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Research Article

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Protein modeling of COX 2 and evaluating docking for prediction of binding affinities of Ru(II)/Co(III) polypyridyl complexes with COX 2 and CDK2 proteins

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ABSTRACT

*Computer aided drug discovery has been broadly used in the pharmaceutical industry to determine new compounds that show significant inhibitory activity against a biological target. In this context, the 3D structure of COX 2 (prostaglandin G/H synthase 2) protein was modeled using homology modeling method by Discovery Studio. Ru(II)/Co(III) polypyridyl complexes of $[Ru(phen)_2dmbip]^{2+}$ (**1**), $[Ru(bpy)_2dmbip]^{2+}$(**2**), $[Co(phen)_2dmbip]^{3+}$ (**3**), $[Co(phen)_2dmbip]^{3+}$ (**4**), $[Ru(phen)_2fyip]^{2+}$ (**5**), $[Ru(bpy)_2fyip]^{2+}$(**6**), $[Co(phen)_2fyip]^{3+}$(**7**) and $[Co(phen)_2fyip]^{3+}$(**8**) were docked into the active site pocket of COX 2 and CDK2 (Cyclin-dependent kinase-2) proteins using LibDock algorithm in Discovery Studio 2.1. Results indicated that Ru(II)/Co(III) polypyridyl complexes interacted with both the proteins COX 2 and CDK2. Among all, the complex **1** exhibited highest binding affinity with both proteins than all other complexes.*

Keywords: COX 2, CDK2, Homology modeling, Polypyridyl complexes, Molecular docking.

INTRODUCTION

Inorganic compounds particularly transition metals have played an important role in the development of new metal based drugs. The planar aromatic ligand present in the transition metal complexes may be responsible for stacking interactions with the aromatic molecules present in biological system [1, 2]. On the other hand, the complexes may be stabilized through hydrogen bonds to associate with the surrounding amino acids of proteins [3-7]. Current research has shown important progress in utilization of transition metal complexes as drugs. Transition metals exhibit different oxidation states and can interact with a number of negatively charged biomolecules. Small molecules are usually designed to interfere with the enzymatic activity of the target protein. In cancer cells the proteins of COX 2 and CDK2 plays an important role in regulating various events of cell cycle and over expression of these proteins should cause the abnormal regulation of cell cycle. Therefore, CDK2 was regarded as a potentially therapeutic target for cancer therapy. Therefore, cytotoxicity can be achieved by inhibition of CDK2 instead of cell arrest [10-14].

Until now, our group have been synthesized many Ru(II)/Co(III) polypyridyl complexes, and some of them have been found to have good *in vitro* cytotoxicity [15-20]. In this context, the 3D structure of COX 2 protein was modeled using homology modeling method by Discovery Studio and Ru(II)/Co(III) polypyridyl complexes **1-8** [15, 16] were docked into active site pocket of both the proteins (COX 2 and CDK2) using LibDock module in Discovery Studio. These results indicated that Ru(II)/Co(III) complexes bind with both the proteins. The complex **1** exhibited highest binding affinity than all other complexes.

EXPERIMENTAL SECTION

Protein homology modeling of COX 2

The query sequence of prostaglandin G/H synthase 2 (COX 2), was taken from Swissprot database with an accession of P35354. In order to construct a homology modeling, the first step is to find out a template structure with high sequence similarity to COX 2 using blast algorithm. Sequence that showed maximum identity with high score and less e-value was aligned and was used as a reference structure to built a 3D model for COX 2. The PDB code of the crystal structure is 1CVU, which was released in 2000 with a resolution of 2.40 Å. The entire sequence of 1CVU contains 552 amino acids. Discovery Studio was used to construct the 3D models of COX 2 protein. The model having least RMSD value by aligning it with the template is selected for further analysis. The final refined model obtained was analyzed by Ramachandran's plot analysis using RAPPER and the quality of model was also validated by ProSA [21] server (https://prosa.services.came.sbg.ac.at/prosa.php). The protein was subjected to energy minimization by applying CHARMm force field. This model was used to identify the active site and for docking with complexes **1-8**.

