The physical studies and interaction with anti-apoptotic proteins of 2-(bis(cyanomethyl)amino)-2-oxoethyl methacrylate molecule

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In this work 2-(bis(cyanomethyl)amino)-2-oxoethyl methacrylate (CMA2OEM) molecule has been characterized theoretically. First, the potential energy surface has been calculated to find the lowest energy state of the molecule. After the most stable state of the molecule, Mulliken atomic charge and nonlinear-optical properties were investigated. Also in the study, binding poses of CMA2OEM molecule and anti-apoptotic proteins, such as BCL-2, BCL-w, MCL-1, AKT1 and BRAF were investigated. The molecular docking results showed that the most stable complex was obtained with this molecule and BRAF protein. The molecular docking results showed that the most stable complex was obtained with this molecule and serine/threonine-protein kinase protein. This study suggested that molecular docking approach may be a potential tool to identify the hydrogen bond interactions in order to treat a disease. Finally, this new ligand could pave the way to experimental studies.

Key words: Mulliken atomic charge, nonlinear-optical properties, molecular docking, anti-apoptotic, CMA2OEM

1. Introduction

Acrylate derivatives which are soluble in many organic solvents are widely used in bath tubs in glass materials [1]. The reason for this is the versatility of acrylic monomers, which indicates their adaptability. In recent studies, this turned out to be urgent for amino methacrylate derivatives in areas such as waste water treatment, biochemical sensor and protein purification [2]. Due to their physical properties, acrylates are used in medicine, orthopedics, tooth and filling materials, drug delivery systems, biochemical sensors and soft tissue studies [3]. A number of studies have been performed in our team on the synthesis of methacrylate monomer and their polymerization. 2-(bis(cyanomethyl)amino)-2-oxoethyl methacrylate (CMA2OEM) is one of the important monomers that can be obtained in 2 steps [4].

Apoptosis is an effective mechanism for eliminating damaged or unnecessary cells by multicellular organisms [5]. Anti-apoptotic proteins are overexpressed in most cancers and, due to this feature, are highly remarkable as the target of anti-cancer agents [6]. Therefore, the study has been performed to determine the anticancer potential of 2-(bis(cyanomethyl)amino)-2-oxoethyl methacrylate (CMA2OEM) targeting anti-apoptotic proteins, thereby inducing apoptosis in cancer cells.

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In this study, physical characterization of 2-(bis(cyanomethyl)amino)-2-oxoethyl methacrylate (CMA2OEM) compound was carried out by Sas et al. [4], in which synthesis and some theoretical studies were carried out. Firstly, the potential energy surface is calculated to find the most optimized state of the molecule. After the molecular optimization is performed by the DFT-B3LYP method, the Mulliken charges, nonlinear optical properties and dipole moment are presented. We demonstrated suitable confirmations of the new synthesis CMA2OEM compound as a ligand within anti-apoptotic proteins binding sites in silico by using Autodock vina114 [7]. In silico studies further provided predictive binding properties of selected ligands for inhibition of target protein.

2. Materials and methods

2.1. Docking procedure

CMA2OEM compound was docked into active sites of anti-apoptotic proteins, BCL-2, BCL-w, MCL-1, AKT1 and BRAF in Autodock Vina software [7]. The structure of anti-apoptotic proteins are freely available from the RCSB Protein Data Bank as a 3D theoretical model (PDB ID; BCL-2: 4man, BCL-w: 2y6w, mcl-1: 5fdo, AKT1: 4gv1, BRAF: 5vam). 2D structure of the ligand was converted to energy minimized 3D-structure. All proteins and ligand were validated before performing the in silico computations. Interaction of amino acid residues of proteins with ligand was analyzed using LIGPLUS tool [8, 9].

