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**Original Article**

# Molecular modeling of 5-Oxo-3-substituted pyrrlidine -2-carboxylic acid derivatives pure as protease inhibitors

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## ABSTRACT

*Ab initio* molecular orbital theory with the HF/6-31G basis has been used to investigate the geometries and preferred conformations for L- proline, novel derivatives of pyrrolidine 2-carboxylic acid derivatives and a few N-acetyl derivatives. Fifteen products were tested and predicated their some physical parameters for the MM2 molecular mechanics program as well as protease inhibitors. Huckel's molecular orbital theory is a convenient method of expressing the energy levels generated by the *p*- orbitals of carbon atoms (HOMO& LUMO). The aim was designated to study the molecular docking at the gorge site and PAS of Protease inhibitors (PI) by the program MOE as well as predication of ADME/T. In conclusion, the selected compound of pyrrlidine -2- carboxylic acid derivative compound 9 show 100% inhibition of protease.

**Keywords**: *Ab intitio*, protease inhibitors, ADME/T, BBB, MM2 molecular mechanics.

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## INTRODUCTION

Protease inhibitors (PI) are new class of drug that inhibit cleavage of protein into functional and structural, they play an important rule in many diseases. Now, there is considerable interest in investigation the potential of angiotensin converting enzyme inhibitors, which are one of the ten front-line therapy for essential hypertension. Many other types of protease inhibitors are used as HIV viral protease, antithrombocin (II), Serine protease and Cysteine protease [1].

The aim of this study was to calculate and molecular modeling of 5-oxo (thio) -3-substituted pyrrolidine-2-carboxylic acid derivatives as new protease inhibitor agents using different theoretical methods. [2]The *Ab initio* methodology was employed for 3-D structure and estimate their minimized energy, charge [3], steric effects[4], chemical hardness ( ή ) [5], chemical potential ( μ )and molecular philicity (ω) [6]. The target predications for single protein were derived from 3-D Swiss target predication reports [7]. All hypothetical agents were tested and obey to Lipinski rule [ 8 ], as well as to discuss some of thier physicochemical properties and ADME/T of low molecular weight compounds and finding pure inhibitors aganist protease.

## METHODOLOGY

### Calculation of Quantum parameters

**Molecular Modeling:** The molecular modeling for the 3-Oxo-3-substiteuted pyrrolidiny-2-carboxylic acid derivatives were performed by using chem-3D ultra 12 version (Cambridge soft corporation, Cambridge, MM, USA ) implemented with molecular orbital computations software and molecular dynamic computation software (MM2). These agents were designated as protease inhibitors using Swiss target prediction protocols[4].

**Computation Studies:** Quantum chemical calculations were performed using Gaussian 16 suite programs. The calculations were carried out at the Hartrre-Fock energy level HF / 6-31G basis set and DFT/ B3LYP/6-31G. Initial geometry optimization was carried out using molecular mechanics by the MM2 force. The lower energy conformers were optimized to fine an appropriate geometry and calculate the physical properties of the compounds, HOMO, LUMO energy levels, heat of formations ($H_f$), hardness (η), electronic chemical potential (μ) and global philicity index (ω)[7].

**HOMO & LUMO energy levels:** Huckel's molecular orbital theory is a convenient method of expressing the energy levels generated by the p-orbitals of carbon atoms. Energies will be in units of β and α where α is the coulomb integral. The energy of α can be arbitrarily standardized as zero. Then the lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO) can be identified. The molecular energy level with the same energy as α is known as the nonbonding molecular orbital, the molecular energy level with a higher energy than α is known anti bonding molecular orbital. The energy level diagram obtained is sometimes referred to as an energy level spectrum.

