بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Study of QSAR ,Molecular Modeling and Docking of Some N,N-dimethyl Carbamate Derivatives as Acetyl cholinesterase Inhibitor

(دراسة العلاقة الكميه بين البنيه والفعاليه والنمذجه الجزيئيه لبعض مشتقات N,N ثنائي ميثيل الكارباميت مثبطا لاستيل كولينستريز)

A Dissertation Submitted in Partial Fulfillment for the Requirements the Degree of Master of Science in Chemistry

By

MALAZ ALSIDDIG MOHMMED DAFALLA

B.Sc. (Honours)scientific laboratory- Chemistry

Supervisor

Prof.Dr. Ahmed Elsadig Mohammed Saeed

November ,2020

استهلال

بسم اللّه الرحمن الرحيم

قال تعالى:

﴿اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ

(3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)﴾

صدق الله العظيم
سورة العلق، الآيات (1-5)

Dedication

Dedicated this work to:

My mom Her affection, Love , encouragement and prays of a day and night make me able to get Such success and honor

My father For earning an honest living for us and for supporting and encouraging me to believe in myself.

Special thanks to my brother and sisters

Acknowledgements

To start with, praise to Allah and thank for giving me the strength and patience to complete this project.

I would like to express my special thanks of gratitude, to my supervisor

Prof.Dr. Ahmed Elsadig Mohammed Saeed

Thanks to Mr . Najm Aldeen Margane Ahmed Babiker

Abstract

N,N- dimethyl carbamate has been commonly used as acetyl cholinesterase inhibitor , a series of N,N-di benzyl amino derivatives were subjected to two dimension (2D) quantitative structure activity relationship and (2D-QSAR)studies . The 2D-QSAR models were constructed on a for word of partial least sequence (PLS) and step wise multiple regression (SW-MLR) . By using MOE procedure and by choosing randomly descriptors as independent variable and plotted against biological activity as dependent variable a number of QSAR equations were obtained the best of them were:

- PIC50= 5.89187-0.06775 LogP-0.01059 PEOE-VSA-1

  R2 = 0.86 RMSE = 0.12

As a result from this equation forty compound was designed and made a dock in to the active site of selected receptor (1EVE) which was retrieved from protein data bank. The most active compounds 19 and 20 that have free energy interaction better than compound 5 as reference compound , and were find that the electron-withdrawal substituent's on aromatics rings of the dibenzylamino group reduce the inhibitory power.

المستخلص

يستخدم N، N- ثنائي ميثيل الكربامات بشكل شائع كأسيتيلمثبط الكولينستريز ، سلسلة من مشتقات N,N-di benzyl amino خضعت لدراسات ثنائية الأبعاد (2D) وعلاقة نشاط البنية الكمية (2D-QSAR). تم تصميم نماذج2D-QSAR على نمط التسلسل الجزئي الأدنى (PLS) والانحدار المتعدد التدريجي (SW-MLR). باستخدام إجراء MOE واختيار الواصفات العشوائية كمتغير مستقل ومخطط مقابل النشاط البيولوجي كمتغير تابع ، تم الحصول على عدد من معادلات QSAR كان أفضلها:

$$PIC50 = 5.89187-0.06775\ LogP-0.01059\ PEOE\text{-}VSA\text{-}1$$

$$R2 = 0.86 \qquad RMSE = 0.12$$

نتيجة لهذه المعادلة ، تم تصميم أربعين مركبًا وجعله رصيفًا في الموقع النشط للمستقبل المحدد (1EVE) والذي تم استرداده من بنك بيانات البروتين. أكثر المركبات نشاطًا 19 و 20 التي لها تفاعل طاقة حر أفضل من المركب 5 كمركب مرجعي ، ووجدت أن بدائل سحب الإلكترون الموجودة في الحلقات العطرية لمجموعة ديبنزيلامينو تقلل من القوة المثبطة.

Table of Content

List of tables

List of figures

List of abbreviations

No	Abbreviation	Detail
1	**ACHE**	Acetyl cholinesterase inhibitor
2	**QSAR**	Quantitative structure activity relation ship
3	**R2**	Correlation coefficient of QSAR model
4	**RMES**	Root main square
5	**F**	Fisher value
6	**P**	Statistical confidence level
7	**Q2**	Cross validate
8	**St**	Stander error of the estimate
9	**Log p**	Partition coefficient
10	**PEOE-VSA-1**	Total negative Vander walls surface area
11	**Dipole x**	Dipole moment x
12	**SMR-VSA2**	Bin 2 molar refractivity
13	**IC50**	The half maximal inhibitory concentration
14	**ACD/chem sketch**	Molecular modeling program
15	**1EVE**	crystal structure of Torpedo California AChE complexed with donepezil
16	**TcAChE**	Torpedo California Acetyl cholinesterase inhibitor

1-Introduction:

There is no substitute for practical laboratory experience, but computer modeling methods play an important role both as an aid in interpreting experimental resultsand as a means of explaining these results. Molecular modeling is now used not justin chemistry but in a wide range of subjects such as pharmacology and mineralogy.