Molecular docking studies

Protein preparation of CDK2 and COX 2

Crystal structure of human cyclin dependent kinase2 (CDK2) as the target protein (PDB ID: 1G5S) was downloaded from Protein Data Bank. Water molecules were removed and the chemistry of the protein is corrected for missing hydrogens. The prepare protein protocol available in Discovery Studio is employed to prepare the protein for further processing by standardizes atom names, inserting missing atoms in incomplete residues, modeling missing loop regions, calculate pKa and protonate the protein and default parameters were used. Following the above steps of preparation, the protein was then refined by energy minimization with appropriate parameters by applying CHARMm force field and using steepest descent algorithm followed by conjugant gradient algorithm until the convergence gradient is satisfied.

The protein preparation for COX 2 was employed by using Discovery Studio protocols. The protein was then refined by energy minimization with appropriate parameters by applying CHARMm force field and using steepest descent algorithm followed by conjugant gradient algorithm until the convergence gradient is satisfied.

Preparation of Ru(II)/Co(III) complexes

The 3D structures of Ru(II)/Co(III) polypyridyl complexes **1-8** were drawn by using chemsketch. The drawn complexes were saved in Mol file format and it was imported to the Discovery Studio. The complexes energy was minimized by applying CHARMm force field, minimization is carried out with the steepest descent algorithm which follows by the conjugant gradient algorithm till it satisfies the convergence gradient. The 3D structure is generated by catalyst algorithm in Discovery Studio. The prepared complexes were used for docking.

Docking Studies

Molecular docking continues to hold a great promise in the field of computer based drug design which screens small molecules by orienting and scoring them in binding site of a protein. First conformational search of Ru(II)/Co(III) complexes were carried out, and all relevant low energy conformations were then rigidly placed in the binding site. The grid box with a dimension of 10Å points was used around the active site pocket of proteins. All the designed complexes were used to dock with into the active site pocket of proteins. Receptor–Ligand interactions, were performed by using Lib Dock module in Discovery Studio. The resulting poses with higher LibDock score were investigated.

RESULTS AND DISCUSSION

Protein homology modeling

The sequence alignment is essential to the success of homology modeling. After alignment of target sequence (COX 2) with template (PDB: 1CVU) the percentage of identity is 73.2% and similarity is 79.4% (Fig 1) and an overall RMSD between the modeled and the experimental structure falling around 1 Å. Protein Homology model Structure of Human COX 2 was constructed (Fig 2) and overlapping homology models of the COX 2 fragment built from the 1CVU template structure (Fig 3). The quality of the 3D model was evaluated using the PROCHECK program and assessed using the Ramachandran's plot. It is evident from the Ramachandran's plot that the predicted model has most favorable regions, the allowed regions, the generic regions and the disallowed regions. Fig 4 and 5 were shown Ramachandran's Plot analysis of Modeled structure of human COX 2 and template 1CVU, respectively. Ramachandran's plot shows that the predicted model is of good quality and the model show all the main chain and side chain parameters to be in the 'better' region. The quality of COX 2 protein as evaluated by ProSA web server (https://prosa.services.came.sbg.ac.at/prosa.php) provided a z-score of -8.64 and it confirm the quality of the homology model of COX 2 (Fig 6).