**Physical properties calculation:** Quantum mechanics calculation methods provide definitions of important universal concept of molecular structure stability and reactivity [2]. An approximation for absolute hardness (η) was developed [2], as follows.

$$\eta = \frac{1}{2}(I - A) = \frac{1}{2}(E_{LUMO} - E_{HOMO}) \quad \text{.....(1)}$$

where (I) is the ionization energy, (A) the electron affinity. According to the Koopmen's theorm [3] the ionization energy and electron affinity can be expressed by the following relation:
I = - E HOMO and A= - E LUMO

Where HOMO is the energy of the highest occupied molecular orbital and LUMO is the energy of the lowest unoccupied molecular orbital. A higher (or less -ve) HOMO energy corresponds to the more reactive molecule in reaction with electrophiles, while lower LUMO energy is essential for molecular reaction with nucleophiles [4]. The hardness corresponds to the gap between these two orbitals in the molecule and it measures the resistance of a molecule to a change in their electron distribution. The global electron affinity can also be used in combination with ionization energy to calculate another global reactivity descriptor, the electronic chemical potential (μ), which can be defined [2] as follows:

$$\mu = \frac{1}{2}(I + A) = \frac{1}{2}(E_{HOMO} + E_{LUMO}) \quad \text{.....(2)}$$

While the global philicity index (ω) can be evaluated using the electronic chemical potential (μ) and chemical hardness (η) [5] as follow:

$$w = \frac{\mu^2}{2\eta} \quad \text{.....(3)}$$

**Physicochemical properties of 5-Oxo-3-subsitued pyrrolidinyl-2- carboxylic acid derivatives:** Some physicochemical properties

were predicated with the free version of MedChem Designer log *p*, number of hydrogen atom donor ( H-Do ) or acceptor ( H-Acc) , violations to the rule of five ( $R_o^5$ )[8]. The percentage of protease inhibitors including different enzymes were estimated using Molinspiration bioactivity score (2016) or Swiss target predication score (2013)[9].

**Molecular Docking:** Molecular docking(MD) is a computationally intensive structure- based virtual screening (SBVS) technique that generates and scores putative protease 1a 30 and organic small molecules complexes according to their calculated binding affinities using Mcule program. The crystal structure of the target was given [ 9 ], molecular docking automatically samples ligand conformations and protease–ligand interactions with a specified region of the protein surface were measured. It has been successfully used for identifying active compounds by filtering out those that do not fit into the binding sites. In this study, molecular docking was performed at the gorge site and PAS of Protease inhibitors ( PI ) by the program MOE. Also, the binding of an amino acid in the enzyme with different functional groups of pyrrolidino derivatives[10].

**ADME/TOX:** Absorption, distribution, metabolism, excretion and toxicity (ADMET) profile of FDA approved that the protease inhibitors drugs, poor pharmacokinetic properties are one of the main reasons for terminating the development of drug candidates[11]. Computed physicochemical properties associated with compounds that have good oral bioavailability, less or decrease toxicity and the capacity to be penetrate the BBB are key decision filter for CNS drug discovery[12]. For further evaluation by Lipinski's rule and some other physico-chemical filtering relevant for CNS activity. The more than 1000 compounds were further submitted to an in silico evaluation for bioavailability and blood brain barrier BBB penetration filter. Specifically, some key computed parameters that we examined as part of multiple property filter of lead compounds are toxicity, octanol /water partition coefficient (Log *P*), total polar surface area ( TPSA), BBB penetration and CNS activity can be expressed *in vivo* blood- brain barrier penetration ( Concentration in the brain / concentration in blood ) [13], to prioritize compounds with higher probabilities of possessing favorable tissue absorption distribution profiles, bioavailability and BBB penetration. On applying all the above mentioned properties[14].

The *in vitro* Caco-2 Permeability assay uses an established method for predicting the *in vivo* absorption of drugs across the gut wall by measuring the rate of transport of a compound across the Caco-2 cell line [15]. The Caco-2 cell line is derived from a human colon carcinoma. The cells have characteristics that resemble intestinal epithelial cells such as the formation of a polarised monolayer, well-defined brush border on the apical surface and intercellular junctions[16][17]..

## RESULTS AND DISCUSSION

The computational approach is one of the newest and fastest developing techniques in pharmacokinetics, ADME (absorption, distribution, metabolism, excretion) evaluation, drug discovery and toxicity[18]. However, to date, the software packages devoted to ADME prediction [19], especially of metabolism, have not yet been adequately validated and still require improvements to be effective[20].The organic chemical structure of studied compounds were depicted in Table (1)[21].

