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Molecular Docking Studies on Novel Phenyl Hydrazine Derivatives of Piperidones for Anticancer Efficiency

P. RAJESH[1,*], M. DINESH KUMAR[1], R. JAMUNARANI[1] and J. MANIKANDAN[2]

[1]Department of Chemistry, Government Arts College (Autonomous), Coimbatore-641 018, India
[2]Department of Chemistry, PSG College of Arts and Science (Autonomous), Civil Aerodrome, Coimbatore-641 014, India

*Corresponding author: E-mail: gacchemistryrajesh@gmail.com

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Phenyl hydrazine derivatives of some 2, 6-diphenyl-4-piperidones **(3-4)** were synthesized by the reaction of piperidone derivatives with phenyl hydrazine. The synthesized compounds **(3-4)** were characterized using IR, ^{1}H NMR and ^{13}C NMR techniques. ADME-Tox properties of the two compounds showed the drug likeliness property and found to obey Lipinski's rule of five. Molecular docking study for both the synthesized compounds **(3-4)** exhibited significant binding potency to the target protein structures. The glide score (G.Score) was least observed with dihydrofolate reductase protein which was -7.88 and -5.44 for compounds **(3)** and **(4)** respectively. The hydrogen bonds were formed with the residues THR56, SER59 of 2.1 Å, 2.4 Å bond. The Glide score was observed nearly equal to the quinazolinone derivative against dihydrofolate reductase. Therefore further studies could be carried out for the synthesized compounds as lead molecule for cancer drug development.

Keywords: Cancer, Piperidones, Molecular docking, Glide score, Dihydrofolate reductase protein.

INTRODUCTION

Cancer is considered to be a heterogeneous disease both morphologically and genetically. In general tumor development occurs when the environment enhances its growth. This may be due to the defect in nascent neoplastic cells thereby arresting the cell proliferation which lead the cell to be insensitive for signaling[1]. The androgen receptor (AR) also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4) is a transcription factor which comes under the type of nuclear receptor. In normal condition the expression of the genes maintains the function and development of male sexual organs[2]. Therefore defects may develop prostate cancer.

The discovery of Bcl-2 gene is believed to be one of the milestones achieved in tumor biology. The name is derived from B-cell lymphoma 2 and found regulating major types of cell death, including apoptosis, necrosis and autophagy, thus operating as nodal points at the convergence of multiple pathways with broad relevance to oncology[3]. In majority of the cancer types, the expression of proapoptotic members of Bcl-2 was found in defected state; therefore the cell loses its tumor suppressor function. mRNA expression of Bcl-2 subfamily members were found highest in many cancer types such as lung, prostate, breast, ovarian, renal and glioma cancer cell lines[4]. Since the multiple tumor types shows the over- expression of Bcl-2, targeting Bcl-2 may provide therapeutic benefit where the Bcl-2 inhibitors like navitoclax, BH3 are under investigation[5].

Cyclooxygenase (COX), officially known as prostaglandin-endoperoxide synthase (PTGS) is an enzyme responsible for the formation of important biological mediators called prostanoids, also known as prostaglandins, prostacyclin and thromboxane. Normal growth of cell involves the metabolites of arachidonic acid where the conversion to prostaglandins is catalyzed by the cyclooxygenase. Cyclooxygenase enzymes exist in two forms COX-1 and COX-2 where COX-2 is found overexpressed in cancerous growth[6]. COX-2 is also found to mediate the inflammatory effect of cyclooxygenase activity. COX-2 selective inhibitors are potent suppressor of colon polyps in colorectal cancer and even the treatment with celecoxib has shown promising results in cancer prevention. Harris and his colleagues[7] had reported COX-2 inhibitors also provide the same magnitude of protection for prostate and lung cancer.

