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RESEARCH ARTICLE

DFT Method, Molecular Orbital's, and Electronic Spectra Study for Characterization the Biological Activity of New Ebselen [2-phenyl-1, 2- Benzisoselenazol-3(2H)-one] Derivatives

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Abstract

Glutathione peroxidase (GPx) is one of the most potent antioxidant enzymes that is based on the selenium metal ion in the active site and plays an important role in reducing hydrogen peroxide and lipids. As low levels of GPx lead to an increase in hydrogen peroxide levels and thus cause direct damage to living tissue and many diseases infection. The number of selenium-containing compounds has been developed for their ability to treat or minimize peroxides. Ebselen "2-phenyl-1, 2- benzisoselenazol-3(2H)-one" is one of the most promising compounds as an antioxidant and have been shown to mimic the glutathione peroxidase activity in vitro. In this paper, seven different chemical derivatives of ebselen (a compound scientifically proven as an antioxidant) were designed by replacing or adding chemical groups and studying the effect of these changes on the behavior and effectiveness of the compound by studying the electron density of the central atom (selenium), HOMO, and LUMO. The stable geometrical shape of the compounds was also determined by calculating frequencies, bond energies and ion extraction for derivative compounds. On the other hand, the polarity of these derivatives and their solubility were calculated as well as the calculation of the Mulliken charge and electronic spectra measurements "infra Red (IR), UV-visible". Some of the compounds were characterized by having a better biologic effect than Ebselen while some of the derivatives were reversed.

Keywords: *Glutathione peroxidase (GPx), Ebselen, HOMO, LUMO, Density Functional Theory (DFT), Becke3-Lee-Yang-Parr (B3LYP).*

Introduction

In organisms, Reactive Species (RS) are produced either to perform a specific purpose or as a by-product of metabolic processes. These molecules are quick reacting and can sometimes be suitable for treating some types of dysfunction that may affect the living system such as "killing invading organizations" and "promoting vasodilatation", as though they can be destructive to cells depending on [1]:

- Generated amounts.
- Effectiveness of antioxidant enzymes.
- Cellular changing conditions.
- Time of action.

Deficiencies in the enzymes lead to imbalance and change in the "oxidation balance" system in the cells. As a result, many different drugs have been conducted to find alternative compounds (co-enzyme drugs) used to restore or mimic the work and activity of some antioxidant enzymes [1]. Reactive species that are oxygen-centered are called "Reactive Oxygen Species (ROS)". Reactive oxygen species include "superoxide anion radicals ($\bullet O_2^-$), singlet oxygen ($^1O_2$), hydroxyl radicals ($\bullet OH$), perhydroxyl radicals ($\bullet HO_2$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl)" [2].Another type of reactive species that are derived from nitric oxide ($\bullet NO$) since they also contain nitrogen-centered and they are known as "Reactive Nitrogen Species (RNS)". Besides nitric oxide, reactive nitrogen species include "nitrogen dioxide ($\bullet NO_2$), dinitrogen trioxide (N_2O_3), peroxynitrite (ONOO-), and peroxynitrous acid (ONOOH)" [2].

Free radicals excess has the potential to damage most of the cell's components, such as lipids, proteins, nucleic acids and carbohydrates. The most important types of damage that free radicals (reactive oxygen

species) may be cause are "modifications/mutations of DNA, oxidation /denaturation of proteins, and lipid peroxidation" [3, 4]. Aerobic organisms have antioxidant defense systems to protect themselves from toxic molecules, which include enzymes and chemical reagents that inhibit the reactions of these substances and thus control the regulation of the ROS concentration. Defense systems can be divided into two main types: i) primary defenses system: which reduce the concentration of free radicals and prevent initiation or inhibit their chain reactions, and ii) secondary defenses system: which block free radicals and reduce their damage they cause by reducing oxidized proteins and DNA repair [5]. Primary defenses include a number of antioxidant enzymes such as "Super Oxide Dismutase (SOD), Catalase (Cat) and glutathione peroxidases (GPx)" and molecules such as "hypotaurine, glutathione (GSH) ascorbic acid, taurine, and uric acid", Figure (1). The secondary defenses include enzymes such as "glutathione transfersases with peroxidase activity" that protect against lipid peroxidation, and "thioredoxin and other oxidoreductases" which react as antioxidants by expedite the reducing of oxidized protein groups such as "thiol" [5].