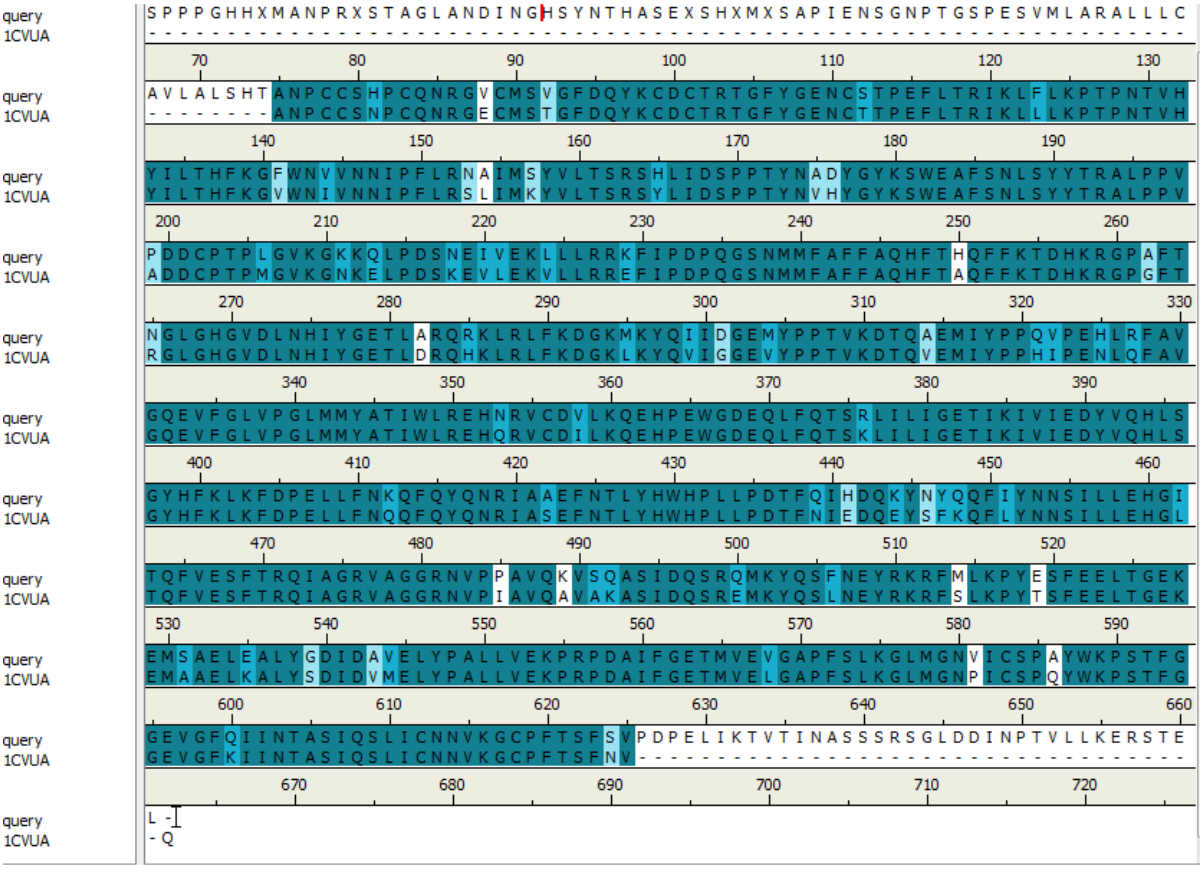

Fig 1. Multiple sequence and structure alignment between protein sequence of Human COX 2 and the selected template (PDB: 1CVU). Conserved regions between template and query are highlighted in dark colour

