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RESEARCH ARTICLE

Natural Bond Orbital (NBO) Analysis and Binding Affinity towards Protein Kinase 2: DFT and Docking Studies of Coumarin Derivatives

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Abstract: The density functional theory (DFT) method has been carried out to investigate the natural bond orbital (NBO) analysis and to find the binding affinity towards protein kinase 2 of different coumarin derivatives. NBO analysis was observed in the range (~ 1.9 e) which indicates the intramolecular charge transfer between the bonding and antibonding orbitals. Molecular docking study has been performed with CK2 enzyme to analysis their anticancer aspects due to interaction with CK2 enzyme. The binding affinity was calculated for different coumarins.

Keywords: Coumarin, DFT, Docking, Natural bond orbital

Introduction

Coumarins are naturally occurring compounds found in all parts of plants and mainly can be found in grasses, legumes and among others[1]. Various studies of coumarins have been conducted in past due to their wide range of applications. They are widely used as antifungal[2], antibacterial[3], chemotherapeutics[4], anti-inflammatory, antioxidant agents and HIV inhibitors. Hence, because of wide range applications coumarins are very interesting compounds to study since a long time. In particular, one of the studies has shown that 7-dihydroxycoumarin has antitumor activity due to inhibition of cyclin D1 which is known to over express in many tumours[5]. Also, their anticancer activity has been identified as the attractive inhibitors of protein kinase (CK2) by virtual screening methodology[6]. CK2, a pleiotropic enzyme, is involved in variety of cellular functions[7] and it has been also reported that they display an antiapoptotic effect in cancer cell lines[8]. They are invariably more abundant in tumours compared with normal tissues and their over expression causes alterations in the expression of cellular oncogenes or tumour suppressor genes[9]. Hence, CK2 is a potential

target for antitumor drugs. Various *in silico* studies have been reported in last few years for screening inhibitors of CK2[10-11]. From the one of the virtual screening approaches, important inhibitor features of coumarins for CK2 has been identified[6].

Molecular modeling studies have been widely employed for coumarin derivatives. The electronic descriptors and vibrational data of molecules are the important parameters for the investigation of biological property. The present study is focused on exploration of electronic properties including molecular structure and NBO analysis and vibrational assignments of coumarin derivatives. A molecular docking study has been performed with CK2 enzyme to gain information about their anticancer aspects due to interaction with CK2 enzyme.

Experimental

All the chemicals used in the present work were purchased from Sigma Aldrich Chemical Co., USA and were used without further purification. The molecular structure and numbering system adopted in this work is shown in Figure 1 and the molecular structure of all the coumarin derivatives used in the present work are given in Table 1.

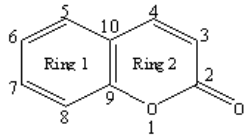
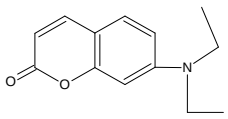
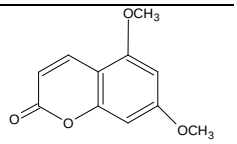
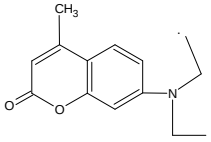
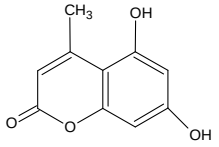
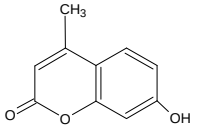
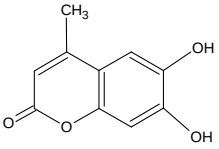
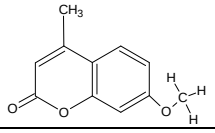


Figure 1. Molecular structure of coumarin

Table 1. Coumarin Derivatives used in present work and their molecular structures

Molecule Name	Structure	Molecule Name	Structure
7-diethylamino coumarin		5,7-dimethoxy coumarin	
7-diethylamino-4-methylcoumarin		5,7-dihydroxy-4-methylcoumarin	
7-hydroxy-4-trifluoromethyl coumarin		6,7-dihydroxy-4-Trifluoromethyl coumarin	
7-methoxy-4-trifluoromethyl coumarin			

DFT calculations

All calculations were performed on a Linux workstation equipped with eight parallel Intel Xeon X5460 processors (3.16 GHZ) with 16 GB total RAM. DFT calculations were performed with the Jaguar v7.9 quantum chemistry module of Schrödinger software[12].

Coumarin derivatives were fully optimized in vacuum at DFT level without imposing constraints using Becke's three-parameter and Lee-Yang-Parr functional (B3LYP) level hybrid functional with basis set *ps*6-311G with ** polarization. In general ** option places polarization functions on all atoms except for transition metals, H and He and *ps*6-311G basis set is used for C, H, N, O and P. Effective core potential used for heavy atoms and Non-ECP atoms uses 6-311G basis.