The values of chemical hardness, chemical potential and global philicity were calculated using equation (1, 2 , 3) respectively and tabulated in table (2) for DFT method and table (3) for HF method[22]. The general structure of pyrrolidine derivative figure1[1&2].

Fig. 2. Complex structure between compound 9 ( Ligand ) with $Zn^{+2}$

The results were listed in (Tables 2&3) respectively, and the values of the calculated properties in both methods were identical. It was noted that compound no.9 gave the lowest values for chemical hardness and philicity and highest value of chemical potential **[**3]**.** Fifteen compounds as 5- oxo-3 –substituted pyrrolidine -2- carboxylic acid derivatives were designated and calculate some parameters as protease inhibitors using different programs and software. Our results were explained a four structures that can inhibited the Protease and demonstrate the most characteristic ligand-protein interactions throug docking study. In general, it may be evidenced the process of inhibitors takes upon an unfolded shape and form a bridge between the 'anionic' (Tyr59) and 'peripheral' (Trp42) sites of 1a 30protease [ 9 ] . Some calculated QSAR properties of the compounds at MM2 level were listed in (table 2). These observiation parameters are charge, total energy, heat of formation, total dipole moment, frontier molecular orbital energy gap (calculated as LUMO–HOMO energy difference), ionization potential, , polarizability, surface area, and volume [ 2][6 ]. All suggested compounds are in ground state not in exited state.

The logarithm of the partition coefficient log *P* give the information about the solubility of these compounds whether it is hydrophilic or hydrophobic molecule. If the log *P* value is positive, the molecule will be hydrophobic and more soluble in the organic solvents. If the log *P* value is negative, the molecule will be hydrophilic and more soluble in aqueous buffer[23].

Most modeling compounds were obey Lipinski rule, the introduction of acetyl group ( $CH_3CO$-) at nitrogen of pyrroldine ring can play an important rule by increase potency towards inhibition of protease enzyme as shown in( tables 3&4) respectively. The docking of compound 9 with 1a 30 protease show good affinity and lowest energy figure 3[9][10]. The enzyme-based models have been useful in rationalizing the QSAR models generated in order to understand further the Protease-inhibitor interactions [11]. Such information available from QSAR [12][13], and enzyme-docking models used successfully to predict more potent analogues within a particular series [ 14][15].

The expected proposed mechanism can explain via the binding of $Zn^{+2}$ ion with compound 9 to form a complex as shown in( figure2) and show 100% as protease inhibitor( table 1). The sulphydryl (SH) group act as a strong chelating moiety with the zinc ion. Consequently, the sulfhydryl group can show adverse effect, which were the same as those caused by mercapto-containing captopril [16].

This postulation that proline was esterified with glycine as spacer, then the $Zn^{+2}$ will form a complex with the sulfhydryl group ( -SH) and carboxylic acid group ( -COOH ) of the compound 9 ( Ligand) and the expected complex [ $Zn^{+2}$-L].$2H_2O$ as shown in (table 2) (figure 2). Our initial goal back in revolutionize ADME screening and to produce large quantities of standardized high quality data for predictive modeling purposes and for delivering results to clients to help them make decisions on how to progress their compounds. The ideal oral drug will be rapidly and completely absorbed from the alimentary canal and will find its way directly and specifically to its site of action. It will not bind to or interact with related receptors, and it will not bind non-specifically to passing serum proteins[21]. By replacing R group with beta-lactam ring compounds 12-15 were listed in (table 3) , and expected to decrease their potency towards inhibition of protease and increase their toxicity. The predication of ADME/Tparmeters were listed in (table 4). Compound 9 show no blood brain barrier as compare with other compounds and can't pass to the central nerve system. While, this compound was showed normal permeability and negative carcinogenicity in rat [22] as shown in (table 4).

Finally, numerous methods have been used for the selected organic compound 1-15 to predict the molecular modelling using different parameters. Among them, Caco2-cell model has been recommended as a reliable *in vitro* model for the prediction of oral drug absorption [20]. In distribution, blood brain barrier (BBB) penetration can give information of therapeutic drug in the central nervous system (CNS), plasma protein binding model in its disposition and efficacy [10][23].

