



Efficacy of some natural flavanoids against PDK-1 Kinase: *An insilico* Molecular docking and Molecular Dynamic Simulation study

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Abstract:

PDK-1, 63Da serine thionine kinase has a well-known role in cell growth regulation and differentiation. It is over expressed in many cancer cells and considered as a novel target of many anti-cancer drugs. In the present study we have explored the affinity and interaction of some plant derived natural flavanoids against PDK-1 with insilico molecular docking and molecular dynamic simulation approach. Ten plant derived natural flavanoids were docked in to the ATP binding site of PDK-1, using the CDOCKER molecular docking program implemented in Discovery studio 2.5 to identify the potential hits against PDK-1. Myricetin, Epigallocatechin, Quercetin and Hesperitin showed high docking score as well as the interaction with hinge region and active sites amino acids. Finally highest docking hit (myrecetin) was subjected to molecular dynamic simulation study, evaluating the binding stability of complex under body environmental conditions. GROMACS 4.5 program. RMSD profile of PDK-1 alone as well as PDK-1 and myricetin complex during 10 ns simulation were always less than 5.0 Å (0.5 nm) during entire simulation, suggesting the stability of simulation system during whole simulation. Molecular docking and molecular dynamic simulation results revealed that natural flavanoids (especially myricetin) act as PDK-1 inhibitors to combat cancer.

Keywords: PDK-1, anticancer drug, CDOCKER, GROMACS 4.5, 10ns.

Introductions:

Protein kinases are significant components of cell signaling pathway [1]. Protein kinases are considered as second largest group of drug target [2]. Phosphoinositide-dependent kinase-1 (PDK-1), a 63 kDa serine/threonine kinase, is a major player in the PI3-kinase signaling pathway that regulates gene expression, cell cycle, growth, and proliferation [3-10]. Many researches discover that 3-phosphoinositide- dependent protein kinase-1 (PDK-1) is overexpressed in many cancer cells [11]. PDK-1 has been termed the “Master kinase” because it phosphorylates highly conserved serine or threonine residues in the T-loop (or activation loop) of numerous AGC kinases, including PKB/AKT, PKC, p70S6K, SGK,

and PDK-1 itself [12]. Although the precise regulatory mechanisms vary, in the case of PKB/AKT, activation by PDK-1 is critically dependent upon prior PI3 kinase activation and the presence of phosphatidylinositol-(3,4,5)- triphosphate (PIP3).

A significant proportion (40–50%) of all tumors involve mutations in PIP3-3-phosphatase (PTEN) [13-16], which results in elevated levels of PIP3 and enhanced activation of PKB/ AKT, p70S6K, and SGK. Inhibitors of PDK-1 could potentially provide valuable therapeutic agents for the treatment of cancer. Although a enormous number of natural compounds have been reported to possess anticancer properties, their mechanisms of action are not well understood. Natural Flavonoids are class of compounds, widely spread in the vegetables and fruits, presently consumed in huge amount in the daily diet. In recent years, flavonoids and their synthetic analogues have been intensely investigated in the treatment of ovarian, breast, cervical, pancreatic, and prostate cancer. To some extent, chemical structures of flavonoids will influence their anticancer activities.

Thus, the present study was on the inhibitory action of some selected natural flavanoids against PDK-1. These selected compounds were initially subjected to the molecular docking study by using CDOCKER program. Finally the best hit (Myrecetin) was further subjected to the molecular dynamic simulation study to evaluate the binding stability of the PDK-1-Myrecetin complex during entire simulation using GROMACS 4.5.

Material and methods:

Molecular docking:

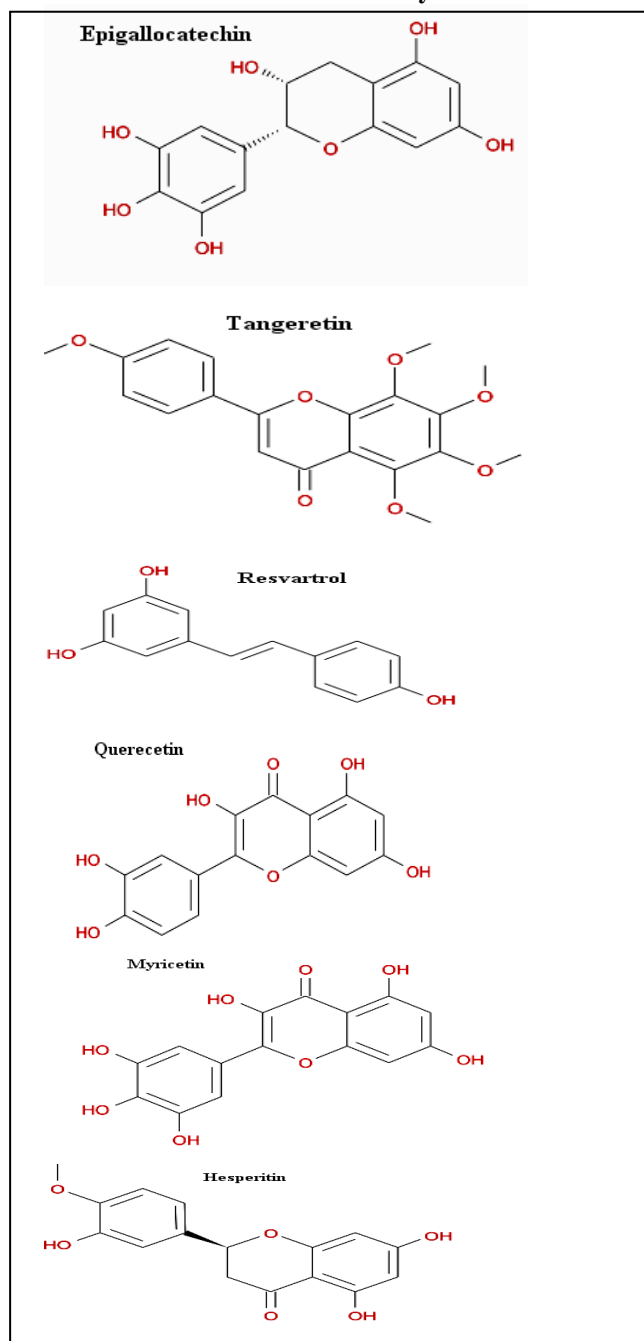
Molecular docking was performed by CDOCKER docking method implemented in Discovery Studio 2.5 [17]. All the docked molecules are analysed on the basis of binding energy and hydrogen bond interactions with active site binding residues and hinge region of amino acids.

Compounds used for study:

The compounds from plant origins like, resvartrol, myricetin, epigallocatechin, tangeretin, quercetin and hesperitin were used. 3D structure of all the compounds were downloaded from the Pubchem database. Chemical

structures of all the studied compounds were shown in Figure.1.

Figure 1: Chemical structure of compounds used for study



Preparation of ligands:

The three dimensional structures of all natural inhibitors taken in this study were downloaded from Pubchem

database. Then CharMM based force field [18] was applied to them and further, were subjected to single step minimization using steepest descent method for 500 steps at RMS gradient of 0.01.

Protein preparation:

The crystal structure of PDK-1 (PDB id 1UU7) taken in this study was extracted from protein data bank, all the HETATMS were removed. The protein was subjected to two steps energy minimization to remove the bad steric clashes using steepest descent and conjugate gradient methods for 1000 steps at RMS gradient of 0.1 and 0.05 respectively. During the energy minimization process the backbone and side chain were fixed by applying the fixed atom constraint, and only hydrogen atoms were minimized. The CharmM force field was applied to the receptors. Fixed atom constrained were removed after the minimization. The receptor protein is divided into the protein part and crystal ligand part.

The protein part was only selected and selections were made to “define selected molecule as receptor” under define and edit binding site, sub panel of the “Tool panel”, where in, the protein is defined as receptor molecule. By selecting only the ligand part and clicking on “Define sphere from selection” so that the crystal ligand is used to define the binding site of 15 Angstroms on the receptor molecule. This ‘input receptor molecule’ was used as input parameter in the CDOCKER protocol parameter explorer. The minimized structure of these compounds were used as input ligand in the protocol explorer of CDOCKER. Each of them is given as input in another parameter meant for ‘input ligands’ and the protocol was run as many times as the number of inhibitors were selected for the experiment. The various conformations for ligand in this procedure were generated by using molecular dynamics. The generated initial structures for the ligand were further refined using simulated annealing. The CDOCKER energy (-(protein-ligand interaction energies)) of best configuration docked into the receptor of all the selected natural inhibitors, which were calculated and compared with that of interacting residues at active site region with the crystallized inhibitors, PDK-1 kinase protein.