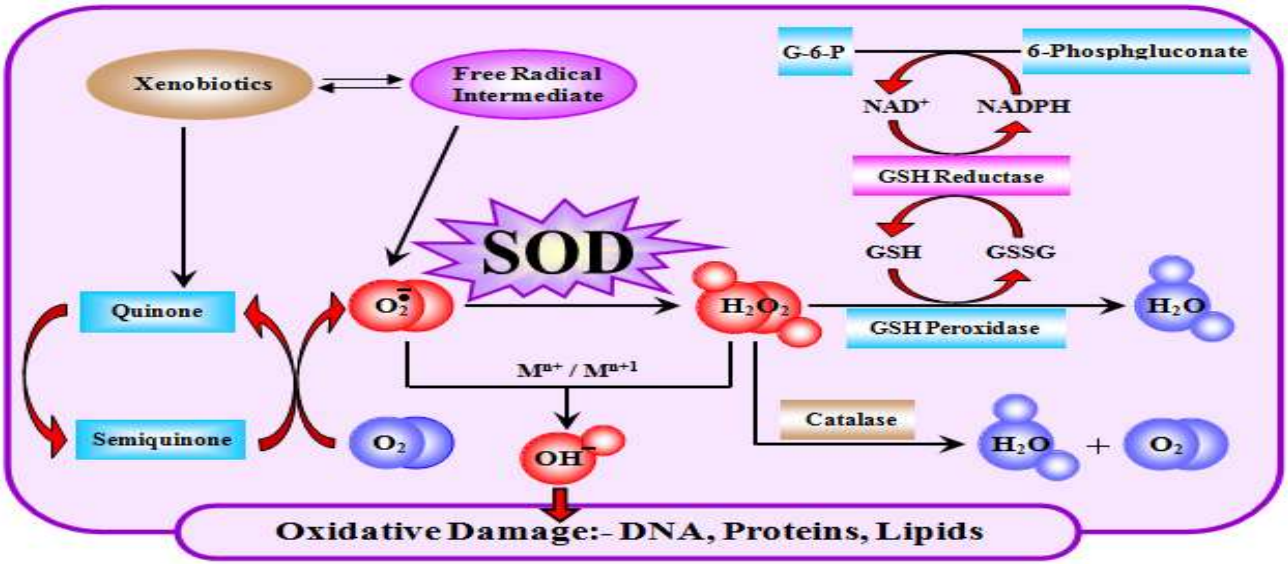

Figure 1: Mechanism of antioxidant enzymes to conversion free radical to molecular oxygen and water

SODs (Superoxide dismutases) are enzymes that have main function in all aerobic organisms to catalytically convert ($O_2^{\bullet -}$) to oxygen (O_2) which produced in the "mitochondria, cytosol, and endoplasmic reticulum" of cells. However, Superoxide dismutases can contribute to the production of other toxic substances such as hydrogen peroxide (H_2O_2), which is toxic to the cells and for the purpose of disposal of the risk of this toxin there are other enzymes becomes necessary are antioxidant, such as (CAT) and (GPx) enzymes, Figure (2) [6, 9].

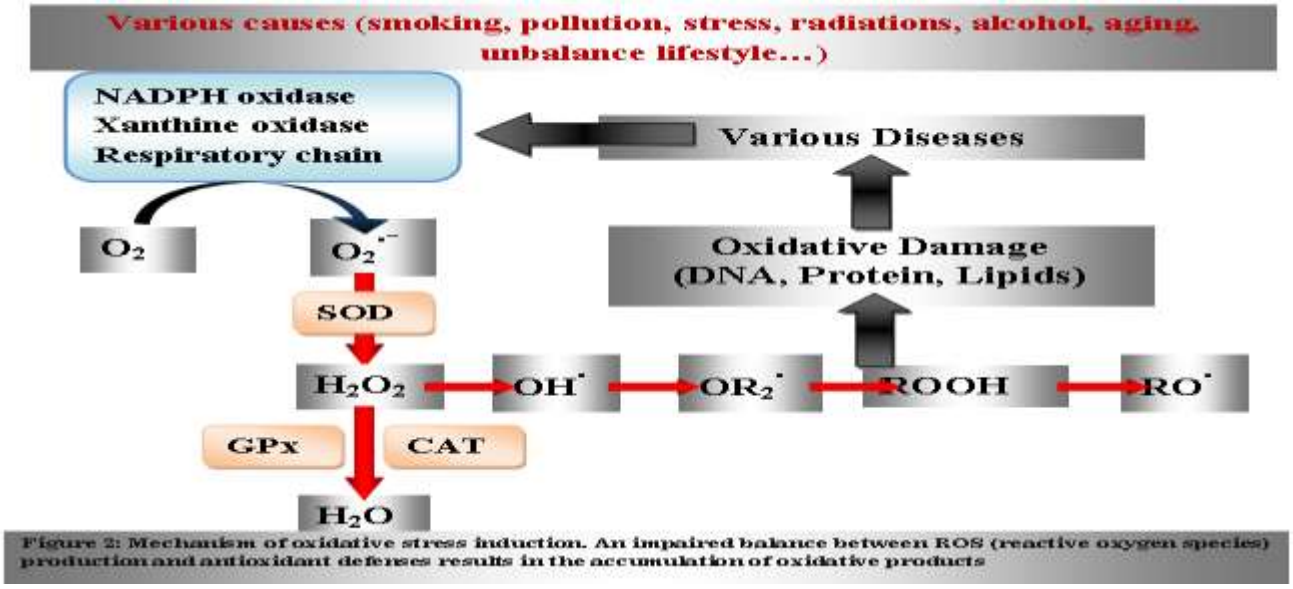

Figure 2: Mechanism of oxidative stress induction. An impaired balance between ROS (reactive oxygen species) production and antioxidant defenses results in the accumulation of oxidative products

Glutathione peroxidase (GPx) is one of the most potent antioxidant enzymes that is based on the selenium metal ion in the active site and plays an important role in reducing hydrogen peroxide and lipids [10]. As low levels of GPx lead to an increase in hydrogen peroxide levels and thus cause direct damage to living tissue and many diseases infection [11, 12].

GPx is classified into four subtypes that stimulates hydrogen peroxide reduction depending on where it is located in the tissue, GPx1 "is ubiquitous and found in the cytosol of most cells, including red blood cells (RBCs)", GPx2 "is also cytosolic but is confined to the gastrointestinal tract", GPx3 "occurs in the plasma as a glycoprotein", and GPx4 "interactions with complex lipids, such as cholesterol and lipoproteins damaged by free radicals, and is found in mitochondria" [13].

There are several proposed mechanisms for GPx action as an antioxidant. The most acceptable mechanisms is shown in Figure (3), includes the take apart of selenol (ESeH) of the selenocysteine which is represent the active form of the glutathione peroxidase to enter the redox cycle reaction involving the selenolate anion and reduces the organic peroxides and hydrogen peroxide.