2.2. Computational procedure

The potential energy surface was calculated using the DFT/B3LYP/6-311++G(d,p) method to determine the most stable state of the CMA2OEM molecule [10-11]. To study the charge distribution of the molecule, Mulliken charges calculated using the same method are shown on the graph (figure 3). Molecular polarizability, hyper polarizability and dipole moment values are calculated in the output file CMA2OEM molecule and tabulated. Gaussian 09 package program was used in these calculations [12].

3. Results and discussion

3.1. Potential energy surface (PES) scan and optimized structure

The PES (potential energy surface) is calculated around the single bonds so that the optimal structure of a molecule can be found. Firstly, to find the lowest energy state of the title molecule, torsional angles were calculated between C and N atoms. Rotation around C15-N17 bond (C12-C15-N17-C21). On this calculation, torsion angle was varied from 0 degrees to 360 degrees by changing every 10 degrees, 36 steps were taken. A second torsion angle was calculated after the lowest energy state of the molecule was found. Rotation around C9-C5 bond (O11-C9-C5-C1). On this calculation, torsion angle was also varied from 0 degrees to 360 degrees by changing every 10 degrees, 36 steps were taken. Synthesis reaction of CMA2OEM [4] and its potential energy surfaces (PES) were shown in figures 1 and 2, respectively.

The molecular structure of CMA2OEM in the ground state was optimized with B3LYP method with 6-311++G(d,p) basis set using the Gaussian 09 software after finding the lowest energy state [10-12].

3.2. Mulliken atomic charges

The Mulliken charge distributions of CMA2OEM were calculated with B3LYP/6-311++G(d,p) method and shown in figure 3.

Mulliken charges provide physical information for the electronic distribution of the molecule. Among the carbon atoms in the molecule, C5 (0.78e) has the highest positive value, C1 and C6 (−0.64e and −0.66e) have the lowest negative value. For this reason, we can say that carbon atoms of C5 and C6 regulate the molecular distribution of the charge. The oxygen and nitrogen atoms in the molecule are...
linked by carbon atoms. As a result, when the carbon atoms take on a variable value (0.18\(e\), −0.29\(e\), 
−0.26\(e\), etc.), the nitrogen and oxygen atoms take on a value of about −0.2\(e\), except for the oxygen atom 
between the two carbon atoms (C9 and C12). This distribution may be due to the fact that these atoms 
are less electronegative than nitrogen and oxygen because they concentrate on carbon atoms.

3.3. Nonlinear optical properties and dipole moment

In organic materials, optical properties are determined by polarizability. The polarizability of an atom 
or molecule is a measure of how easily the nucleus and electrons can displace their stable state. It is the 
valence electrons farthest from the nucleus of electrons that are easily displaced in an atom or molecule. 
For this reason, the valence electrons have a great contribution to the polarizability. The bonds between 
carbon atoms and other elements are of two kinds, \(\sigma\) and \(\pi\) bond. The nonlinear optical properties of 
molecular systems depend on the polarizability of electrons in the \(\pi\)-bond. Polarizability discloses the 
electronic structure of the molecule precisely and comprehensibly. Nonlinear optical properties (NLO) 
viz., molecular polarizability (\(\alpha\)), anisotropy of polarizability (\(\Delta\alpha\)), molecular first hyperpolarizability 
(\(\beta\)) and electronic dipole moment (\(\mu\)) for the study compound were evaluated. The polarizability (\(\alpha\)), 
anisotropy of polarizability (\(\Delta\alpha\)), molecular first hyperpolarizability (\(\beta\)) are obtained by the following
The calculated parameters and electronic dipole moment for CMA2OEM are tabulated in Table 1. It is well known that the higher values of dipole moment, molecular polarizability, and hyperpolarizability are important for more active NLO properties. CMA2OEM has a relatively homogeneous charge distribution and it does not have a large dipole moment. The calculated value of dipole moment was found to be 3.3475 D. If we compare the common values of urea ($\alpha$ and $\beta$ of urea are 1.3732 D and 372.89 \times 10^{-33} \text{ esu}$) the hyperpolarizability and dipole moment values of CMA2OEM are larger than those of urea.