2 D Plot of protein ligands interactions:

For study of hydrogen bond as well as the hydrophobic interactions of all docking results, 2D diagram were drawn by Accelrys Discovery Studio 3.5 viewer. The two dimensional structure showing the hydrogen bond as well hydrophobic interactions were produced.

Molecular dynamic simulation study of Myricetin in water:

MD simulation of the complex was carried out with the GROMACS4.5.4 package using the GROMOS96 43a1 force field^[19] The lowest binding energy (maximum negative) docking conformation generated by CDOCKER was taken as initial conformation for MD simulation. The topology parameters of proteins were created by using the Gromacs program. The topology parameters of Myricetin was built by using the Dundee PRODRG server^[20] The complex was immersed in an octahedron box of extended simple point charge (SPC) water molecules^[21,22] The solvated system (PDK-1, Myrecetin and water) was neutralized by adding 3 Cl ions as the PDK-1 have +3 charge. To discharge the conflicting contacts, two step energy minimization was performed by using the steepest descent method of 10000 steps followed by the conjugate gradient method for 10000 steps. MD simulation studies consist of two phases, equilibration phase and production phases. In the first stage of equilibration, the solute (protein, counterions, and myricetin) was fixed and the position-restrained dynamics simulation of the system, in which the atom positions of protein were restrained at 300 K for 200 ps. Finally, the full system was subjected to MD production run at 300 K temperature and 1 bar pressure with isotropic molecule based scaling for 10000 ps. For analysis, the atom coordinates were recorded every 0.5 ps during the entire MD simulation.

Results and Discussions:

Among the all selected natural flavanoids docked with in the active side of the PDK-1, Myricetin was found to exhibit the most efficient binding with highest docking score. Epigallocatechin, Qercetin and Hespetin were also effective. All the comparison was made on the basis of dock score given by CDOCKER as well as the hydrogen bond interctions. To verify the precision of the docking program and the docking parameters used for docking, the crystal ligand inhibitor from our reference crystal protein (pdb id:1UU7) was removed and again subjected to molecular docking within the active site of PDK-1 using the same parameters as that taken for docking the natural compounds. It was observed that the crystal ligand had a dock score of -20.7 kcal/mol with RMSD value of 0.84 . This shows that the original binding mode may be reproduced by CDOCKER docking program. Crystal ligand is used as reference molecule to examine the effectiveness of all other inhibitors. Myricetin, a compound derived from grapes, berries , herbs as well as other plants shows promising response and can be used as a potent inhibitor against PDK-1 for curing cancer. Some more compounds have been revealed by this study which

may be used as potent inhibitors against cancer. All the docked inhibitors were bound to the ATP site of PDK-1. The active site of PDK-1 was surrounded by a G-loop, a hinge region, and a hydrophobic pocket. Myricetin has the highest dock score of -41.38 kcal/mol followed by the Epigallocatechin, Querecetin and Hesperitin. In Myricetin, the maximum number of residues involved in hydrogen bonds, where in total 5 residues are involved in making of six number of hydrogen bonds shown in Figure. 2(A). The major interactions as shown in the binding site are the important H-bonds with Asp223, Lys111, Glu 166 and other two residues Ala162 and Ser160 in the hinge region. The groups involved in H-bonding were carboxylate, hydroxyl (hydrogen donor), NH (hydrogen donor) and oxygen atoms (hydrogen acceptor) of side chain or backbone of residues. It is also observed that some amino acid residues play an important role in the binding of inhibitors within the active site of PDK-1. V96, A109 and L159 were involved in making hydrophobic contacts in all the cases, and they were presumed to be a key players. (Table-1).