QSAR studies is the computer-aided prediction of clinical outcomes of drugs by means of the quantitative modeling of the relationships existing between the chemical, structural and biological features. This challenging objective requires the use of a appropriated datasets and the application of sophisticated modeling strategies. The datasets required for the aforementioned modeling have to integrate molecular data, as well as a wide range of biological information resulting from in *vitro* and in*vivo* experiments, along with clinical observations. The computational models to be developed require multi-dimensional and often multi-scale approaches. The systems biology perspective has frequently to be taken into account. Several examples will be shown such as the predictive modeling of the antipsychotics side-effects ,the chemo- and bioinformatics substantiation of possible adverse drug effects detected bypharmacoepidemiological methods and the prediction of the cardiotoxicity by multi-scale modeling.

Acetyl cholinesterase (AChE) plays a pivotal role in the termination of synaptic transmission by rapid hydrolysis of theneurotransmitter acetylcholine (ACh) into acetate and choline inthe synaptic cleft, after the neurotransmitter release from thepresynaptic nerve terminal. AChE is present in both the central and peripheral nervous systems, including at the neuromuscular junction.

Alzheimer's disease (AD) is one of the lethal diseases, mainlyaffecting older people. The unclear root cause and involvement of variousenzymes

in the pathological conditions confirm the complexity of the disease. Quantitative structure activity relationship (QSAR) techniques are of great significance in the design of drugs against AD.(Ambure and Roy, 2014 **)**

1-1 Computational Chemistry

Computational Chemistry is a branch of chemistry that uses computer simulation to assist in solving chemical problems. It uses methods of theoretical chemistry, incorporated into efficient computer programs, to calculate the structures and properties of molecules and solids. It is necessary because, apart from relatively recent results concerning the hydrogen molecular ion, the quantum many-body problem cannot be solved analytically, much less in closed form. While computational results normally complement the information obtained by chemical experiments, it can in some cases predict hitherto unobserved chemical phenomena. It is widely used in the design of new drugs and materials. Examples of such properties are structure the expected positions of the constituent atoms ,absolute and relative (interaction) energies, electronic chargedensity distributions, dipoles and higher multiple, vibrational frequencies, reactivity, or other spectroscopic quantities, and cross sections for collision with other particles.

Computational pharmaceutics involves the application of computational modeling to drug delivery and pharmaceutical nano technology. In combination with existing branches of pharmaceutics, it offers rapidly growing potential for developing rational, deductive and knowledge-based strategies in pharmaceutics.(Ouyang and Smith, 2015)

1-1-1 Molecular modeling

encompasses all methods, theoretical and computational, used

to model or mimic the behavior of molecules. The methods are used in the fields of computational chemistry, drug design, computational biology and materials science to study molecular systems ranging from small chemical systems to large biological molecules and material assemblies. The simplest calculations can be performed by hand, but inevitably computers are required to perform molecular modeling of any reasonably sized system. The common feature of molecular modeling methods is the atomistic level description of the molecular systems. This may include treating atoms as the smallest individual unit (a molecular mechanics approach), or explicitly modeling electrons of each atom (a quantum chemistry approach).

1-1-2 Molecular mechanics

Is one aspect of molecular modeling, as it involves the use of classical mechanics (Newtonian mechanics) to describe the physical basis behind the models. Molecular models typically describe atoms (nucleus and electrons collectively) as point charges with an associated mass. The interactions between neighboring atoms are described by spring-like interactions (representing chemical bonds) and Van der Waals forces. The Lennard-Jones potential is commonly used to describe the latter. The electrostatic interactions are computed based on Coulomb's law. Atoms are assigned coordinates in Cartesian space or in internal coordinates, and can also be assigned velocities in dynamical simulations. The atomic velocities are related to the temperature of the system, a macroscopic quantity. The collective mathematical expression is termed a potential function and is related to the system internal energy (U), a thermodynamic quantity equal to the sum of potential and kinetic energies. Methods which minimize the potential energy are termed energy minimization methods (e.g., steepest descent and conjugate

gradient), while methods that model the behavior of the system with propagation of time are termed molecular dynamics.(Heinz,*etal*,2016).