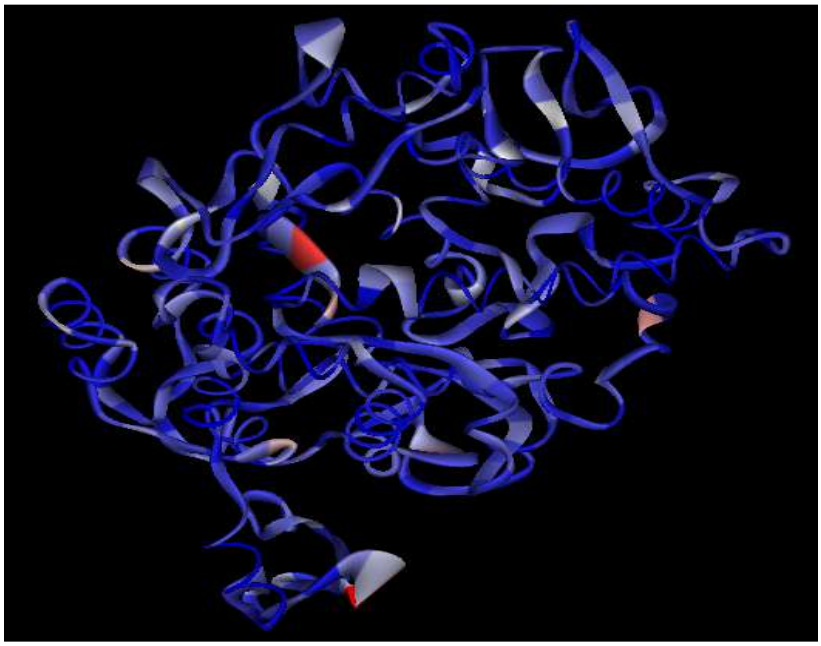

Fig 2. Shows Modeled Structure of Human COX 2. The red coloring and increase in thickness of the ribbon, as seen for the upper loops, makes determining their location trivial

