACTION SCHEME OF BDBC



SCHEME.2.REACTION SCHEME OF MDNP

(i) STUDY OF ANTICANCER ACTIVITY OF THE COMPOUNDS

The cytotoxicity of the synthesised compounds was evaluated against normal Vero (African green monkey kidney) and MCF-7 (human breast cancer) cell lines by MTT Assay method. MTT assay is a rapid and high accuracy colorimetric approach that is widely used to determine cell growth and cell cytotoxicity, particularly in the development of new drug. It measures cell membrane integrity by determining mitochondrial activity through enzymatic reaction on the reduction of MTT to formazan which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

MICRO CULTURE TETRAZOLIUM (MTT) ASSAY METHOD

Cells (1×10^5/well) were plated in 24-well plates and incubated in 37°C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl) -2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours and 1ml of DMSO was added later in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula

% cell viability = [A570 of treated cells / A570 of control cells] × 100

Graphs are plotted using the % of Cell viability along Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

CYTOTOXIC EFFECT OF THE COMPOUNDS ON NORMAL *VERO CELL* LINE

The results of cytotoxicity evaluation of the compounds are shown in Fig.1, 2 and Table 1. It is observed from the results that as the concentration of the compounds increases, cell viability decreases. Even for higher concentration as 1000µg/ml of the compounds, percentage of cell viability for BDBC and MDNP are 80.00% and 77.77%, respectively. Hence, IC_{50} value for the compounds may be obtained for concentration greater than 1000 µg/ml and so it cannot be calculated from the graphs. As cytotoxicity is inversely proportional to the cell viability, compounds are almost non- toxic to normal Vero cell line because of their higher IC_{50} values. Results show that the cell viability is 97.77% for BDBC and 95.55% for MDNP at the concentration 7.8 µg/ml of the compounds. Because of low cytotoxic effect exhibited by the synthesized compounds, they may be considered as potential candidates at a concentration less than 7.8µg/ml for pharmaceutical applications.

ANTICANCER EFFECT ON BREAST CANCER CELL LINE (MCF7)

Results are tabulated in Table.2 and graphically represented in Fig. 3 & 4. Recorded information show the decrease in cell viability against the increase in the concentration of the compounds. The concentration corresponding to the IC_{50} values of the compounds BDBC and MDNP are 15.6 and 31.2 µg/ml, respectively, found from the graph. Lower IC_{50} values with the MCF7 cell lines indicate that the compounds have got good anticancer effect on MCF7 breast cancer cell lines.

TABLE.1 CYTOTOXICITY OF COMPOUNDS ON VERO CELL LINE

Concentration (µg/ml)	Dilutions	COMPOUND **BDBC**		COMPOUND **MDNP**	
		Absorbance (O.D)	Cell Viability (%)	Absorbance (O.D)	Cell Viability (%)
1000	Neat	0.36	80	0.35	77.77
500	01:01	0.39	86.66	0.36	80
250	01:02	0.4	88.88	0.37	82.22
125	01:04	0.41	91.11	0.39	86.66
62.5	01:08	0.42	93.33	0.41	91.11
31.2	01:16	0.42	93.33	0.41	91.11
15.6	01:32	0.43	95.55	0.42	93.33
7.8	0.08611	0.44	97.77	0.43	95.55
Cell control	-	0.45	100	0.45	100

TABLE.2 ANTICANCER EFFECTS OF THE COMPOUNDS ON MCF7 CELL LINES

Concentration (µg/ml)	Dilutions	COMPOUND **BDBC**		COMPOUND **MDNP**	
		Absorbance (O.D)	Cell Viability (%)	Absorbance (O.D)	Cell Viability (%)
1000	Neat	0.09	15.25	0.08	13.55
500	01:01	0.13	22.03	0.14	23.72
250	01:02	0.17	28.81	0.2	33.89
125	01:04	0.19	32.2	0.23	38.98
62.5	01:08	0.22	37.28	0.28	47.45
31.2	01:16	0.27	45.76	0.33	55.93
15.6	01:32	0.32	54.23	0.37	62.71
7.8	0.08611	0.34	57.62	0.41	69.49
Cell control	-	0.59	100	0.59	100