The conversion coefficients are as follows: polarizability $\alpha = 1 \text{ au} = 0.1482 \times 10^{-24} \text{ esu}$, first hyperpolarizability $\beta = 1 \text{ au} = 0.863993 \times 10^{-32} \text{ esu}$.

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### Table 1. Dipole moments $\mu$ (D), polarizability $\alpha$, anisotropy of polarizability $\Delta \alpha$, and first hyperpolarizability $\beta$ of CMA2OEM.

| Parameter | Value |
|-----------|-------|
| $\mu_x$  | $-2.9967 \times 10^{-2}$ |
| $\mu_y$  | $-1.3147 \times 10^{-2}$ |
| $\mu_z$  | $0.7051$ |
| $\mu_0$  | $3.347508475$ |
| $\alpha_{xx}$ | $23.510075$ |
| $\alpha_{yy}$ | $19.352970$ |
| $\alpha_{zz}$ | $19.913798$ |
| $\alpha$ | $20.925614$ |
| $\Delta \alpha$ | $40.90764548$ |
| $\beta_{xxx}$ | $2201.5729$ |
| $\beta_{xyy}$ | $-469.5798$ |
| $\beta_{xzz}$ | $688.1708$ |
| $\beta_{yzz}$ | $329.6725$ |
| $\beta_{zzz}$ | $262.9360$ |
| $\beta$ | $2400.53445$ |
| $\beta_y$ | $-2.193229717$ |
| $\beta_z$ | $62.26591199$ |
| $\beta$ | $2597.206142$ |
3.4. Molecular docking

Molecular docking calculations were obtained from two different programs: Autodock Vina and VMD [16]. Water molecules and cofactors were removed from the proteins to provide an interaction between only ligand and receptor. The Lamarckian Generic Algorithm was used as a score function to guess the best interaction between ligand and anti-apoptotic proteins. The highest binding score refers to the most stringent binding between protein and ligand. The docking results calculated by Vina were presented in table 2. According to these results, the highest binding score was obtained between CMA2OEM molecule and anti-apoptotic BRAF protein with $-6.5$ kcal/mol affinity energy.

CMA2OEM was found to be docked at all tested anti-apoptotic proteins with good confirmation. BRAF and CMA2OEM interactions can be observed in figure 4. In the BRAF and CMA2OEM compound (table 3), hydrogen bonds with oxygen atom of the ester and THR 458, GLN 562 and GLY 478 residues were identified. Van der Waals interactions with amino acid residues GLU 715, ALA 712, ASP 565, LEU 711, HIS 477, LYS 475 and TRP 476 were also determined.

Bcl-2 and ligand interaction can be observed in figure 5. CMA2OEM molecule formed one hydrogen bond with ARG 124 amino acid residue and seven Van der Waals interactions with different amino acid residues. In Bcl-w and CMA2OEM interaction, hydrogen bond occurred in the same amino acid with the different residue (ARG 95) (figure 6, table 3).

Mcl-1 and CMA2OEM interactions can be observed in figure 7. In the Mcl-1 and CMA2OEM compound, hydrogen bond with oxygen atom of the ester and LEU 267 residue was identified. Van der Waals interactions with amino acid residues MET 250, PHE 254, VAL 253, THR 266, ARG 263, PHE 228 and PHE 270 were also determined. AKT1 and CMA2OEM interaction is shown in figure 8. For AKT1 protein and CMA2OEM, an interaction of hydrogen bond with THR 195 was identified (table 3). Van der Waals interactions with amino acid residues PHE 161, GLY 294, GLU 191, HIS 194, LEU 181, LYS 179, ASP 292 and LEU 295 were also defined.