Table 1: Dock Scores of natural compounds with target protein PDK-1 along with details of the residues involved in binding

Compoun -ds	Docking Energy (kcal/mol)	Hydrogen bonding residues	Hydrophobic interactions
Myricetin	-41.38	K111,D223,S160,A162,E166	T222,V96,L159,L88,A109,V143,L212
Epicallach etin	-33.7	K111,S160,A162,T222,D223	A109,L212,L159,V96,L88,G165,D166
Qercetin	-33.56	K111,S160,A162,T222	S94,L159,G89,V96,L88,E166,G165,L212,V143,A109
Hesperitin	-32.46	K111,D223,S160,A162	N210,T222,L159,V143,A109,L88,G165,V96,L212,D210
Resvartrol	-20.12	A162, S160 , T222,N210	L159,V143,A109,E166,L212,V96,G89,L88,D208
Tangereti n	-13.6	No hydrogen bonds formed	V96,L159,A109,E166,D208

S160, A162 and T222 were involved in making of maximum number of hydrogen bond followed by D223,K111 and E166. Further, epigallocatechin also had higher dock score. It was observed that Epigallocatechin involved in making of total five hydrogen bonds with

five amino acids shown in Figure. 2(B). Quercetin and Hesperetin were involved in making of total six and four hydrogen bonds respectively shown in Figure. 2(C) & Figure. 2(D).

Figure 2: (A-D): 2D representation of the top four docked ligands within the active site of PDK-1. Fig 1(A) Binding orientation of Myricetin within the active site of PDK-1.

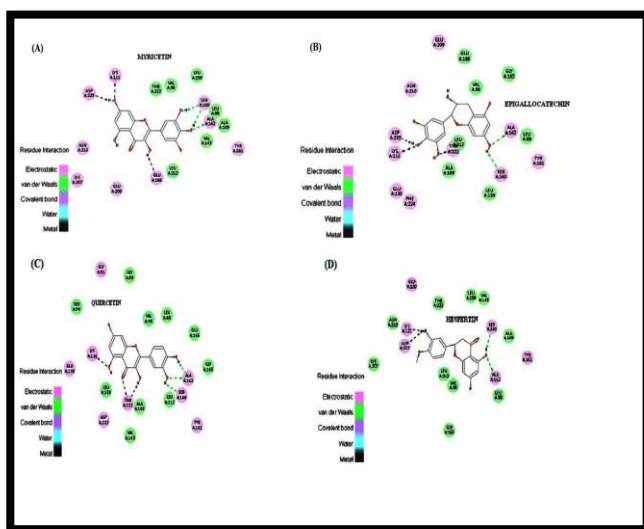
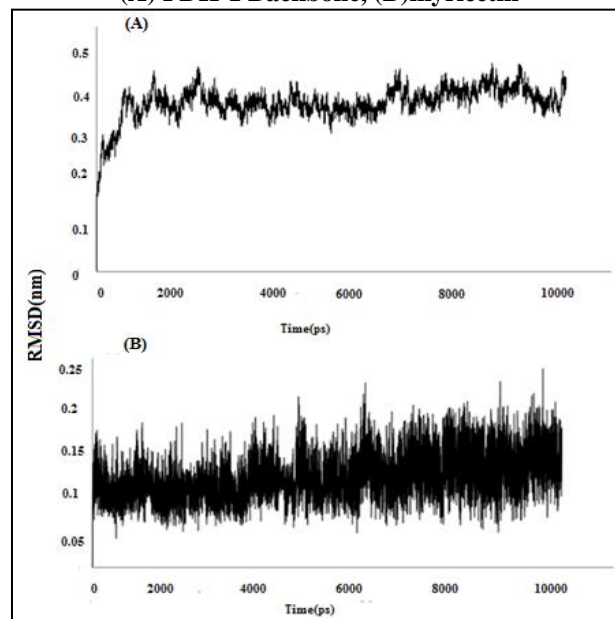


Fig 2(B) Binding orientation of Epigallocatechin within the active site of PDK-1. Fig 2(C) Binding orientation of Quercetin within the active site of PDK-1 Fig 2(D) Binding Orientation Hesperetin of within the active site of PDK-1

Finally molecular dynamic simulational study of PDK-1 and myricetin has been revealed interesting results. All-atom MD simulations represent a convenient method to investigate differences in motions of residues/atoms that led to structural and chemical changes. The time dependent behavior of MD trajectories for PDK-1 and myricetin was analysed to include the root mean square deviation (RMSD) for all backbone atoms, coulombic interaction energy as well as van der waal interaction energy. The Root Mean Square Deviation (RMSD) of backbone atoms with respect to the initial conformation was calculated as a function of time to assess the conformational stability of the protein during the simulations. Figure. 3(A) depict that the RMSD trajectories for PDK-1 backbone as well as the myrecetin were always less than 5.0 Å (0.5 nm) during entire simulation, suggesting the stability of simulation system. Figure 3A shows an initial steep rise was observed in the RMSD profile for the first, ~1000 ps and after then