FIG.1 CYTOTOTOXICITY OF COMPOUND BDBC ON VERO CELL LINE

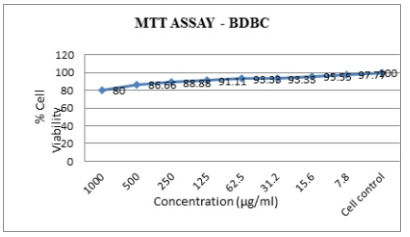


FIG.2 CYTOTOTOXICITY OF COMPOUND MDNP ON VERO CELL LINE

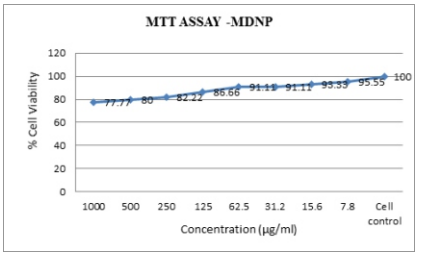


FIG. 3. ANTICANCER ACTIVITY OF COMPOUND BDBC ON MCF7 CELL LINE

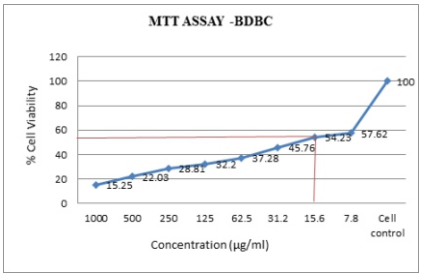
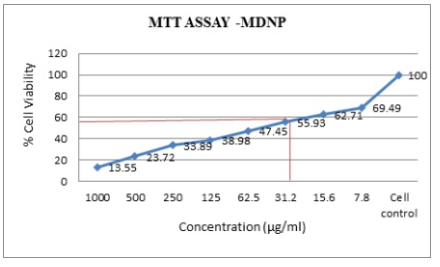


FIG. 4. ANTICANCER ACTIVITY OF COMPOUND MDNP ON MCF7 CELL LINE



RESULTS

To be a good drug, the IC_{50} value of such agent should be sufficiently low to avoid any possible unspecific effects. IC_{50} value on MCF-7 cell line for the compounds BDBC and MDNP are 15.6 and 31.2 µg/ml, respectively, while on Vero cell line that are greater than 1000 µg/ml, which suggests that the compounds have strong inhibition against the MCF-7 cell lines and weak inhibition on normal healthy body cell. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bio guided studies, if it exerts an IC_{50} value 30 µg/ml (Suffness, M. and

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Pezzuto, JM., 1990). IC_{50} values 15.6 µg/ml and 31.2 µg/ml obtained for the compound BDBC and MDNP is satisfying the said condition (less than 30µg/ml), may be used for pharmaceutical applications as potential anticancer agents. Also therapeutic index value greater than 16, suggest that they can be used as chemotherapeutic or a chemopreventive agent based on their ability to induce cytotoxicity in cancer cells with relatively low toxicity to normal cells. Further studies with *in vivo* and clinical trials needs to be conducted to establish these as safe agents for cancer therapy.

(iii). DOCKING STUDIES OF THE SYNTHESIZED COMPOUNDS WITH HUMAN AROMATASE ENZYME AS TARGET PROTEIN

Aromatase (CYP19), a cytochrome P450, is the enzyme that synthesizes estrogens. Estrogens are female sex hormones involved in the development and growth of breast tumors. Aromatase activity appears to be enhanced in certain estrogen-dependent local tissue next to breast tissue, endometrial cancer, endometriosis, and uterine fibroids. Aromatase is expressed at a higher level in human breast cancer tissue than in normal breast tissue. Aromatase inhibitors, which stop the production of estrogen in postmenopausal women, have become useful in the management of patients with breast cancer whose lesion was found to be estrogen receptor positive. Exemestane (AROMASINR), 6-methylideneandrosta-1, 4-diene-3,17-dione, are prominent drugs currently used for the treatment of estrogen-dependent breast cancer. The three dimensional crystal structure of human Aromatase complexed with ligand exemestane, ($C_{16}H_{14}F_3NO_3S$) (PDB id: 3s7s) was extracted from the Protein Data Bank (PDB).

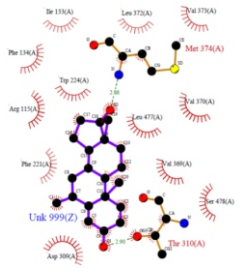
Molecular docking studies of the synthesized compounds has been carried out to understand the binding mechanisms of these bioactive compounds with human aromatase enzyme complexed with ligand exemestane as target protein (receptor) using XP docking program of Maestro, version 9.4, Schrödinger software. The objective of the present study is to demonstrate that the synthesized compounds bind to active site amino acids and to evaluate whether these molecules can be used as potential drug candidates.