Dihydrofolate reductase (DHFR) is an enzyme which catalyzes the reduction of dihydrofolic acid to tetrahydrofolic acid using NADPH as electron donor. It plays an essential role in cell metabolism, cellular growth and in regulating the amount of tetrahydrofolate in the cell. Tetrahydrofolate and its derivatives are essential for purine and thymidylate synthesis[8]. Therefore DHFR is considered to play a central role in the synthesis of nucleic acid precursors and has been

shown that a mutant cell completely lacks DHFR therefore it has to be supplied with glycine, purine and thymidine to grow. Estrogen receptor is activated by the hormone 17β-estradiol (estrogen) and it exists in two forms, one as a member of nuclear hormone family of intracellular receptors. Also as an estrogen G protein-coupled receptor GPR30 (GPER) belongs to G protein-coupled receptor. ERs are widely expressed in different tissue types however exhibits some notable diffe-rences in their expression patterns in which ERα found in breast cancer cells are one such example[9]. The expression of ER is the main indicator of potential responses to endocrine therapy (ET) and approximately 70 % of human breast cancers are hormone-dependent and ER-positive[10].

The glucocorticoid receptor (GR or GCR) also known as NR3C1 (nuclear receptor subfamily 3, group C, member 1) is expressed in almost every cell regulating the genes to control the development, metabolism and immune response[11]. Glucocorticoid receptor is found involved in the prostate cancer, breast cancer, solid tumors and acute lymphoblastic leukemias (ALL). Mechanism of cancer cell resistance in acute lymphoblastic leukemias may due to the glucocorticoid receptor-mediated apoptosis[12].

The mammalian target of rapamycin (mTOR) also known as mechanistic target of rapamycin or FK506 binding protein 12-rapamycin associated protein 1 (FRAP1) is a protein which in humans is encoded by the FRAP1 gene. Mammalian target of rapamycin is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis and transcription. Mammalian target of rapamycin inhibition clinically showed promising activity against particular cancers including cell lymphoma, endometrial cancer and renal cell carcinoma.

EXPERIMENTAL

The compounds **(3)** and **(4)** were synthesized by mixture of corresponding ketone (0.1 mol), benzaldehyde (0.2 mol) and dried ammonium acetate (0.1 mol) were dissolved in ethanol (50 mL)[13]. These contents were heated until the yellow colour turns into orange and were kept in room temperature for 14 h. Concentrated ammonia (30 mL) was added followed by conc. HCl (30 mL) and then cooled in ice water. Then the precipitate was neutralized with dry ether (30 mL). Finally, the neutralized product 3-methyl-2, 6-diphenyl-piperidin-4-one **(1-2)** was filtered and recrystallized from absolute ethanol. Phenyl hydrazine derivative of 2,6-diphenyl-piperidin-4-ones **(3-4)** were synthesized by the reaction of corresponding 2, 6-diphenyl-piperidin-4-ones (0.01 mol) with phenyl hydrazine (0.01 mol) and sodium acetate (0.01 mol) in ethanol (30 mL) with continuous stirring and heating for 3 h. The contents were kept undisturbed for 24 h. The precipitate was filtered, washed with ether and recrystallized from absolute ethanol. All the compounds **(3-4)** having very good yield. The synthetic scheme of the preparation of compounds **3** and **4** is given in Fig. 1. Purity test for the compounds were performed by TLC using glass plates coated with silica gel of 0.25 mm thickness. Spots were visualized using iodine chamber and characterized by ^{1}H NMR and IR spectroscopy analysis.