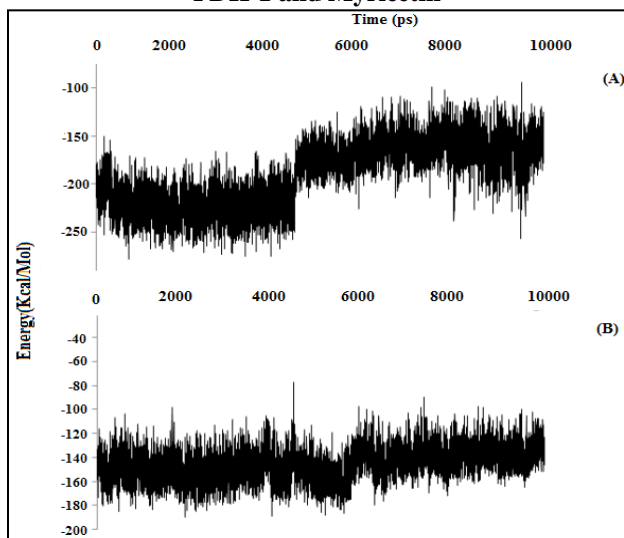
subsequently almost a constant profile was achieved for the simulation system of PDK-1 myricetin complex. While RMSD profile of myrecetin was observed below 0.25 nm with noticeable fluctuation shown in Figure. 3(B). RMSD profile of myrecetin shows stability of compound during entire MD simulation.

Figure. 3. RMSD profile of (A) PDK-1 Backbone, (B)myricetin



To observe in-depth the binding ability PDK-1 and myricetin during entire MD simulation, the short range (SR) coulombic and van der waals (vdw) interaction energies were calculated and displayed (Figure. 4 A and 4B). The energy profile of SR coulombic interaction energy between myrecetin and PDK-1 was showed in Figure. 4A. It was observed that SR coulombic interaction energy showed slight increase (more negative) during first ~4000 ps after then a decrease (less negative) of ~100 KJ/mol was observed (Figure 3(A)). This indicates a decrease in polar interaction after ~4000 ps. In the case of the **vdw** interaction energy, a more stable profile was observed as compared to SR coulombic interaction energy throughout the simulation (Figure 3(B)). The average value of coulombic interaction energy was found -159.68 KJ/mol whereas for vdw interaction energy was -149.25 KJ/mol. The total number of H-bonds (cut off 0.35 nm) which were formed during entire molecular dynamic simulation between myricetin and PDK-1, was found to be fluctuated from 0 to 3 with an average value of 1.02/0.5 ps. To found the other important residues involved in making of hydrogen bonds during entire simulation.

Figure 4. Profile of (A) columbic interaction energy, (B) van der waals (vdw) interaction energy between PDK-1 and Myricetin



Conclusions:

This study involved a comparative evaluation of various natural flavanoids as potential anticancer compounds wherein the effectiveness has been studied with reference to PDK-1 Kinase inhibition. The molecular docking results showed the effectiveness of three natural flavanoids among all the compounds taken for the study. Finally best hit ie myreccetin was subjected to molecular dynamic simulation study, evaluating the binding stability of complex under body environmental conditions. The simulation results have been evaluated with reference to Protein Ligand interaction and molecular dynamic simulation in water. These studies depict that the role of some crucial amino acids involved in proper binding of inhibitors with the active site of PDK-1 can be useful for designing better drugs to combat cancer. Further, these studies support the potential role of myreccetin in cancer which should be further studied for modulation of other signaling pathways as PDK-1 kinase being master regulators of other down stream regulators protein PIP3 kinase, AKT kinase.

References:

[1]. Fabian MA, Biggs III WH, Treiber DK, Atteridge CE, Azimioara MD, Benedetti MG, Carter TA, Ciceri P, Edeen PT, Floyd M, Ford JM, Galvin M, Gerlach JL, Gortzfeld, RM, Herrgard S, Insko DE, Insko MA, Lai AG, Lelias JM, Mehta SA, Milanov ZV, Velasco A.M, Wodicka LM, Patel HK, Zarrinkar PP, Lockhart DJ (2005) A small

molecule –kinase interaction map for clinical kinase inhibitors. Nature Biotech 23: 329-336.