Docking

For the docking study, X-ray crystallographic coordinates of Protein kinase CK2 in complex with DBC, PDB entry 2QC6 (downloaded from RCSB) having resolution of 2.20 Å was used. Docking analysis was performed with Glide after preparing the protein structures with Preparation Wizard[12]. During pre-processing, bond orders were assigned; hydrogens were added to all atoms in the protein structure. Water molecules beyond 5 Å from hetero (het) groups were deleted and missing loops were filled up. States were generated for these hetero atoms at pH=7.0 ± 3.0. In the presence of force field OPLS2005, hydrogen atoms of protein structure were minimized, orientations of retained water molecules were sampled and pK_a's were determined. Grid calculations were performed for the protein active site by generating the grid at the centroid of DBC. No constraints were applied and rotations of rotatable groups were disallowed.

The coumarin derivatives were prepared using the Ligprep v2.6 module[11]. All the possible protonation states at pH=7.0 ± 2.0 were generated using ionization tool. Specified chiralities of these ligands were retained while ligand preparation. Low energy conformers were obtained in the force field OPLS2005 without any constraints. These conformers were retained for docking studies. Five thousand poses were kept in the initial phase of the docking keeping the default scoring window cut-off value at 100. Ligand Van der Waals radii were scaled to a factor of 0.80 (default value) for non-polar atoms with a partial charge cut-off level of 0.15 (absolute value). Post docking minimizations were performed by including five poses per ligand.

Glide v5.8 [11] was used for docking analysis. Glide's workflow predicts the binding mode of the ligand with high accuracy. The docking study was initiated by bringing specified prepared proteins and ligand molecules together. Glide docking uses a series of hierarchical filters to find the best possible ligand binding locations in a prebuilt receptor grid space that represents the shape and properties of receptors. It evaluates the energy interactions of the ligand with the protein in terms of G score value which is used for predicting binding affinity.

Results and Discussion

Natural bond orbital (NBO) analysis

The NBO analysis has been performed on coumarin derivatives using DFT at B3LYP level using 6-311G** basis set. Delocalization schemes were obtained by use of second-order bond-antibond (donor-acceptor) NBO energetic analysis. The change in electron density in the (σ^*, π^*) antibonding orbital and electronic energy have been calculated by NBO analysis using DFT method to give clear evidence of stabilization originating from various molecular interactions.

NBO analysis provided the accurate 'Natural Lewis structure' picture of molecule, because all the orbital are mathematically chosen to include the highest possible percentage of the electron density. The most important role of NBO analysis is that it gives information about interactions in both filled and virtual orbital spaces that could enhance the analysis of the intermolecular interaction and intramolecular interaction. The interaction result is the loss of occupancy from the localized NBO of the idealized Lewis structure into an empty non-Lewis orbital. Delocalization of electron density between occupied Lewis type (bond or

lone pair) NBO orbital and formally unoccupied (antibonding) non-Lewis NBO orbital corresponds to a stabilizing donor-acceptor interaction. Since the non-covalent delocalization effects coumarin derivatives are associated with $\sigma \rightarrow \sigma^*$ interaction between filled (donor) and unfilled (acceptor) orbitals[13-15]. The NBO analysis at C_3-C_4, C_2=O_2, O_1-C_9, C_{10}-C_5 and C_7-C_6 orbitals of coumarin derivatives showed strongly delocalized acceptance. The electron density of seven conjugated single bond of aromatic ring (~1.9 e) demonstrates strong delocalization[15] as shown in Table 2. The C-C bond of the seven coumarin compounds in benzene ring leads to the σ bond and have approximately 1.9 electron density thus these bonds act as electron donor. In case of 5, 7-dimethoxycoumarin, second order perturbation energy was found to be highest value of 20.2 kcal/mol due to involvement of O_1-C_{10} natural bond orbitals. Similarly, for all the coumarin derivates with the groups attached at 5th and 7th positions highest second order perturbation was found of the order of 21.2 kcal/mol.

Table 2. NBO analysis of coumarin derivatives

Molecule Name	C3-C4* (ED/e)	C2-O2 (ED/e)	O1-C9 (ED/e)	C10-C5 (ED/e)	C6-C7 (ED/e)
5,7-Dihydroxy-4-methyl coumarin	1.97727	1.99700	1.98937	1.97379	1.97820
6,7-Dihydroxy-4-trifluoromethyl coumarin	1.97835	1.99512	1.98949	1.96679	1.97460
7-Diethylamino-4-methyl coumarin	1.97817	1.99575	1.98954	1.97059	1.97308
7-Diethylamino coumarin	1.98385	1.99527	1.98961	1.97217	1.97492
7-Hydroxy-4-trifluoromethyl coumarin	1.97882	1.99506	1.98952	1.97175	1.98157
7-Methoxy-4-trifluoromethyl coumarin	1.97883	1.99505	1.98995	1.97198	1.98228
5,7-Dimethoxy coumarin	1.98994	1.99690	1.99166	1.97964	1.98918