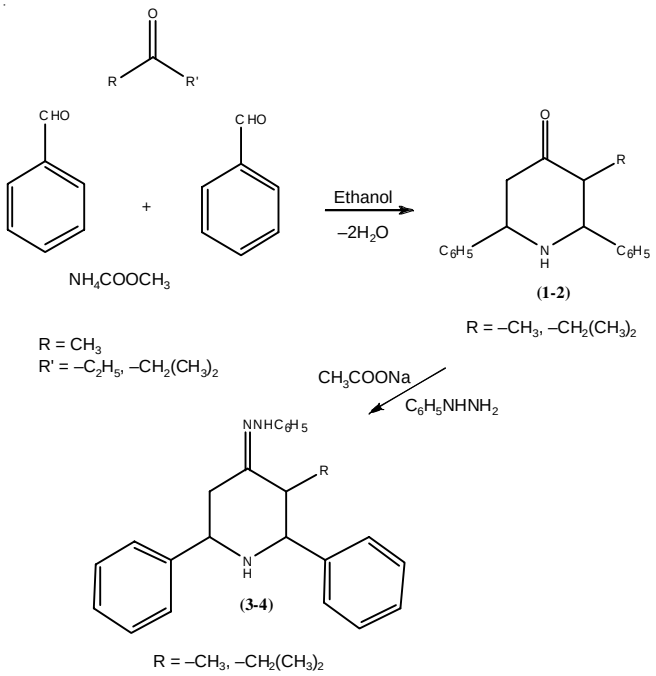


Fig. 1. Synthetic scheme for phenyl hydrazine derivatives of 2,6-diphenyl-piperidin-4-ones (**3-4**)

The conversion of active compounds into qualified clinical candidates has proved to be a challenge. *In silico* ADME-Tox analysis focuses mainly on predicting the absorption, distribution, metabolism, excretion and toxicity properties of chemical compounds, therefore assist in modifying the small molecules accordingly to develop into a potent drug. Bioavailability depends mainly on absorption and liver first-pass metabolism. The volume of its distribution, together with its clearance rate, determines the half-life of a drug and therefore its dosage is a key point. This information is valuable for the refinement of the *in silico* ADME-Tox models. Both the compounds were studied for its mode of interaction with cancer targets like androgen receptor, Bcl2 protein, cyclooxygenase II, dihydrofolate reductase, estrogen receptor, glucocorticoid receptor and mammalian target for rapamycin through molecular docking analysis.

The 3D structures of the synthesized molecules were drawn using Chemsketch 11.0. The ADME toxicity was analyzed for both the compounds using the QikProp module of Schrodinger suite. The protein structures were retrieved from PDB (Protein Data Bank). Molecular docking was carried out using Glide module of Schrodinger suite. Small molecules were prepared with LigPrep which produces a single, low-energy, 3D structure with correct chirality for eachsuccessfully processed input structure. The ligand structures were prepared adding hydrogen atoms and minimized with OPLS 2005 force field. The salts and water molecules which did not show interaction with the protein was removed. The resulting energy minimized prepared structures were used for further docking studies. The proteins were prepared by removing the water molecules which do not have the particular role in ligand binding.

The receptor grid can be set up and generated from the Receptor Grid Generation panel.Glide ligand docking requires a set of previously calculated receptor grids and one or more ligand structures.The Settings tab defines the basic options

TABLE-1
ADME-Tox PROPERTIES OF THE SYNTHESIZED COMPOUND (**3**) and (**4**)

Properties	Normal Range	Compound (**3**)	Compound (**4**)
Molecular weight	130.00-725.00	355.482	383.535
Solute as donor- hydrogen bond	0-10	2	2
Solute as acceptor- hydrogen bond	0-5	3.5	3.5
Octanol-water partition coefficient	<5	5.1	5.8

for docking ligands specifying the grid, selectingthe precision and setting flexibility options.Docking was carried out to perform XP ligand docking with the receptor Grid generated.

RESULTS AND DISCUSSION

The IR,^{1}H NMR, ^{13}C NMR spectra of compounds **3** and **4** have been studied and reported in this study.

N-(3-Methyl-2,6-diphenyl-piperidin-4-ylidine)-N'-phenyl-hydrazine (3): Brown solid; Yield: 68 % m.p.: 115 °C; IR (KBr) λ_{max} (cm^{-1}): 3339, 3309 (NH) 2253 (C=N) 1369 (C-N) 1113 (N-N); ^{1}H NMR (300 MHz, $CDCl_3$) 3.54-3.57 (d, 1H, *J* 10.2 Hz), 2.92-2.98 (m, 1H, *J* 16.8 Hz), 2.57-2.65 (dd, 2H, *J* 23.4 Hz), 3.89-3.94 (m, 1H, *J* 14.7 Hz), 1.01-1.03 (d, 2H, *J* 6.6 Hz), 2.09-2.25 (dd, 1H, *J* 45.9 Hz), 8.45 (s, 1H), 7.05-7.51 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) 12.32, 34.55, 44.83, 60.83, 69.43, 112.76-129.15, 143.00, 143.66, 146.01, 148.59.