- [2]. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The Protein Kinase Complement of the Human Genome. Science 298: 1912-1934.
- [3]. Akritopoulou-Zanze I, Hajduk PJ (2009) Kinase-targeted libraries: the design and synthesis of novel, potent and selective kinase inhibitors. Drug Discov Today 14: 291-297.
- [4]. Anderson SL, Stokoe D, Erdjument-Bromage H, Painter GF, Holmes AB, Gaffney PR, Reese CB, McCormick F, Tempst P, Coadwell J, Hawkins PT (1998) Protein Kinase B Kinases That Mediate Phosphatidylinositol 3,4,5-Trisphosphate-Dependent Activation of Protein Kinase B. Science 279 :710–714.
- [5]. Alessi DR, Deak M, Casamayor A, Caudwell FB, Morrice N, Norman DG, Gaffney P, Reese CB, MacDougall CN, Harbison D, Ashworth A, Bowles M (1997) 3-Phosphoinositide-dependent protein kinase-1 (PDK-1): structural and functional homology with the *Drosophila* DSTPK61 kinase. Curr. Biol. 7: 776–789.
- [6]. Currie RA, Walker KS, Gray A, Deak M, Casamayor A., Downes CP, Cohen P, Alessi DR, Lucocq J (1999) Role of phosphatidylinositol 3,4,5-trisphosphate in regulating the activity and localization of 3-phosphoinositide-dependent protein kinase-1. Biochem J 337: 575–583.
- [7]. Kobayashi T, Cohen P (1999) Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK-1) and PDK2. Biochem J 339:319–328.
- [8]. Kobayashi T, Deak M, Morrice N, Cohen P (1999) Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. Biochem J 344:189–197.
- [9]. Park J, Leong ML, Buse P, Maiyar AC, Firestone GL, Hemmings BA (1999) Serum and glucocorticoid-inducible kinase (SGK) is a target

of the PI 3-kinase-stimulated signaling pathway.
EMBO J 18:3024–3033.

- [10]. Pullen N, Dennis PB, Andjelkovic M, Dufner A, Kozma SC, Hemmings BA, Thomas G (1998) Phosphorylation and Activation of p70^{s6k} by PDK-1. *Science* 279: 707–710.
- [11]. Alessi DR, Kozlowski MT, Weng Q-P, Morrice N, Avruch J (1997) 3-Phosphoinositide-dependent protein kinase 1 (PDK-1) phosphorylates and activates the p70 S6 kinase *in vivo* and *in vitro*. *Curr Biol* 8:69–81.
- [12]. Mora A, Komander DMF van Aalten, DR Alessi (2004) PDK-1, the master regulator of AGC kinase signal transduction. *Semin Cell Dev Biol* 15:161–170.
- [13]. Dahia PL (2000) PTEN, a unique tumor suppressor gene. *Endocr Relat Cancer* 7:115–129.
- [14]. Sansal I, Sellers WR (2004) The Biology and Clinical Relevance of the *PTEN* Tumor Suppressor Pathway. *J Clin Oncol* 22: 2954–2963.
- [15]. Cully M, You H, Levine AJ, Mak TW (2006) Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer* 6:184–192.
- [16]. Accelrys Discovery Studio 2.5; Accelrys: San Diego, CA, 2009. <http://www.accelrys.com>.
- [17]. Berendsen, H.J.C.; Van der Spoel, D.; Van Drunen, R. *Phys. Commun.* 1995, 91, 43
- [18]. Lindah, E.; Hess, B.; Van der Spoel, D. *J. Mol. Model.* 2001, 7, 306.
- [19]. Schuttelkopf, A.W.; Aalten, V *Acta Crystallogr.* 2004, 60, 1355.
- [20]. Van Gunsteren, W.F.; Billeter, S.R.; Eising, A.A. et al. *Vdf Hochschulverlag AG: Zurich*1996.
- [21]. Van Gunsteren, W.F.; Daura, X.; Mark, A.E. Von Rague Schleyer, P., Ed.; Wiley and Sons: Chichester, U.K. 1998, 2, 1211-1216.F