**The numbering is according to Figure 1. O_1 is the Oxygen atom in the pyrone ring and O_2 is the carbonyl oxygen atom*

Docking analysis

From the previous work of docking analysis of coumarin derivatives[6], coumarin skeleton was found to be attractive for CK2 protein. Coumarin derivatives are known to bind at hydrophobic ATP binding site like other known inhibitors and coumarin molecules with ionisable hydroxyl group at 7th position was found to be critical for binding. In the active site of CK2 the co-crystallized ligand *i.e.* 3, 8-dibromo-7-hydroxy-4-methylcoumarin (DBC) is surrounded by hydrophobic residues and do not bind to hinge region. In our case we have performed docking studies of seven coumarin derivatives (Table 1). In each case ligands were found to be in hydrophobic ATP binding pocket which is having Val116, Ile66, Ile174, Phe113 and Met163 residues. Hence, we found that these coumarin derivatives were involved in hydrophobic interactions along with polar interactions with Asn117, Asn118 residues and electrostatic interactions with positively charged residues Lys68, Asp175. Lys is a positively charged and Asp is a negatively charged residue which helps ligands to form electrostatic interactions. In the study of seven coumarin derivatives of our study, we have observed their interactions with backbone Lys68 and nearby water molecule. Also, OH group is involved in the interactions and thus makes coumarins with OH group at 7th position more selective for CK2 binding. Π-cation interactions also occur with Leu85 and Ile174 residues.

For co-crystallized ligand DBC, binding affinity was found to be -4.969 kcal/mol which includes hydrophobic interactions with Br groups and OH group at 7th position. These receptor-ligand interactions are depicted in Figure 2. In case of 5, 7-dimethoxy coumarin, hydrophobic interactions were present due to two methoxy groups at 5th and 7th position and overall binding energy was found to -6.969 kcal/mol. Whereas hydroxyl group at 5th and 7th and methyl at 4th positions resulting in increased binding energy of -7.970 kcal/mol due tohydrogen binding interactions with three water molecules in active site. For 7-hydroxy-4-trifluoromethyl coumarin, high binding energy of -8.810 kcal/mol was observed due to strong hydrophobic interactions with in the ATP binding site as shown in Figure 3. In case of 7-methoxy-4-trifluoromethyl coumarin very low binding energy of -4.23 kcal/mol was obtained due to methoxy group at 7th position which resulting into low binding affinity. Hence, at 7th position only ionisable group like hydroxyl group is preferred due to its involvement interactions with positively charged residue Lys68 and hydrogen bonding with water molecules. On comparing 7-diethylaminocoumarin and 7-diethylamino-4-methyl-coumarin we observed -6.969 and -3.911lkcal/mol binding energy respectively due to increased hydrophobicity due to methyl group at 5th position.

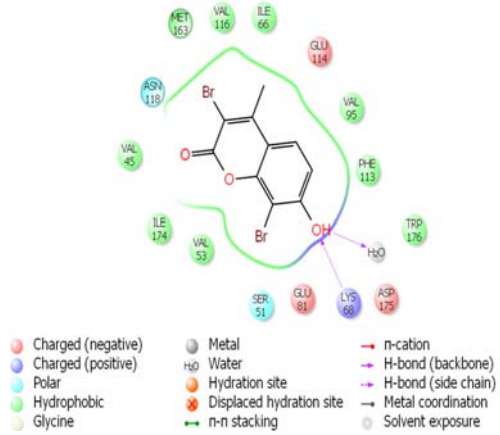


Figure 2. Hydrophobic receptor-ligand interaction of DBC

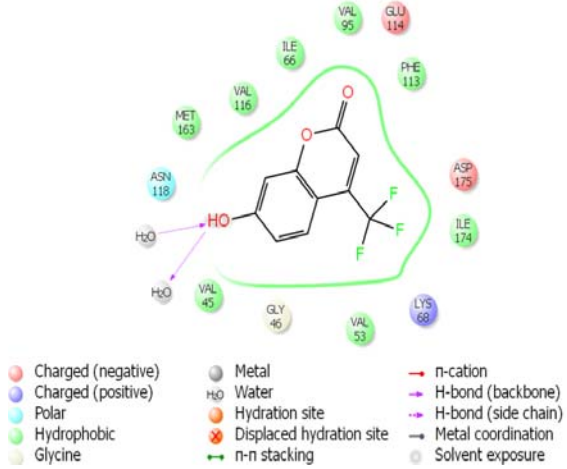


Figure 3. Hydrophobic receptor-ligand interaction of 7-hydroxy-4-trifluoromethyl coumarin

Conclusion

DFT level of theory at *ps*6-311G basis sets predicted the optimized structure of coumarin derivatives of our study. NBO analysis predicted the delocalization in the coumarin ring. The optimized structures were employed for docking studies to predict their binding affinity towards an important target of protein kinase 2. Electronic properties predicted for these coumarin derivatives found to be well correlated with affinity values obtained. Due to substitutions at 4^{th} and 7^{th} position of coumarin ring leads to better binding towards the target and thus revealed importance of hydrophobic substituents at these positions. Hence, further modification in this coumarin scaffolds will lead to development of better inhibitors of protein kinase 2 and thus will show better anticancer activity.

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