N-(3-Isopropyl-2,6-diphenyl-piperidin-4-ylidine)-N'-phenyl-hydrazine (4): Brown solid; Yield: 45 % m.p.: 138 °C; IR (KBr) λ_{max}, (cm^{-1}): 3228, 3427 (NH) 2238 (C=N) 1352 (C-N) 1116 (N-N); ^{1}H NMR (300 MHz, $CDCl_3$) 3.98-4.04 (t, 1H, *J* 19.8 Hz), 2.64-2.72 (m, 1H, *J* 24.6 Hz), 2.58-2.59 (dd, 2H, *J* 16.8 Hz), 4.07-4.12 (d, 1H, *J* 14.4 Hz), 1.03-1.26 (m, 6H, *J* 68.1 Hz), 2.53-2.54 (m, 1H, *J* 3.3Hz) 1.62-1.70 (m, 1H, *J* 25.5 Hz), 8.79 (s, 1H), 7.05-7.51 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) 17.52-20.85, 25.76, 37.81, 51.74, 60.99, 64.53, 115.75-128.44, 136.66, 141.98, 142.67, 148.59.

ADME-Tox refers to absorption, distribution, metabolism, excretion and toxicity properties which should be considered to develop a new drug, because they are the main cause of failures for candidate molecules in drug design. The synthesized compounds were subjected for analyzing the ADME-Tox properties and the results are given in Table-1. Compound **3** has obeyed the Lipinski's rule of five rather compound **4** has violated the octanol-water partition co-efficient showing 5.8.The rule has following criteria: no more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, a molecular mass less than 500 daltons, an octanol-water partition coefficient log P not greater than 5[14]. Both the compounds were found with molecular weight of about 355 and 383 KDa respectively which were less than 500 KDa. The number of donor and acceptor are below the normal range, is about 2 and 3.5 for both the compounds.

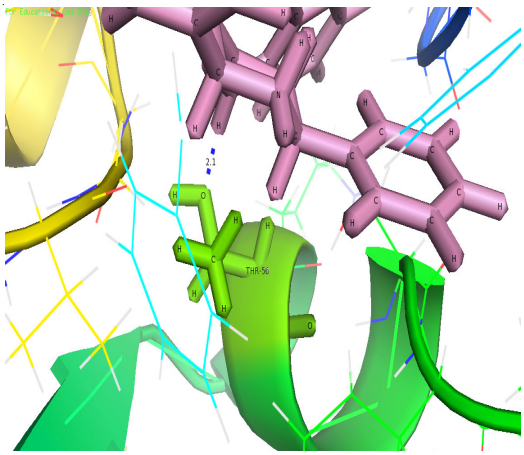
The proteins androgen receptor (2AM9), Bcl2 protein (1GJH), cyclooxygenase II (4OTY), dihydrofolate reductase (1DRF), estrogen receptor (3ERD), glucocorticoid receptor (1R4O) and mammalian target for rapamycin (1AUE) were retrieved from PDB with respective to PDB ID. The active sites were predicted using the online tool LigSite.The interaction of the molecules with each protein was observed using XP visualize and the glide score, interacting residues along with their bond length are given in Table-2.