## CONCLUSION

The information derived from the molecular modeling displays are useful in molecularly designing new potential protease inhibitor agent. The 5-oxo-3-subsituted pyrrolidine-2- carboxylic acid derivatives show different activities against protease. Quantum chemical calculations were performed using Gaussian 16 suite programs and software as well as the predication of ADME/TOX parameters, only compound 9 was showed pure protease inhibitor. Our postulation that the functional groups (-SH,-COOH) will form a complex with $Zn^{+2}$ ion. The carboxyl and thiol groups are essential, whereas the N-acetyl moiety

and the spacing of the essential groups influence the potency of inhibition.

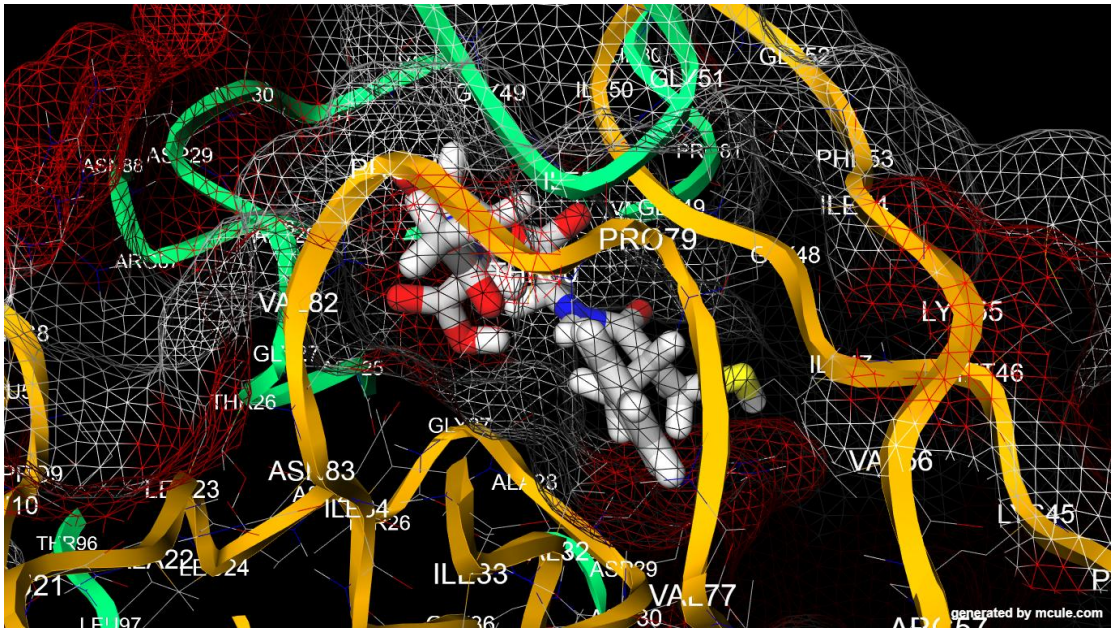
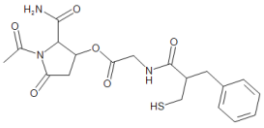



Fig.3. Docking structure between 1a 30protease and compound 9.