The selenolate, after that is oxidized to selenic acid and reacts with reduced glutathione (GSH) to form a selenosulfide adduct (ESeSG). Another molecule of glutathione is then reacted with selenosulfide to form the oxidized glutathione (GSSG). Thus, the mechanism product that the hydroperoxide is converted to corresponding alcohol (reduced), while two equivalents of glutathione are converted to the water and disulfide (oxidized) [14].

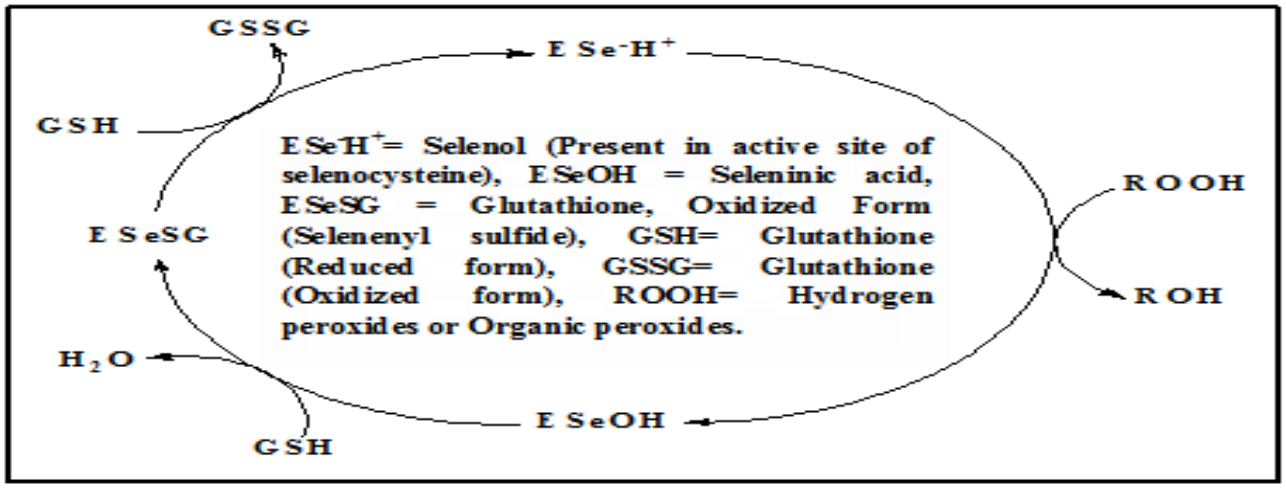

Figure 3: Catalytic mechanism of GPx

Based on the above, the number of selenium-containing compounds has been developed for their ability to treat or minimize pyroxides. Ebselen [2-phenyl-1, 2- benzisoselenazol-3(2H)-one] is one of the most promising compounds as an antioxidant and have been shown to mimic the glutathione peroxidase activity in vitro [15]. Several studies have been performed by Marinio et al., to stimulate the reaction of glutathione peroxidase by ebselen. Results have shown that selenol is responsible for the glutathione peroxidase activity of ebselen, Figure (4) [16].

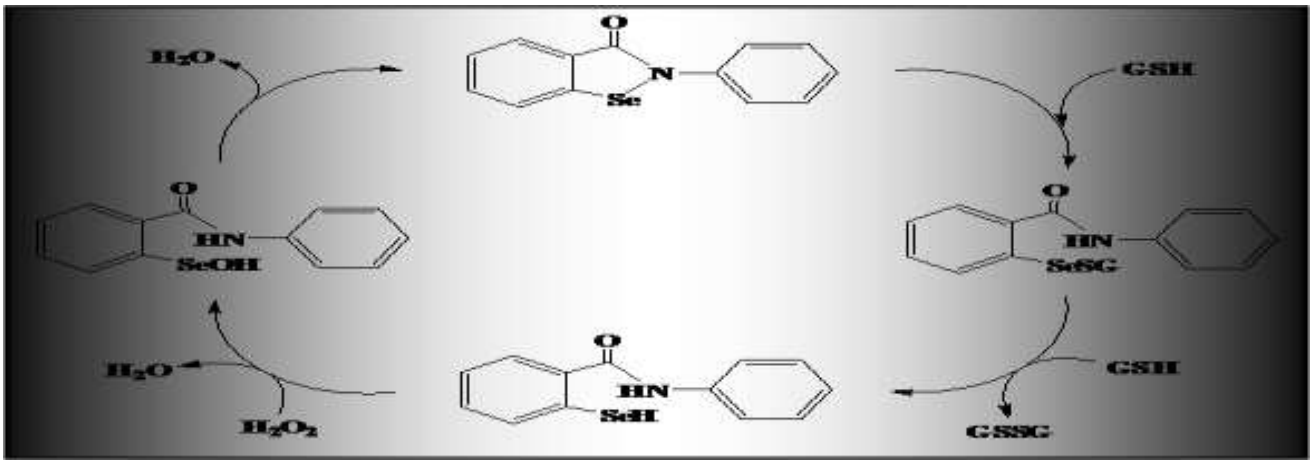

Figure 4: Kinetic study of the catalysis of the GPx reaction by ebselen

In recent years, many studies and scientific discoveries have been conducted, including the use of metals (including selenium) in the design of a large number of different pharmaceutical compounds. Many of these studies have been successful in the design, installation, and use of these drugs in the treatment of many diseases and health problems [17].

In this paper, seven different chemical derivatives of ebselen (a compound scientifically proven as an antioxidant) were designed by replacing or adding chemical groups and studying the effect of these changes on the behavior and effectiveness of the compound by studying the electron density of the central atom (selenium) HOMO, and LUMO.

The stable shape of the compounds was also determined by calculating frequencies, bond energies and ion extraction for derivative compounds. On the other hand, the polarity of these derivatives and their solubility were calculated as well as the calculation of the Mulliken charge and electronic spectra measurements "infra Red (IR), UV-visible". Some of the compounds were characterized by having a better biologic effect than Ebselen while some of the derivatives were reversed.