TABLE-2
INTERACTION PROFILE OF CANCER SPECIFIC PROTEIN WITH THE SYNTHESIZED COMPOUNDS

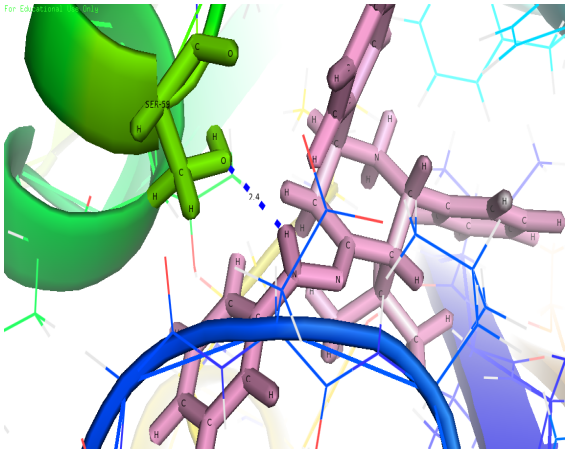
Compd. No.	G.Score	Interacting residues	Bond length (Å)
Androgen receptor			
3	-3.45	PRO682 (O-H)	2.8
		GLY683 (O-H)	2.1
4	-2.68	ASN705 (O-H)	2.1
BCL-2 Protein			
3	-3.92	ALA4 (O-H)	1.9
4	-2.53	ASP103 (O-H)	2.5
COX-2 Protein			
3	-2.97	GLY225 (O-H)	1.8
		ASN375 (O-H)	2.1
		GLN374 (O-H)	2.1
4	-2.93	GLY225 (O-H)	1.8
Dihydrofolate reductase protein			
3	-7.88	THR56 (O-H)	2.1
4	-5.44	SER59 (O-H)	2.4
Estrogen receptor			
3	-5.34	TRP393 (N-H)	2.3
		GLU323(O-H)	2.0
4	-5.15	PRO324 (O-H)	1.9
		GLU323 (O-H)	1.9
Glucocorticoid receptor			
3	-2.47	ASN491 (O-H)	2.6
4	-2.41	ASN491 (O-H)	2.3
Mammalian target of rapamycin			
3	-2.89	ARG 2043 (O-H)	1.9
		GLU 2053 (O-H)	2.0
4	-2.47	GLU2060 (O-H)	2.1

The glide score (G.Score) was least observed with dihydrofolate reductase protein and therefore the interaction of compound **(3)** and compound **(4)** were shown in the Fig. 2. Compound **(3)** has scored -7.88 and compound **(4)** has scored -5.44 of G.score which was found nearly equal to the quinazolinone derivative against DHFR[15]. The hydrogen bond was formed with the residues THR56, SER59 of 2.1 Å, 2.4 Å bond length for compound **(3)** and compound **(4)**, respectively.

The interaction of the compound **(3)** and **(4)** with the rest of the proteins were shown in the Fig. 3. With androgen receptor the glide score was observed to be -3.45 and -2.1. Compound **(3)** forms two hydrogen bonds whereas one bond is formed in the case of compound **(4)**. Both shared the electrons from the atom "O" (representing oxygen) of protein to "H" (representing hydrogen) atom of the ligand. The amino acids proline and glycine located at the position 682 and 683 forms hydrogen bond of length 2.8 Å and 2.1 Å. Compound **(4)** forms bond with ASN705 showing bond length of 2.1 Å. Likewise, the

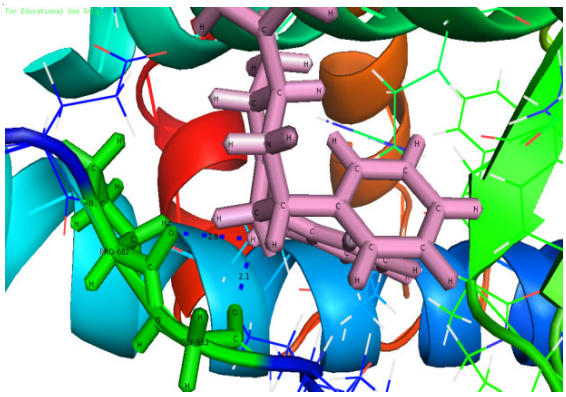


DHFR *vs.* compound (**3**)

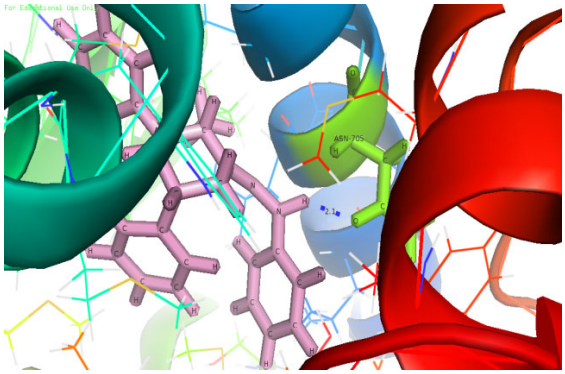


DHFR *vs.* compound (**4**)