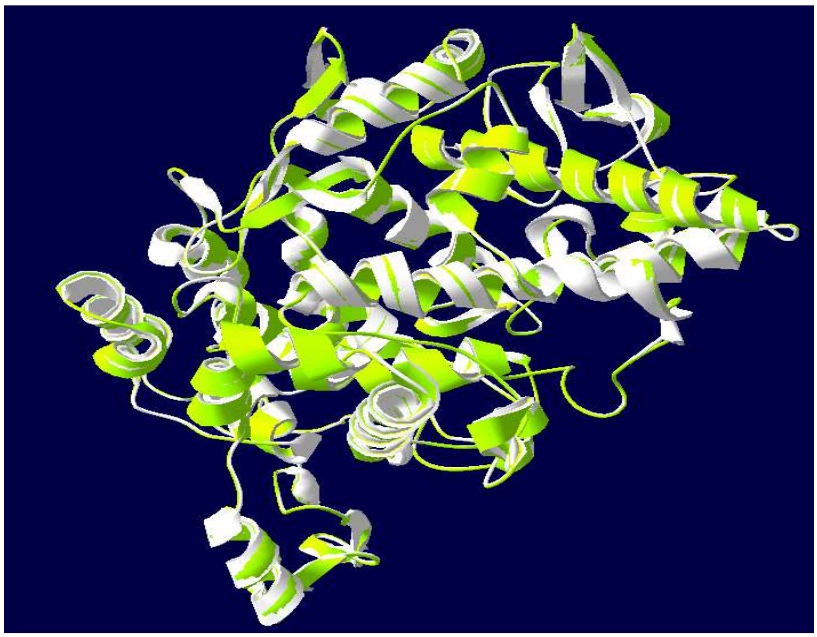

Fig 3. Shows green template structure 1CVU and white modeled COX 2

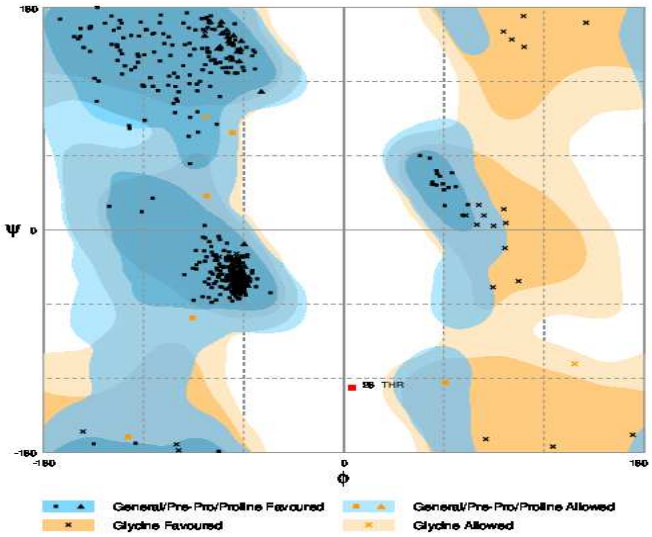

Fig 4. Shows Ramachandran's plot analysis of Modeled structure of human COX 2

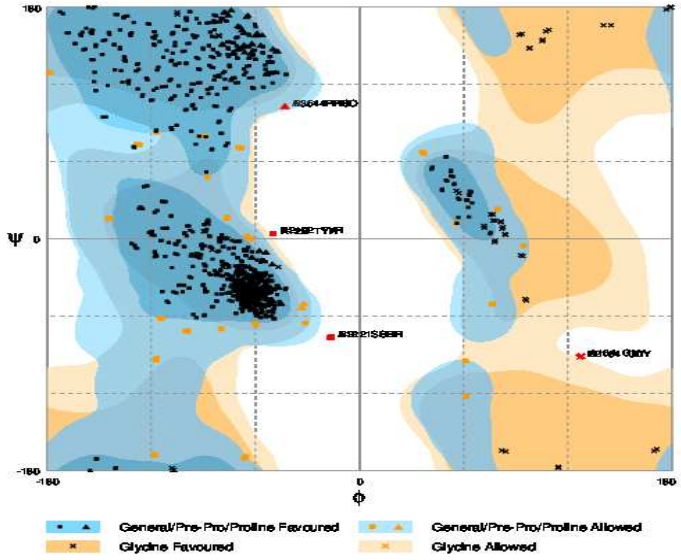

Fig 5. Ramachandran's plot analysis of template 1CVU

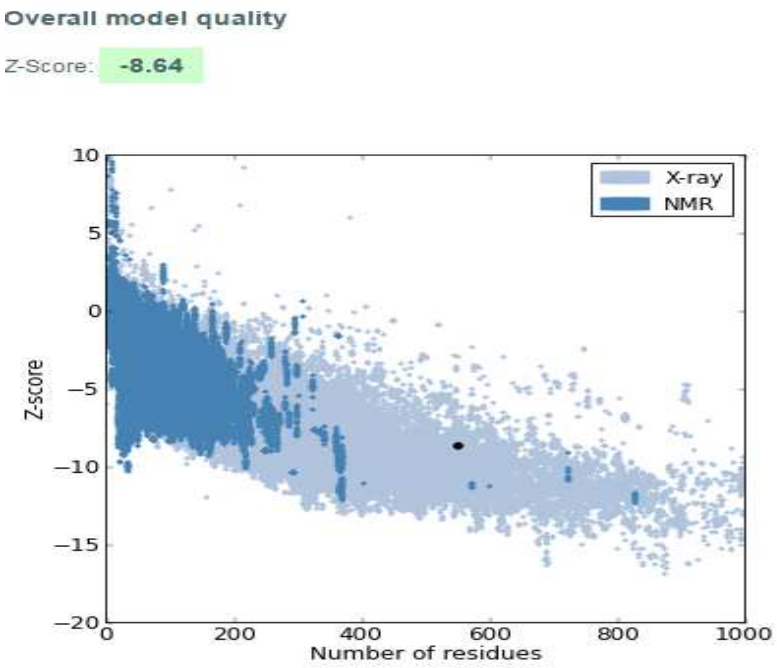

Fig 6. PROSA Result

Docking Studies

One of the most important and useful areas of application of molecular modeling is the approach of docking of small molecules into protein. The LibDock module from Discovery Studio was used to perform the docking. In this docking studies, Ru(II)/Co(III) complexes were docked into active site pocket of COX 2 and CDK2 proteins. These results indicated that all the Ru(II)/Co(III) complexes interacted with both the proteins of COX 2 and CDK2. Fig 7 and 8 were shown the interactions of complex **1** with COX 2 and CDK2, respectively. The LibDock scores were found in the range of 99 to 138 kcal/mole. The amino acid residues of the proteins COX 2 and CDK2 involved in hydrogen bond interactions with the complexes. LibDock score and interacting amino acids were depicted in Tables 1and 2. The more positive the LibDock score is the better the theoretical complex.