Aim of the Work

In this work, we are building, designing, studying, seven Ebselen derivative compounds and characterized the substitution effectiveness of chemical groups on the central atom activity [selenium (I)] of known antioxidant drugs (Ebselen: "2-phenyl-1,2-benzisoselenazol-3(2H)-one").

Calculation Method

The molecular design of the ebselen derivative compounds was constructed and completed using "Gaussian 03 (2003) program package" calculations. The calculations were performed using the method "DFT/ B3LYP" with the base set "6-31 G" [18]. These study included the calculation and investigation of many chemical and physical properties of the derivative compounds and their comparison with the known drug in terms of the biological effectiveness such as calculating the "Balance geometrical shape (Optimization) of molecules, Electronic Density of the atoms in molecules (especially of metal ion and coordinated atoms of ligands), HOMO (Highest occupied molecular orbital), and LUMO (lowest unoccupied molecular orbital)" which represent most critical to describe the chemical reactivity, optical properties, and biological activity of the compounds.

Result and Discussion

Biomedical inorganic chemistry is one of the modern fields of chemistry, which provides a wide range of understanding and treatment of complex and intractable diseases through the offers the potential for the designing new therapeutic and diagnostic cofactors, Figure (5).

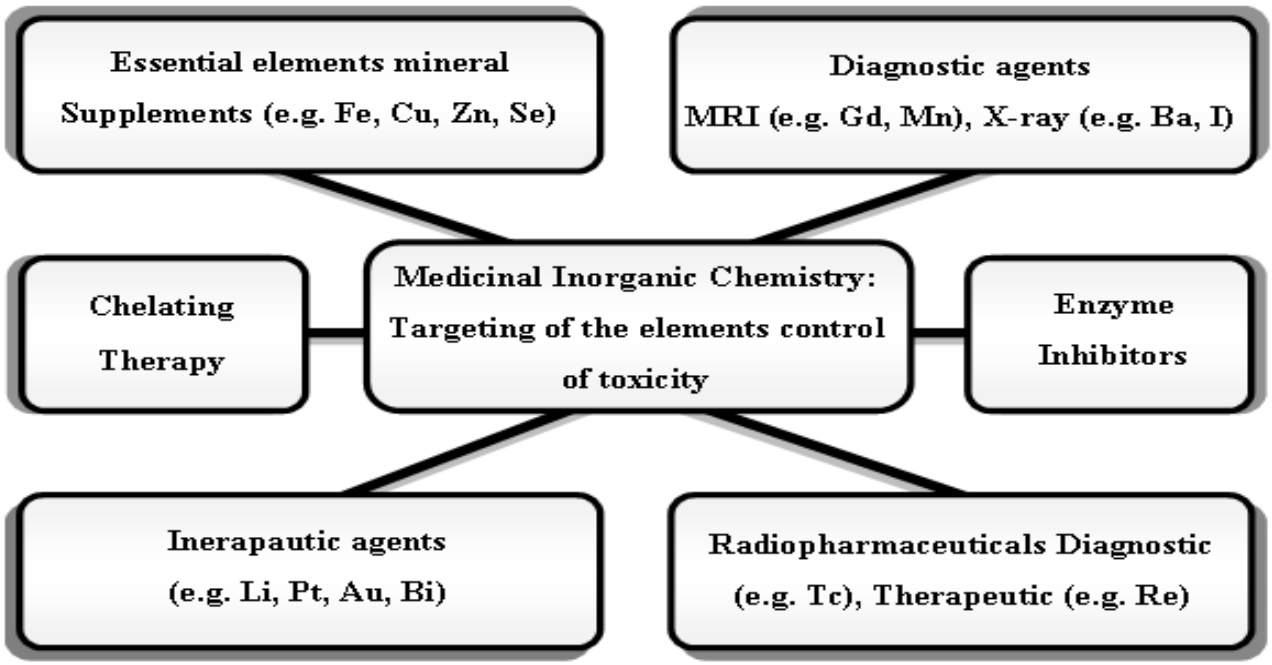

Fig 5: The major key areas of medical inorganic chemistry

It is imperative for organisms to maintain stability and balance through the continuous flow of food and energy in the changing environment for stay alive. This process is very complex as this flow is controlled by some inorganic ions and a compound "cofactors" provides a dynamic flow system. Cofactors are "enzymes" that are protein in nature and about (30%) of enzymes are metallo-enzymes which contain a metal ion in the active site of the entire enzyme .The metallo-enzymes controlling on many processes such as "oxidation-reduction", "acid- catalyzed hydrolyses", and "synthesis of isomerases of hydrocarbon compounds". Chemical compounds can be considered suitable for medical applications if they have three essential characteristics: "ability to be ligand exchange", "the oxidation states range which can be accessible and "the ability of metal in the compound to mimic iron mechanism in binding to particular biological molecules" [19]. Glutathione, a tri-peptide consisting of "glutamic acid - cysteine - glycine", is the substrate for glutathione peroxidase (GPx), which is an important enzymatic component of the intracellular antioxidant defenses [20]. Glutathione peroxidases reduce hydrogen peroxide and alkyl hydroperoxides to alcohols using reduced glutathione as effective reducer.

$$2H_2O_2 \xrightarrow{GPx} 2H_2O + O_2$$

$$ROOH + AH_2 \xrightarrow{GPx} ROH + H_2O + A$$

Where: - "AH_2: an acidic molecules, A: conjugated base".