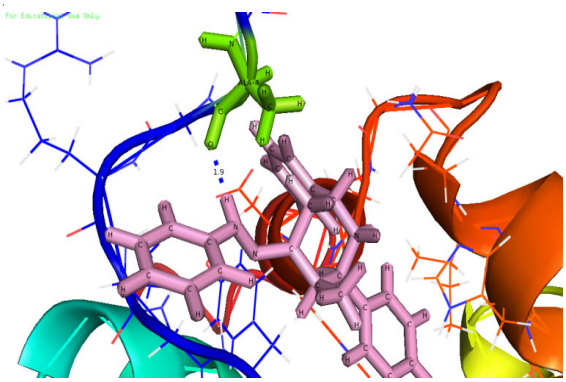
Fig. 2. Interaction of DHFR with compounds (**3**) and (**4**)



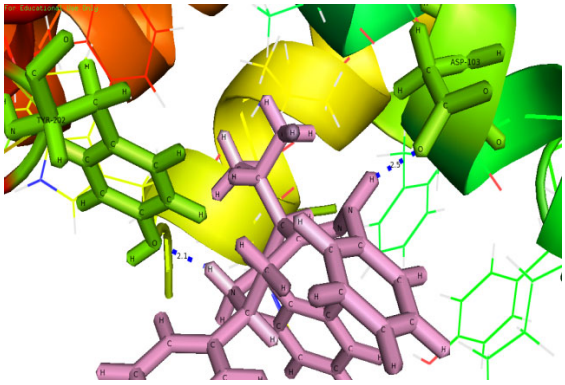
Androgen receptor *vs.* compound (**3**)



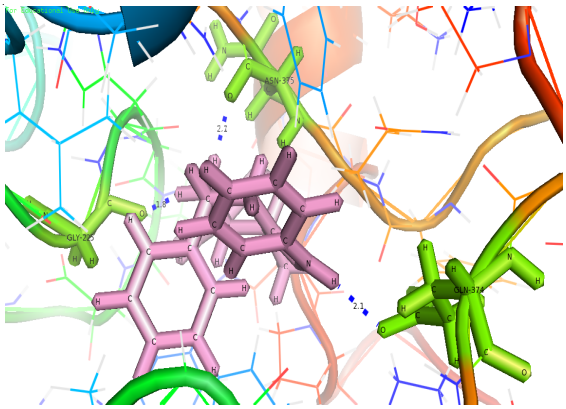
Androgen receptor *vs.* compound (**4**)



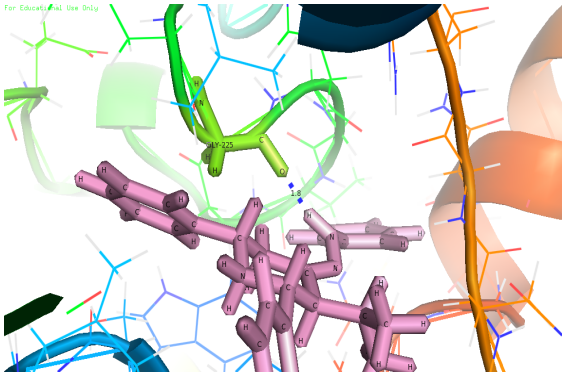
Bcl-2 *vs.* compound (**3**)



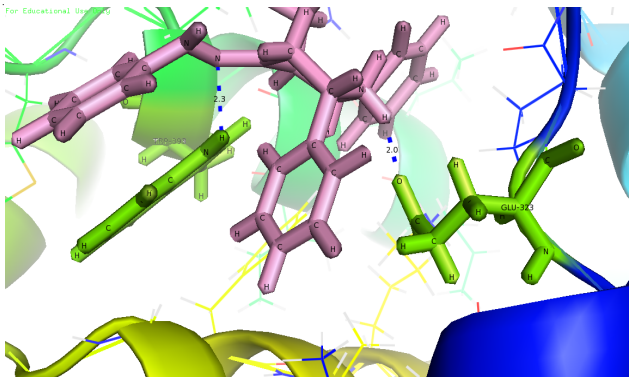
Bcl-2 *vs.* compound (**4**)