**Table 1 Calculated properties of 5-Oxo-3- substituted pyrrolidine -2- carboxylate as protease inhibitor**

| No | Chemical structure | Mwt | logP | H-Don | H-Ac | TPSA $(A)^2$ | $R_o^5$ | % PI |
|---|---|---|---|---|---|---|---|---|
| 1 | | 115.13 | -2.41 | 2 | 2 | 115.06 | Obey | 0 |
| 2 | | 131.13 | -2.41 | 2 | 2 | 131.50 | Obey | 20 |
| 3 | | 145.16 | -0.62 | 2 | 2 | 143.04 | Obey | 0 |
| 3a | | 185.18 | -0.56 | 1 | 2 | 74.68 | Obey | 67 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4 | | 186.19 | -0.60 | 2 | 2 | 143.05 | Obey | 0 |
| 4a | | 187.19 | -0.46 | 2 | 2 | 77.84 | Obey | 80 |
| 5 | | 199.21 | -0.09 | 2 | 2 | 157.07 | Obey | 0 |
| 6 | | 199.21 | -0.09 | 2 | 2 | 157.07 | Obey | 0 |
| 7 | | 218.16 | -2.83 | 2 | 2 | 219.06 | Obey | 0 |
| 8 | | 173.17 | -1.48 | 3 | 2 | 173.06 | Obey | 0 |
| **9** | | 422.12 | 1.94 | 3 | 5 | 130.08 | **Obey** | **100** |
| 10 |  1-acetyl-3-(2-(2-benzyl-3-mercaptopropanamido)acetoxy)-5-oxopyrrolidine-2-carboxylic acid compound with ethene (1:1)).(( | 421.47 | 0.40 | 3 | 5 | 135.80 | Obey | 87 |

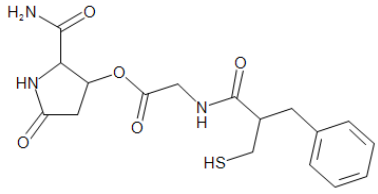
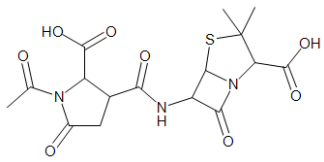
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 11 |  <br>-3-(2-(2-benzyl-3-mercaptopropanamido)acetoxy)-5-oxopyrrolidine-2-carboxylic acid compound with ethene (1:1). | 379.43 | 0.15 | 4 | 4 | 127.59 | Obey | 90 |
| 12 |  <br>acetyl-2-carboxy-5-oxopyrrolidine-3-carboxamido)methyl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid | 413.40 | -0.64 | 3 | 8 | 161.39 | Non | 84 |
| 13 | | 412 | -0.90 | 4 | 8 | 161.18 | Non | 60 |
| 14 | | 370.38 | -1.46 | 5 | 6 | 159.8 | Obey | 60 |
| 15 | | 412.42 | -0.90 | 4 | 7 | 167.18 | Obey | - |

**Table 2 Calculated properties using Gaussian /DFT/B3LYP/6-31G**

| No | S.E | Hf | LogP | cLogP | LogS | pKa |
|---|---|---|---|---|---|---|
| 1 | 14.014 | -398.51 | -0.2148 | -2.413 | -0.1670 | 9.822<br>2.521 |
| 2 | 8.4632 | -571.08 | -0.7424 | -2.413 | 0.0799 | 18.567<br>2.4070 |
| 3 | 12.2211 | -524.40 | -0.5474 | -0.6279 | -0.2244 | 3.8826<br>- |
| 4 | 11.744 | -524.09 | -0.5759 | -0.6279 | -0.2032 | 10.0<br>3.844 |
| 5 | 12.2543 | -544.73 | **-0.0055** | **-0.0951** | -0.6385 | 9.00<br>3.844 |
| 6 | 11.7451 | -542.09 | -0.5759 | -0.6279 | -0.2032 | 10.0<br>3.844 |
| 7 | 12.3405 | -784.73 | -0.4165 | -2.8325 | -0.4992 | -<br>- |
| 8 | 12.5558 | -643.63 | -1.2540 | -1.4853 | -0.2460 | 12.0<br>13.7834 |
| **9** | **-8.3182** | **-1760.71** | **0.30132** | **1.93985** | **-2.4167** | **26**<br>**3.0405** |
| $Zn^{+2}$ –L<br>$2H_2O$ | 39.7064 | ---- | 0 | 0 | 0 | ---- |