In this paper, seven ebselen derivative compounds modeling were performed using "Gaussian 03 (2003) program package" calculations. The calculations were performed using the method "DFT/ B3LYP" with the base set "6-31 G". These compounds deferent about known drugs (C_1: ebselen) by group substituent (such as: OH^-, CH_3, C_2H_5, NO_2, phenyl, and CH_3CO^- groups) instead of hydrogen atom of phenyl ligand groups, Figure (6). To identification the symmetry of a selenium compounds (ebselen derivatives), we need to geometrical entities application (symmetry elements) which is include: "Identity (**E**)", "Proper rotation axis: (**C_n**)", "Symmetry plane: (**σ**)", "Inversion center: (**i**)", and "Improper rotation axis: (**S_n**)" , so the determination of symmetry element and calculation of point group in addition to "vibrational, rotational, and translational" energy calculation for all compounds were to be complete where all the results for each molecules (ligands and their compounds) show out to be restricted to "C_1" because these compounds have not any symmetry element except the identity (E), Figure [(7) and the others like it].

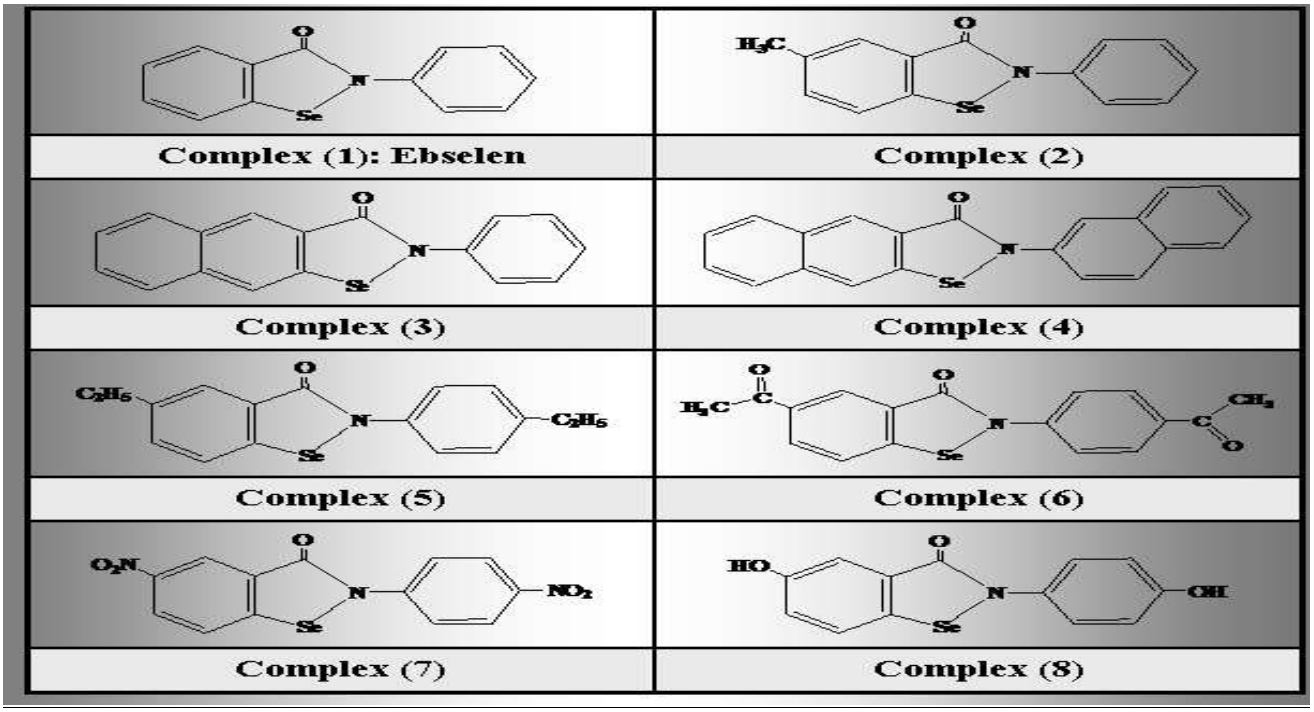

Fig 6: derivative compounds of ebselen

The conformations of the ebselen derivative complexes in addition to known promising prodrug [standard compound (C_1): ebselen] obtained from DFT calculation. To estimate the electronic density of atoms in all compounds, the optimization process was fully done "the global minimum energy information of the compounds is achieved" especially for coordinated atoms of the ligands, Table (1) Figure (7).

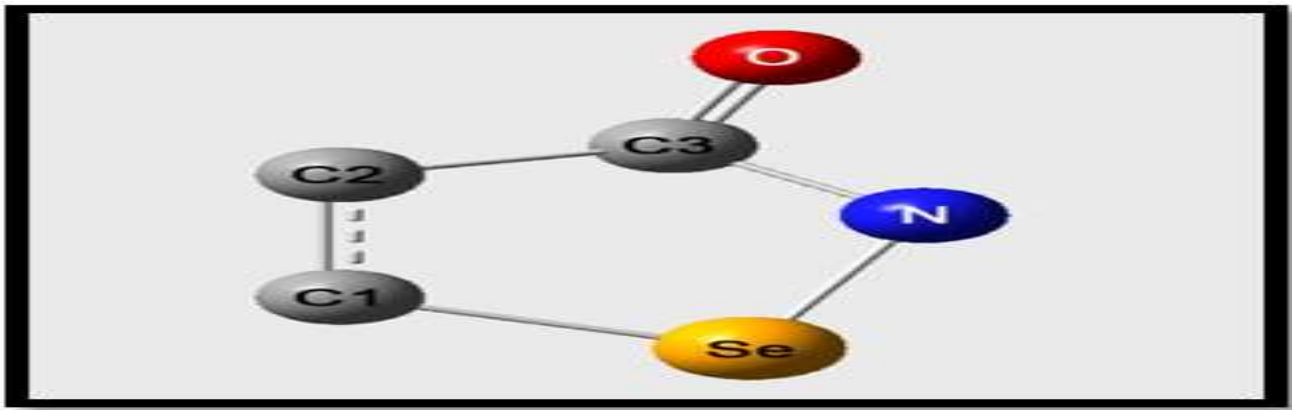

Figure 7: Selenium (I) ion and coordinated atom of the ligands

Table 1: Electronic density of Selenium (I) ion and coordinated atom of the ligands