1-1-3 QSAR

QSAR is one of the earliest approaches to structure-based design. It relies on finding a correlation between the physical properties of a compound and its activity as a drug. A typical physical property used is the partition coefficient between octanol and water-that is, the relative concentrations of a compound in the two solvents when it is allowed to distribute itself between them. This is used as a crude measure of the preference of the compound to dissolve in lipids (fats) in the body. This solubility in lipids is important as it can determine where in the body the drug can reach. The activity towards a target enzyme for a large number of compounds is measured by obtaining the equilibrium constant for the reaction:

molécules + enzyme $\longrightarrow$ molécule-enzyme complex (1.1)

Values of various other physical properties for each of the compounds are then either measured or obtained from the scientific literature or databases of properties. An equation is derived which expresses the activity as in Equation 1.1 or the biological activity as manifested in physiological reactions in terms of the physical properties. The physical properties used and the weight given to them are adjusted until the best fit to all the compounds is obtained.(Moore,2002)

1-1-4 Docking

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behavior plays an important role in rational design of drugs as well as to elucidate fundamental

biochemical processes.(kitchen,*etal*,2004)

1-2Acetylcholine

Acetylcholine **(**ACh**)** is an organic chemical that functions in the brain and body of many types of animals, including humans, as neurotransmitter a chemical message released by nerve cells to send signals to other cells [neurons, muscle cells , and gland cells].(Tiwari,etal,2013)

Its name is derived from its chemical structure: it is an ester of acetic acid and choline. Parts in the body that use or are affected by acetylcholine are referred to as cholinergic Substances that interfere with acetylcholine activity are called anti cholinergics. Acetylcholine is the neurotransmitter used at the neuromuscular junction—in other words, it is the chemical that motor neurons of the nervous system release in order to activate muscles. This property means that drugs that affect cholinergic systems can have very dangerous effects ranging from paralysis to convulsions. Acetylcholine is also used as a neurotransmitter in the autonomic nervous system, both as an internal transmitter for the sympathetic nervous system and as the final product released by the parasympathetic nervous system.(Tiwari,*etal*,2013)

1-2-1 Chemistry

Acetylcholine is a choline molecule that has been acetylated at the oxygen atom. Because of the presence of a highly polar, charged ammonium group, acetylcholine does not penetrate lipid membranes.

Because of this, when the drug is introduced externally, it remains in the extracellular space and does not pass through the blood–brain barrier synonym of this drug is miochol.(Smythies, 2009).

1-2-2 Biochemistry

Acetylcholine is synthesized in certain neurons by the enzyme choline acetyl transfers from the compounds choline and acetyl-CoA. Cholinergic neurons are capable of producing ACh. An example of a central cholinergic area is the nucleus basal is of Meynertin the basal for brainThe enzyme acetyl cholinesterase converts acetylcholine into the inactive metabolites choline and acetate. This enzyme is abundant in the synaptic cleft, and its role in rapidly clearing free acetylcholine from the synapse is essential for proper muscle function. Certain neurotoxins work by inhibiting acetyl cholinesterase, thus leading to excess acetylcholine at the neuromuscular junction, causing paralysis of the muscles needed for breathing and stopping the beating of the heart.(Smythies, 2009)

1-2-3Acetylcholinesterase

Acetyl cholinesterase, encoded by HGNC gene ACHE; EC 3.1.1.7) is the primary cholinesterase in the body. It is an enzyme that catalyzes the breakdown of acetylcholine and of some other choline esters that function as neuro pterans mitters .AChE is found at mainly neuromuscular junctions and in chemical synapses of the cholinergic type, where its activity serves to terminate synaptic transmission. It belongs to carboxy lesterase family of enzymes. It is the primary target of inhibition by organo phosphorus compounds such as nerve agents and pesticides.(Triggiani,2011)

1-2-3-1 Enzyme structure and Mechanism

AChE is a hydrolyses that hydrolyzes cholinesterase. It has a very high catalytic activity each molecule of AChE degrades about 25000molecules of acetylcholine (ACh) per second ,approaching the limit

allowed by diffusion of the substrate .The active site of AChE comprise s sub sites the anionic site and the esteratic sub site. The structure and mechanism of action of AChE have been elucidated from the crystal structure of the enzyme.(Sussman, ,*etal*.1991)