### Table 2. Docking binding energy results of novel CMA2OEM molecule as inhibitor with anti-apoptotic proteins.

| CMA2OEM       | Affinity energy (kcal/mol) |
|---------------|---------------------------|
| BRAF (PDB ID: 5vam) | $-6.5$                    |
| BCL-2 (PDB ID: 4man)   | $-5.9$                    |
| BCL-w (PDB ID: 2y6w)   | $-6.1$                    |
| mcl-1 (PDB ID: 5fdo)   | $-6.4$                    |
| AKT1 (PDB ID: 4gv1)    | $-6.3$                    |

Figure 4. Docking figure of CMA2OEM molecule in BRAF protein cavity.
### Table 3. Interacting amino acid residues of anti-apoptotic proteins with ligand [17].

| Number of hydrogen bonds | Types of amino acid-ligand interaction |
|--------------------------|----------------------------------------|
| 3                        | GLN 562, GLY 478, THR 458              |
| 1                        | ARG 124                                |
| 1                        | ARG 95                                 |
| 1                        | THR 195                                |

**Figure 5.** Docking figure of CMA2OEM molecule in Bcl-2 protein cavity.

**Figure 6.** Docking figure of CMA2OEM molecule in Bcl-w protein cavity.

### 4. Conclusion

In this study, biological and physical characterization of CMA2OEM compound was carried out using DFT/B3LYP/6-311++G(d,p) method. After optimization of the molecule, Mulliken atomic charge and NLO properties were examined. Mulliken charges (charges distribution-chk file) graphics were drawn to understand the properties and the dynamics of the molecule. The NLO properties of the molecule were calculated by the same method and 2 CMA2OEM was seen to be more polarized compared to the polarized molecule urea.

The possible docking alternatives were studied in Autodock Vina between CMA2OEM, as an inhibitor,
and human anti-apoptotic proteins, BCL-2, BCL-w, MCL-1, AKT1 and BRAF. Inhibition capability of this molecule on these proteins was evaluated, and potential inhibition of CMA2OEM molecule was tested \textit{in silico}. The most promising result was obtained from CMA2OEM and BRAF interactions. In this interaction, more stable conformation with lower energy in ligand-protein complex was analyzed. Hence, binding studies have been shown to be a useful tool that reveals electronic affinity and can help to understand ligand-protein interactions.

In the present study, we have designed and analysed a ligand in order to obtain a new drug active molecule. As concerns the previous literature precedence, the aforementioned investigation has not been reported in the literature so far. Thus, this study shows that the molecular interaction affinities between anti-apoptotic targets and compound are based on molecular docking. In aggregate, these molecular docking results will aid in better understanding of its molecular action with anti-apoptotic proteins. Taken together, our findings shed light on the molecular basis of the factors governing the binding of anti-apoptotic proteins and causes major consequences for the development of efficient therapeutic approaches.

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Фізичні дослідження і взаємозв'язок з антиапоптотичними білками молекули 2-(бі(ціаометил)аміно)-2-оксоетил метакрилат

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У цій роботі молекула 2-(бі(ціаометил)аміно)-2-оксоетил метакрилат (CMAOEM) вивчалася теоретично. Спурсь розраховувалася потенційна енергія поверхні, щоб знайти стан молекули з найнижчою енергією. Далі досліджувався найстійкіший стан молекули, атомний заряд Міллікена і нелінійні оптичні властивості. Також вивчалися положення зв'язків молекули CMAOEM і антиапоптотичних білків, таких як BCL-2, BCL-w, MCL-1, AKT і BRAF. Результати молекулярного докінгу показали, що найнайкішій комплекс отримується з цієї молекули і білка серін/треонін-протеїнкіназа. Дане дослідження дозволяє зробити припущення, що варто взагалі досліджувати взаємодію водневих зв'язків з метою лікування захворювань. Нарешті, цей новий лік міг би прокласти шлях до експериментальних досліджень.

Ключові слова: атомний заряд Міллікена, нелінійні оптичні властивості, молекулярний докінг, антиапоптотичний, CMAOEM