Complex	Se	N	O	C1	C2	C3
C_1	33.149722	8.230674	8.089272	5.696170	5.361782	4.927369
C_2	33.144512	8.227416	8.089858	5.705375	5.396613	4.934239
C_3	33.155462	8.228627	8.086319	5.741045	5.440645	4.933502
C_4	33.152592	8.233690	8.086813	5.743292	5.437601	4.929057
C_5	33.145667	8.230087	8.091166	5.701324	5.394924	4.932148
C_6	33.153346	8.241202	8.064078	5.698687	5.491656	4.927048
C_7	33.088050	8.238495	8.057777	5.711497	5.497999	4.917157
C_8	33.142139	8.218692	8.099245	5.720670	5.421577	4.927069

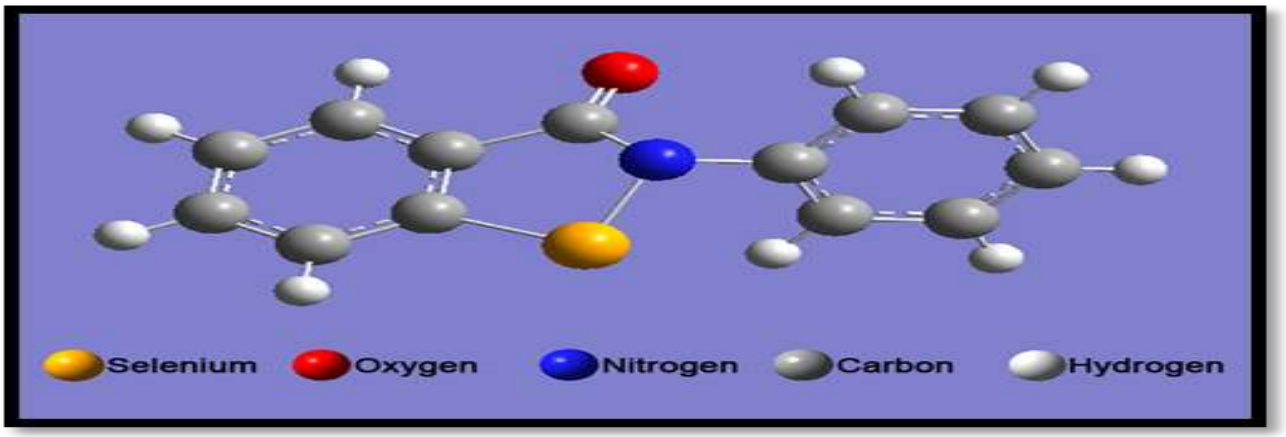

Fig 7: Geometrical Shape of Compound (C_1)

The most essential orbital's to describe the optical properties of the compound, and chemical and biological activity of the chemical species are the frontier highest occupied MO's and lowest unoccupied MO's (HOMO, LUMO). Higher value of HOMO of compound mean it could be oxidation and act as a "Lewis Base: ability to donate electrons to convenient acceptor molecule with low energy", while higher value of lowest unoccupied molecular orbital LUMO of a compound mean it could be reduction and act as a "Lewis Acid: ability to accept electrons from convenient donor molecule". Depending on above definition, the results show that activity of the building compounds (C_2, C_5, C_7, C_8) are higher when compared with that of C_1 compound (as a standard) according to Lowest unoccupied MO (LUMO) which indicate the ability to accept electrons of building compounds, while the other compounds (C_3, C_4, C_6) gave lower than that of C_1 complex, Table (2), Figure 8 and others like it].

Table 2: The energy of Highest Occupied Molecular Orbital's, Lowest Unoccupied Molecular Orbital's, and ΔE

Compound	E_{HOMO} (e.v)	E_{LUMO} (e.v)	ΔE (e.v)
C_1	-0.21360	-0.06254	0.15106
C_2	-0.21122	-0.06060	0.15062
C_3	-0.21855	-0.06652	0.15203
C_4	-0.20044	-0.06707	0.13337
C_5	-0.20690	-0.05906	0.14784
C_6	-0.22994	-0.08132	0.14862
C_7	-0.20062	-0.06163	0.13899
C_8	-0.20452	-0.06058	0.14394