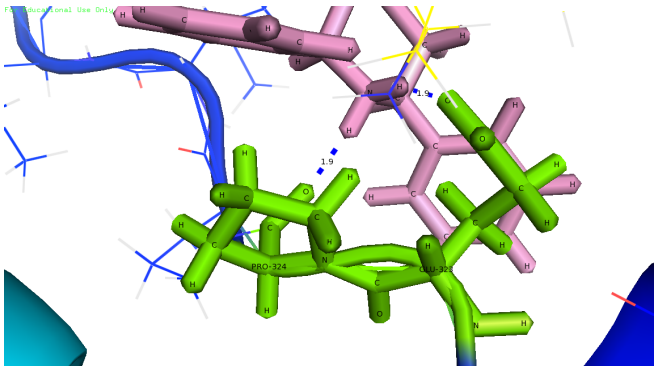
COX2 *vs.* compound (**3**)



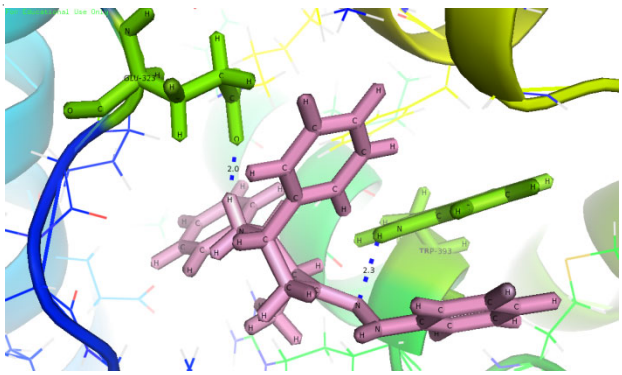
COX2 *vs.* compound (**4**)



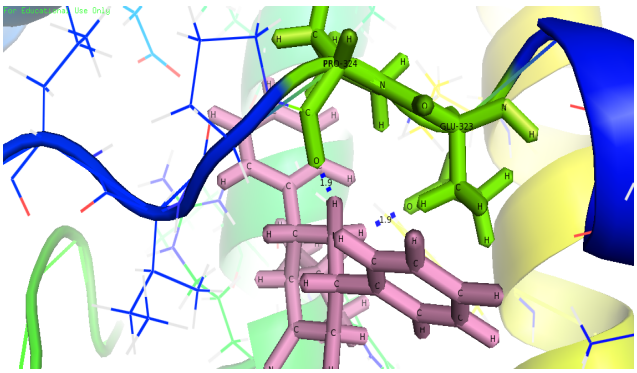
Estrogen receptor *vs.* compound (**3**)



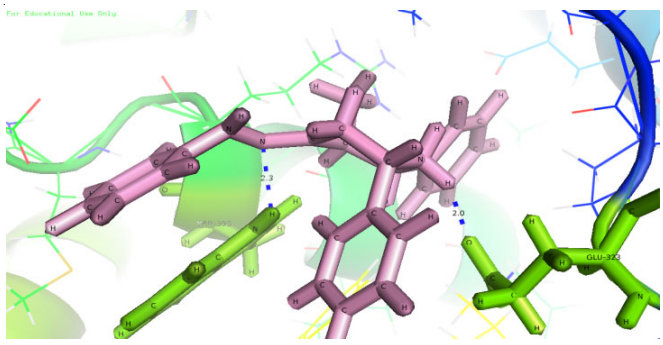
Estrogen receptor *vs.* compound (**4**)



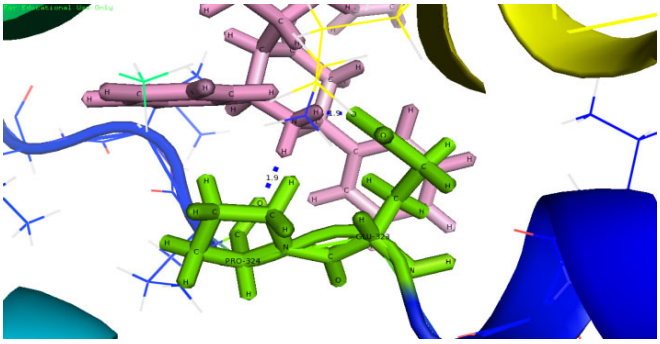
Glucocorticoid receptor *vs.* compound (**3**)