The anionic sub site accommodates the positive quaternary amine of acetylcholine as well as other cationic substrates and inhibitors. The cationic substrates are not bound by a negatively charged amino acid in the anionic site, but by interaction of 14 aromatic residues that line the gorge leading to the active site. All 14 amino acids in the aromatic gorge are highly conserved across different species. Among the aromatic amino acids, tryptophan 84 is critical and its substitution with alanine results in a 3000-fold decrease in reactivity. The gorge penetrates halfway through the enzyme and is approximately 20 angstroms long. The active site is located 4 angstroms from the bottom of the molecule.(Harel, *etal*,1993)

1-2-3-2 Acetyl cholinesterase inhibitor T

The major form of acetyl cholinesterase found in brain, muscle, and other tissues, known as is the hydrophilic species, which forms disulfide-linked oligomers with collage nous, or lipid-containing structural subunits. In the neuromuscular junctions AChE expressesin asymmetric form which associates with ColQ or subunit. In the central nervous system it is associated with PRiMA which stands forProline Rich Membrane anchor to form symmetric form. In either case, the ColQ or PRiMA anchor serves to maintain the enzyme in the intercellular junction,ColQ for the neuromuscular junction and PRiMA for synapses.(Colovic,*etal*, 2013)

1-2-3-3 Acetyl cholinesterase inhibitor H

The other, alternatively spliced form expressed primarily in the erythroid tissues, differs at the C-terminus, and contains a cleavable hydrophobic peptide with a PI-anchor site. It associates with membranes through the

phosphor in ositide (PI) moieties added post translationally .(Dori,*etal*, 2007)

1-2-3-4 Acetyl cholinesterase inhibitor R

The third type has, so far, only been found in *Torpedo* sp. and mice although it is hypothesized in other species. It is thought to beinvolved in the stress response and, possibly, inflammation.(Dori,*etal*, 2007)

1-2-4 Acetylcholinesterase inhibitor:

An acetyl cholinesterase inhibitor (often abbreviated AChEI) or anti cholinesterase is a chemical or a drug that inhibits the acetyl cholinesterase enzyme from breaking down acetylcholine, thereby increasing both the level and duration of action of the neurotransmitter acetylcholine. Acetyl cholinesterase inhibitors are classified as reversible, irreversible, or quasi-irreversible (also called pseudo irreversible**).**

1-3Carbamate

A carbamate is an organic compound derived from carbamic acid (NH_2COOH). Acarbamate group, carbamate ester (e.g., ethyl carbamate), and carbamic acids are functional groups that are inter-related structurally and often are inter converted chemically. Carbamate esters are also called urethanes.

1-3-1 Synthesis

Carbamic acids are unstable, but the salts of these elusive acids are well known. For example, ammonium carbamate is generated by treatment of ammonia with carbon dioxide.

$$2\ NH_3 + CO_2 \rightarrow NH_4[H_2NCO_2]$$

Carbamates also arise via hydrolysis of chloroform amides and subsequent esterification:

$$R_2NC(O)Cl + H_2O \rightarrow R_2NCO_2H + HCl$$

Carbamates may be formed from the Curtis rearrangement, where isocyanates formed are reacted with an alcohol.

RNCO + R′OH → RNHCO2R′

The synthesis of carbamates was carried out following two different strategies (due to the low yields):

N,N–d ibenzylation of the amino alcohol followed by dimethyl carbamoylation (Route A)or dimethyl carbamoylation of the amino alcohol and subsequent N,N –dibenzylation (Route B). The higher yields in carbamates were obtained following Procedure B.