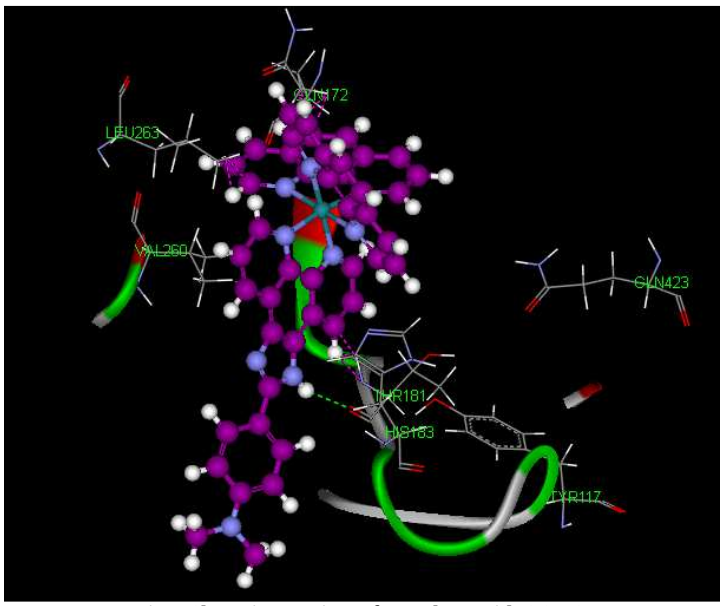

Fig 7. Shows interactions of complex 1 with COX 2

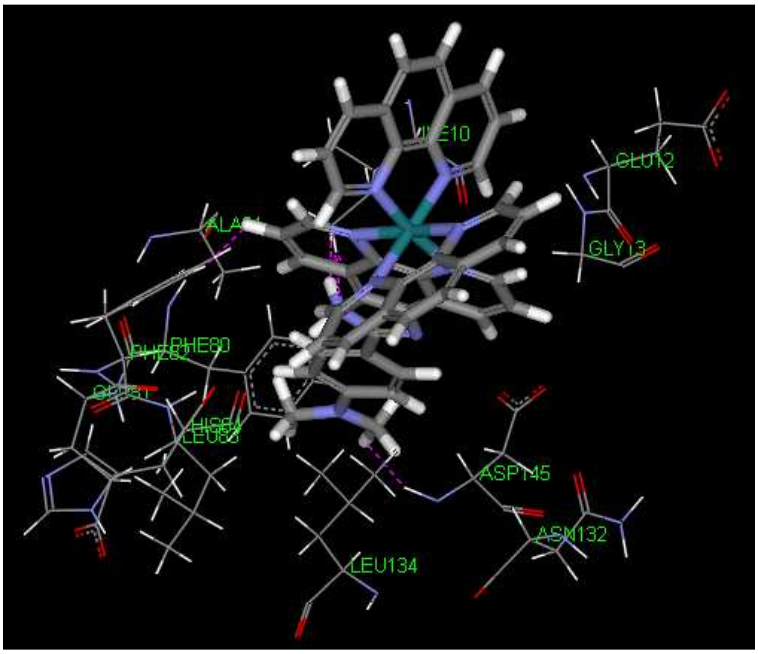

Fig 8. Shows receptor-ligand interactions of complex 1 with Human CDK2

Table 1. Interacting amino acids of CDK2 and Docking score

Complex	Libdock score kcal/mole	Interacting amino acids
1	126.953	GLU12, ILE10, GLY13, THR14, GLN85, LYS89, LYS129, GLN131, LEU134
2	118.521	ILE10, GLY13, VAL18, VAL64, PHE80, HIS84, LYS88, LYS89, LEU134, ASP145
3	114.028	GLU12, ILE10, VAL18, PHE82, ASP86, LYS89, LYS129, LEU134, VAL164
4	110.098	GLU12, ILE10, GLY13, THR14, HIS84, LYS88, GLN131
5	138.064	ILE10; LYS89; ASP145, LEU298
6	109.590	ILE10, ASP86, LYS89, ASN132
7	99.975	THR14, GLN85, LYS89, ASP145, LEU298
8	100.744	GLU8, THR14, PHE82, ASP145