RESULTS OF DOCKING STUDIES

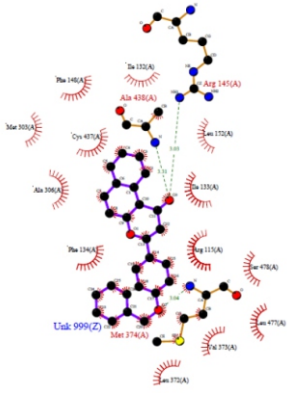
The exemestane (co-crystal) has docked well at the active site of target protein with the glide score of -9.473 and glide energy of -48.055 Kcal/mol. Oxygen atom of the co-crystal interacts with nitrogen group of MET 374 and Oxygen atom of Thr310 by forming hydrogen bonds at a distance of 2.86 and 2.90Å, respectively (Fig.5). Docking studies of the compound BDBC shows that there are three different ligand poses generated with different docking scores and glide energies which are shown in the Table 3. The oxygen atom O2 in the pyran ring of the compound interacts with the nitrogen atoms of ARG145 (N-H...O) and ALA438 (N-H...O) at a distance of 3.03 and 3.31Å, respectively, while the nitrogen atoms of MET 374 (N-H...O) interact with the oxygen atom O1 of the compound at a distance of 3.04 Å. The compound BDBC has docked well at the active site of target protein with the glide score of –8.228 and glide energy of -55.582 Kcal/mol. Many of the residues are in close proximity to the compound and are hydrophobic in nature (Fig.5). The compound MDNP has docked well at the active site of target protein with the glide score of.-7.952 and glide energy of -40.528 Kcal/mol. The oxygen atom O2 in the carbonyl group of the compound interacts with the nitrogen atom of MET 374(N-H...O) forming a hydrogen bond at a distance of 3.10 Å and the nitrogen atom of ALA438 (N-H...O) interacts with the oxygen atom O3 in the methoxy group of the compound at a distance of 3.24 Å. (Fig. 5).

Hydrogen bonding interactions play a very important role in determining how effective the compound in the active site and our study on the synthesized compounds showing hydrogen bond interactions with good glide score and glide energy compared to that of the co-crystal, hence can act as potential drug molecule in inhibiting human Aromatase enzyme.

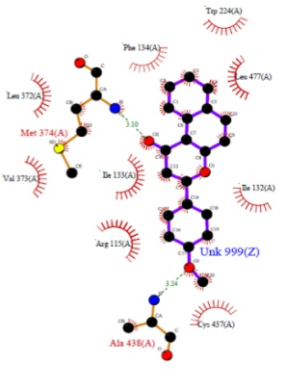
FIG.5. LIGPLOT SHOWING THE INTERACTIONS OF LIGAND AT THE ACTIVE SITE RESIDUES.



COCRYSTAL



BDBC



MDNP

TABLE.3 DOCKING RESULTS OF BDBC AND MDNP

Ligand	No of poses	H-bond interaction D-H...A	Distance (Å)	Glide score	Glide Energy Kcal/mol
Co-crystal	3	1.(MET 374)N-H...O (THR 310)O-H...O	2.86 2.90	9.473	48.055
		2.(MET 374)N-H...O (ASH 309)O-H...O	2.95 3.16	-8.278	-41.263
		3.(ARG 115)N-H...O	3.17	-6.933	-45.834
BDBC	3	1.(ARG 145)N-H...O (ALA 438)N-H...O (MET 374)N-H...O	3.03 3.31 3.04	-8.228	-55.582
		2.(MET 374)N-H...O	2.96	8.031	-52.681
		3.(ALA 438)N-H...O	2.95	-7.453	-43.523
MDNP	3	1.(MET 374)N-H...O (ALA 438)N-H...O	3.10 3.24	-7.952	-40.528
		2.(THR 310)N-H...O	2.90	-6.230	-37.55
		3.(VAL 369)N-H...O	2.94	-6.628	-35.78

CONCLUSION

A series of two new Flavanone derivatives were synthesised and screened for cytotoxicity on MCF7 cancer cell and normal Vero cell lines. The compounds exhibits excellent anti-cancer activity against human breast cancer cells without being significantly cytotoxic to normal cells. The docking studies of the compounds show good docking score and active site interactions and are comparable to that of the co-crystal. This study may lead to development of new therapeutic agents in our fight against cancer. Hence we consider these derivatives may be future leads for anti cancer drug discovery and further detailed studies are recommended.

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