**Table 2 calculattion of other properties using Gaussian /DFT/B3LYP/6-31G**

| No | HOMO/LOMO | H | μ | Ω |
|---|---|---|---|---|
| 1 | -0.2161/ -0.0140 | 0.10105 | -0.11505 | 0.0655 |
| 2 | -0.0.397/0.1368 | 0.2669 | -0.1301 | 0.031708 |
| 3 | -0.2671/-0.0247 | 0.12119 | -0.1459 | 0.08783 |
| 4 | -2.680/-0.0242 | 0.1219 | -0.1461 | 0.08755 |
| 5 | -0.2566/-0.0371 | 0.10973 | -0.14686 | 0.09828 |
| 6 | -0.2603/-0.0241 | 0.11809 | -0.14221 | 0.08562 |
| 7 | -0.2537/-0.1083 | 0.07272 | -.18102 | 0.2253 |
| 8 | -0.2456/-0.0237 | 0.110925 | -0.13467 | 0.08174 |
| **9** | **-0.0629/0.0510** | **0.05695** | **-0.00595** | **0.00031** |
| $Zn^{+2}$ –L<br>$2H_2O$ | -0.3052/0.00047 | 0.15283 | -0.15236 | 0.07723 |

**S.E = Streic energy Hf = Heat of formation.**

**Table 3 Calculated properties using Gaussian / HF/ 6-31G**

| No | $H_f$ (Hartree) | HOMO/LOMO | ή | μ | Ω |
|---|---|---|---|---|---|
| 1 | -398.4462 | -0.36311/0.16022 | 0.26165 | -0.10144 | 0.01968 |
| 2 | -473.2751 | -0.3885/0.15885 | 0.27367 | -0.11482 | 0.02408 |
| 3 | -508.669 | -0.42099/0.14555 | 0.28327 | -0.13772 | 0.03347 |
| 4 | -511.1521 | -0.42166/0.14698 | 0.28432 | -0.13734 | 0.03317 |
| 5 | -550.2632 | -0.40701/0.13749 | 0.27224 | -0.13476 | 0.033353 |
| 6 | -550.2952 | -0.4031/0.14198 | 0.27254 | -0.13056 | 0.031272 |
| 7 | -828.4146 | -0.40536/0.06447 | 0.23491 | -0.17044 | 0.061831 |
| 8 | -625.1188 | -0.39396/0.15012 | 0.27204 | -0.12192 | 0.02732 |
| **9** | **-1752.729** | **-0.1955/0.1812** | **0.18835** | **-.00715** | **0.000135** |
| $Zn^{+2}$ –L<br>$2H_2O$ | --- | -0.32761/0.0005 | 0.16405 | -0.16355 | 0.08152 |

**Table 4 Some ADME/TOXICITY parameters of compounds (1-15)**

| No | BBB C.brain/C.blood | Caco-2 nm/s | Plasma permeability binding % | Log Kp cm/h | Carcinogenicity (Rat) |
|---|---|---|---|---|---|
| 1 | 0.399054 | 20.392 | 83.379678 | -4.20515 | Negative |
| 2 | 0.294675 | 18.7208 | 73.517428 | -4.82636 | Negative |
| 3 | 0.383204 | 20.1792 | 60.271158 | -3.86221 | Postive |
| 4 | 0.231434 | 19.1313 | 36.128900 | -4.57658 | Postive |
| 5 | 0.372056 | 20.4917 | 14.126530 | -3.51309 | Postive |
| 6 | 0.40214 | 20.497 | 27.547085 | -3.5666 | Postive |
| 7 | 0.114893 | 13.4114 | 22.944148 | -3.26301 | Postive |
| 8 | 0.280445 | 21.1094 | 18.660317 | -4.81058 | Negative |
| **9** | **0.047824** | 20.9073 | **61.071758** | -3.10683 | **Negative** |
| 10 | 0.0449153 | 20.8869 | 45.144161 | -3.8866 | Negative |
| 11 | 0.051368 | 20.8486 | 61.51940 | -3.99353 | Negative |
| 12 | 0.0441073 | 0.65291 | 52.80880 | -4.89116 | Postive |
| 13 | 0.0376338 | 0.41886 | 23.00777 | -5.25648 | Positive |
| 14 | 0.0351909 | 0.47966 | 18.8000 | -5.29133 | Negative |
| 15 | 0.0372308 | 0.512417 | 29.0875 | -5.23859 | Positive |

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