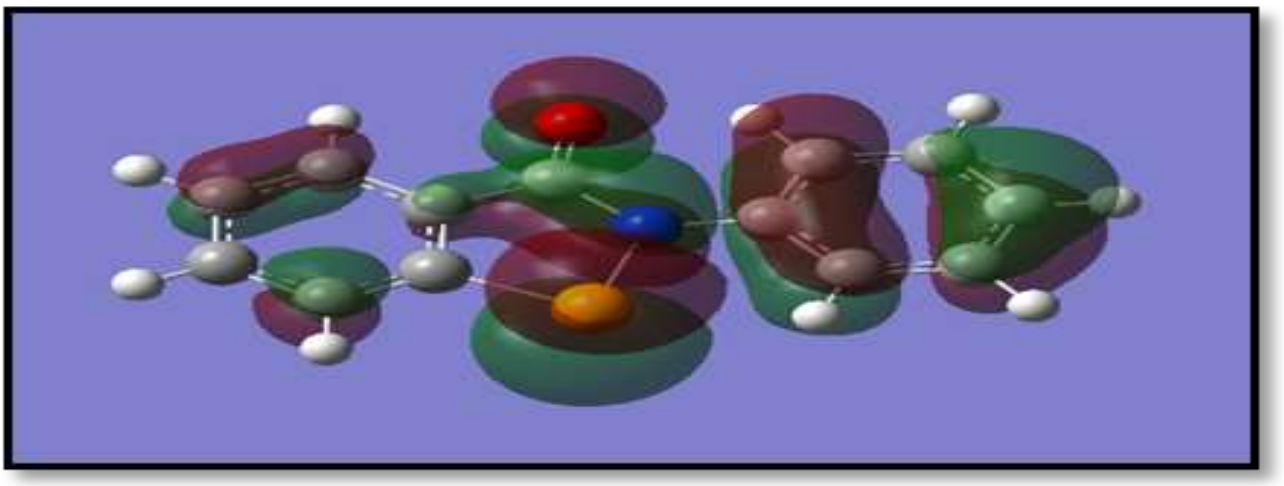

Fig 8: Highest Occupied MO (HOMO) of C_1.

On the other hand, ΔE energy (energy gaps) of all compounds (except C_3) are lower than standard compound (C_1: ebselen) which that indicate stability of building compounds. The "infrared (IR), UV-visible" spectrum technique of a molecule is represented to be a one of a important physical property and is described for the molecules.

In that capacity, these spectrums can be utilized as a unique fingerprint for distinguishing and comparison between the derivative complexes and reference complex (Ebselen).Electronic spectra measurements for the complexes were calculated theoretically by:

- Infrared (IR): using the job type optimization and frequency along with at "DFT/B3LYP" method with "6-31G", figure [(9 and 10 and the others like it]
- UV-Visible: also the job type optimization and Frequency used along with "TD-SCF /B3LYP" method with "6-31G" basis set, figure [11 and 12 and the others like it)].

And when we compare the results of both methods with the previous compounds practical data. It was found that there was a close agreement between the theoretical calculation for each one of the building complex and electronic spectra.

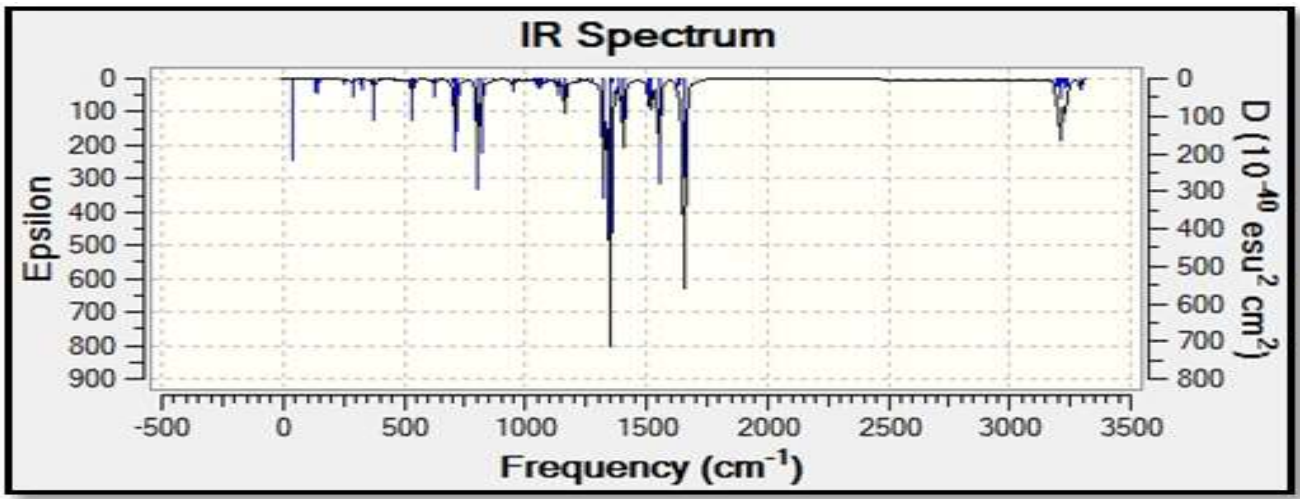

Fig 9: Infrared Spectrum of (C1)

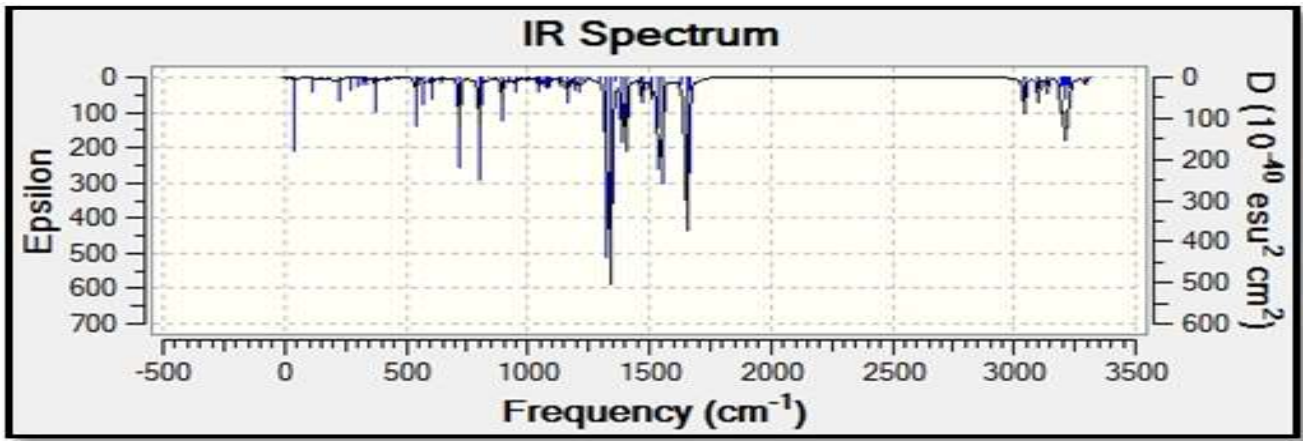

Fig 10: Infrared Spectrum of (C2)