Glucocorticoid receptor *vs.* compound (**4**)



Mammalian target of rapamycin *vs.* compound (**3**)



Mammalian target of rapamycin *vs.* compound (**4**)

Fig. 3. Interaction of compounds (**3**) and (**4**) with respective proteins

synthesized compounds were observed to show interactions with the active site residues of each protein. Next to DHFR, the compounds show least score with estrogen receptor of about -5.34 and -5.15. Both the compounds interacted with the GLU323 of bond length 2.0 Å for compound **1** and 1.9 Å for compound **(4)**. Compound **(3)** also formed hydrogen bond with TRP393 residue, where the bond is shared between the N and H atom is of 2.3 Å length. Compound **(3)** interacts with the residue PRO324 of bond length 1.9 Å.

Conclusion

A series of phenyl hydrazine derivatives of some 2,6-diphenyl-4-piperidones (**3** and **4**) were synthesized and characterized by IR, ^{1}H NMR and ^{13}C NMR techniques.The proteins androgen receptor, B-cell lymphoma-2, cyclooxygenase-2, dihydrofolate reductase, estrogen receptor, glucocorticoid receptor and mammalian target for rapamycin were chosen for the present study. The synthesized compounds were subjected for analyzing the ADME-Tox properties and the compound **(3)** has not shown any violation in the Lipinski rule of five. The compound **(3)** and compound **(4)** were docked and the interaction with each protein was analyzed and it was found that both the compounds exhibited the glide score was least observed with dihydrofolate reductase protein which was -7.88 and -5.44 respectively. Therefore, the current study would be a foundation for developing the compound **(3)** in the treating cancer.

REFERENCES

1. P.N. Kelly and A. Strasser, *Cell Death Differ.*, **18**, 1414 (2011).
2. Z. Culig, H. Klocker and G. Bartsch, *Endocr. Relat. Cancer*, **9**, 155 (2002).
3. K.W. Yip and J.C. Reed, *Oncogene*, **27**, 6398 (2008).
4. W.J. Placzek, J. Wei, S. Kitada, D. Zhai, J.C. Reed and M. Pellecchia, *Cell Death Dis.*, **1**, 1 (2010).

5. L. Gandhi, D.R. Camidge, M. Ribeiro de Oliveira, P. Bonomi, D. Gandara, D. Khaira, C.L. Hann, E.M. McKeegan, E. Litvinovich, P.M. Hemken, C. Dive, S.H. Enschede, C. Nolan, Y.-L. Chiu, T. Busman, H. Xiong, A.P. Krivoshik, R. Humerickhouse, G.I. Shapiro and C.M. Rudin, *J. Clin. Oncol.*, **29**, 909 (2011).
6. A. Ferrandez, S. Prescott and R. Burt, *Curr. Pharm. Des.*, **9**, 2229 (2003).
7. Regular Use of Selective COX-2 Inhibitors Decreases Risk of Breast Cancer, Science Daily, BioMed Central (2006).
8. M.J. Chen, T. Shimada, A.D. Moulton, A. Cline, R.K. Humphries, J. Maizel and A.W. Nienhuis, *J. Biol. Chem.*, **259**, 3933 (1984).
9. M. Marino and P. Galluzzo, *Cancer Ther.*, **6**, 149 (2008).
10. F. Lumachi, A. Brunello, M. Maruzzo, U. Basso and S.M. Basso, *Curr. Med. Chem.*, **20**, 596 (2013).
11. N.Z. Lu, J.B. Collins, S.F. Grissom and J.A. Cidlowski, *Mol. Cell. Biol.*, **27**, 7143 (2007).
12. G. Schlossmacher, A. Stevens and A. White, *J. Endocrinol.*, **211**, 17 (2011).
13. C.R. Noller and V. Baliah, *J. Am. Chem. Soc.*, **70**, 3853 (1948).
14. C.A. Lipinski, *Drug Discov. Today. Technol.*, **1**, 337 (2004).
15. M. Priya, K. Girija, M. Karikalan and N. Ravichandran, *Int. J. Pharm. Chem. Sci.*, **1**, 503 (2012).