Table 2. Interacting amino acids of COX 2 and Docking score

Complex	Libdock score kcal/mole	Interacting amino acids
1	127.280	VAL433, VAL260, LEU263, SER441, GLN189, THR198, ASN368, SER441,TYR117
2	113.908	TYR371, HIS372, TRP373, LEU376, VAL433, HIS200
3	103.671	TRP373, THR198, ASN208, VAL277, LYS197, HIS193
4	112.23	HIS372, TRP373, LEU376, HIS200, VAL433
5	131.003	TRP373, LEU376, HIS193, GLN275, HIS200, THR198, GLN275, GLN189
6	127.021	TYR371, GLN189, VAL433, THR192, PHE196, VAL430
7	122.562	TRP373, LEU377, GLN189, VAL430, TYR390, PHE196,
8	104.92	TRP373, LEU377, GLN189, PHE196, VAL430, VAL277, TYR390

CONCLUSION

The homology modelling was used to determine the 3D structure of Human COX 2. Ru(II)/Co(III)polypyridyl complexes **1-8** were docked with the COX 2 and CDK2 proteins using LibDock module in Accelry's Discovery Studio. The binding energies of Ru(II)/Co(III) polypyridyl complexes are considerably different. The results reveal that of the Ru(II)/Co(III) polypyridyl complexes shows strong binding affinity that can act as the most potential drugs for treating both the proteins COX 2 and CDK2. This can be concluded that these compounds can prove to be good inhibitors of both the proteins (COX 2 and CDK2) and these compounds could be effective for designing novel drugs.

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REFERENCES

[1] JP Rehmann; JK Barton, *Biochemistry*, **1990**, 29(7), 1701–1709.
[2] KE Riley; P Hobza, *J. Phys. Chem. A.,* **2007**, 111(33), 8257–8263.
[3] H Liu; Z Lu; G A Cisneros; W Yang, *J. Chem. Phys.*, **2004**, 121(2), 697-706.
[4] HM Senn; W Thiel, *Curr. Chem*., **2007**, 268, 173–290.
[5] C Lee; W Yang; R G Parr, *Phys. Rev. B.*, **1988**, 37(2), 785–789.
[6] AD Becke, *J. Chem. Phys.*, **1993**, 98(7), 5648–5652.
[7] PR Schreiner, *Angew. Chem. Int. Ed.*, **2007**, 46, 4217–4219.
[10] S Lapenna; A Giordano, *Nat. Rev. Drug Discov*., **2009**, 8(7), 547–566.
[11] M Knockaert; P Greengard; L Meijer, *Trends Pharmacol. Sci*., **2002**, 23, 417–425.
[12] TG Davies; DJ Pratt; JA Endicott; LN Johnson; ME Noble, *Pharmacol.Ther*., **2002**, 93, 125–133.
[13] DO Morgan, *Annu. Rev. Cell Dev. Biol.*, **1997**, 13(1), 261–291.
[14] N Gray; L Detivaud; C Doerig; L Meijer, *Curr. Med. Chem.*, **1999**, 6(9), 859–876.
[15] MR Reddy; PV Reddy; YP Kumar; A Srishailam; N Navaneetha; S Satyanarayana, *J. Fluoresc*., **2014**, 24(3), 803–817.
[16] M Shilpa; PN babu; JNL Latha; AG Devi; Nagarjuna; YP Kumar; S Satyanarayana, *J. Incl. Phenom. Macrocycl. Chem.*, **2011**, 70(1), 187–195.
[17] P Venkat Reddy; M Rajender Reddy; A Srishailam; Y Praveen Kumar; Ch Nagamani; N Deepika; K Nagasuryaprasad; SS Singh; S Satyanarayana, *Anal. Biochem*., **2015**, 485, 49–58.
[18] CS Devi; PN babu; N Sumathi; N Deepika; PV Reddy; NV babu; SS Singh; S Satyanarayana, *Eur. J. of Med. Chem*., **2014**, 72, 160–169.
[19] N Deepika; YP Kumar; CS Devi; PV Reddy; A Srishailam; S Satyanarayana, *J. Biol. Inorg. Chem.*, **2013**, 18(7), 751–766.
[20] A Srishailam; YP Kumar; P Venkat Reddy; N Navaneetha; V Uma; SS Singh; S Satyanarayana, *J. of Photochem. And Photobio. B: Biology*., **2014**, 132, 111–123.
[21] MJ Sippl, *Proteins* **1993**, 17(4), 355–362.