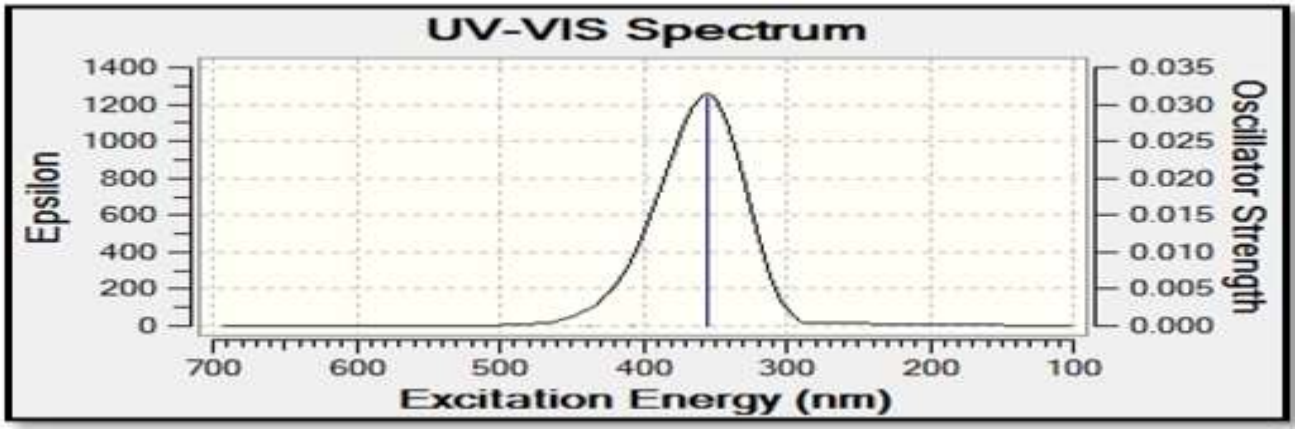

Fig 11: UV-Visible spectrum of (C1)

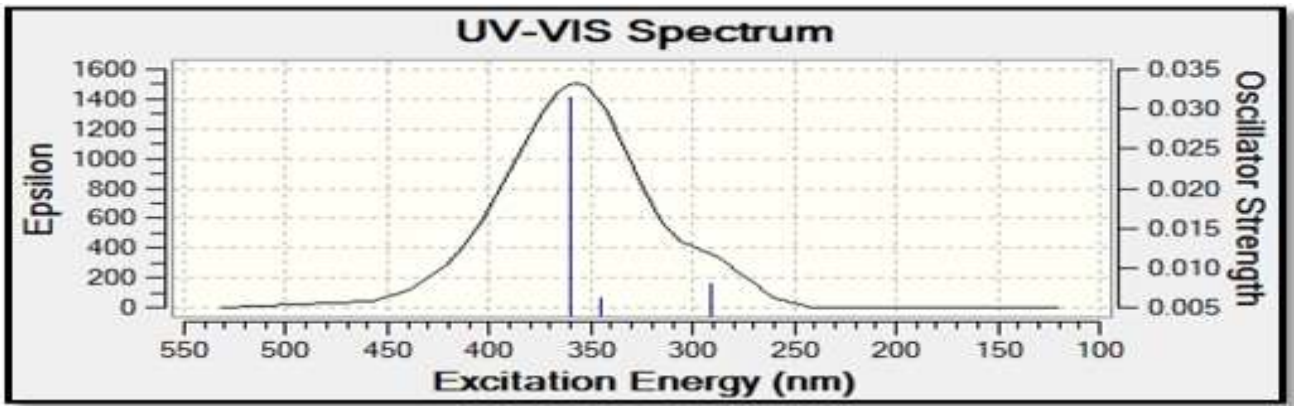

Fig 12: UV-Visible spectrum of (C2)

By calculating the Mulliken atomic charges of the building compounds, we observe that the charge of coordinated atoms of the ligand with the selenium ion (N, C1) is negatively charged while the carbon atoms which linking to coordinated atoms have positive charge (C2, C3). At the same time, the selenium ion charge of the metal is reduced to ($0.6934 \leq Se \leq 0.7550$), these results show that part of the electronic density was transferred and redistribution towards the selenium (I) ion and thus the stable compounds formation, Table (3) Figure (7).

Table 3: Mulliken atomic charges of selenium (I) ion and coordinated atoms of the ligand, Polarity of the compounds

complex	Mulliken atomic charge						Polarity (Debye)
	Se	N	O	C1	C2	C3	
C_1	0.6993	-0.9601	-0.4620	-0.3802	0.0879	0.5349	3.6415
C_2	0.6948	-0.9590	-0.4632	-0.3826	0.0934	0.5334	3.7220
C_3	0.6955	-0.9609	-0.4618	-0.3773	0.0849	0.5393	3.7193
C_4	0.6987	-0.9694	-0.4622	-0.3782	0.0847	0.5401	3.7063
C_5	0.6934	-0.9601	-0.4651	-0.3808	0.0913	0.5339	3.3579
C_6	0.7229	-0.9704	-0.4414	-0.3768	0.0828	0.5453	9.6955
C_7	0.7550	-0.9758	-0.4350	-0.3698	0.0604	0.5517	7.0749
C_8	0.7457	-0.9573	-0.4713	-0.3775	0.0797	0.5318	7.8578

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