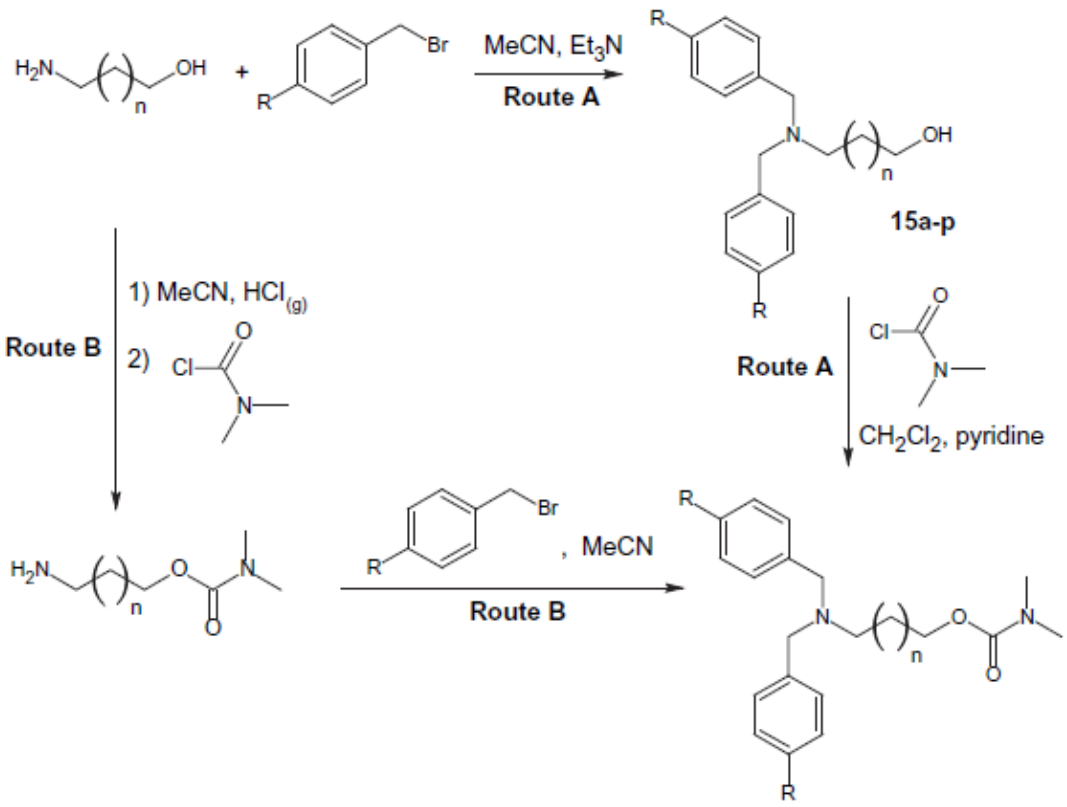


Figure (1.1) : route of carbamate synthesis

1-3-2 Carbamate insecticides

The so-called carbamate insecticides feature the carbamate ester functional group. Included in this group are aldicarb (Temik), carbofuran (Furadan), carbaryl (Sevin), ethienocarb, fenobucarb, oxamyl, and methomyl. These insecticides kill insects by reversibly inactivating the enzyme acetyl cholinesterase.The organophosphate pesticides also inhibit

this enzyme, although irreversibly, and cause a more severe form of cholinergic poisoning.Fenoxycarb has a carbamate group but acts as a juvenile hormonemimic, rather than inactivating acetyl cholinesterase.(Metcalf, 2000)

1-3-3 Carbamate Nerve Agents

While the carbamate acetylcholinesterase inhibitors are commonly referred to as "carbamate insecticides" due to their generally high selectivity for insect acetyl cholinesterase enzymes over the mammalian versions, the most potent compounds such as aldi carb andcarbofuran are still capable of inhibiting mammalian acetyl cholinesterase enzymes at low enough concentrations that they pose significant risk of poisoning to humans, especially when used in large amounts for agricultural applications. Other carbamate based acetyl cholinesterase inhibitors are known with even higher toxicity to humans, and some such as T-1123 and EA-3990 were investigated for potential military use as nerve agents.(Gupta., 2015)

1-3-4 Methyl Carbamate

Methyl carbamate(also called methylurethane, or urethylane) is an organic compound and the simplest ester of carbamic acid (H_2NCO_2H). It is a colour less solid.Methylcarbamate is prepared by the reaction of methanol and urea:

$$CO(NH_2)_2 + CH_3OH \rightarrow CH_3OC(O)NH_2 + NH_3$$

It also forms in the reaction of ammonia with methyl chloroformate or dimethyl .

Methylcarbamate is used primarily in the textile and polymer industries as a reactive intermediate. In the textile industry, it is used in the manufacture of dimethyl ethylcarbamate-based resins that are applied on polyester cotton blend fabrics as durable-press finishes. The treated fabrics have good crease-angle retention, resist acid souring in

commercial laundries, do not retain chlorine, and have flame retardant properties. Methyl carbamate also is used in the manufacture of pharmaceuticals, insecticides, and urethane.(Maronpot,*etal*,1986)

N-Methyl carbamates are widely used as insecticides .They have anticholinesterase activity without a cumulative effect.

1-3-5N-Alkyl and N,N-dialkyl carbamates

It are widely used as insecticides' and as medicinal for the relief of myasthenia gravis and other disorders. In both cases, the ultimate mode of action appears to be the inhibition of cholinesterase. It has been shown that cholinesterase is carbamylated by carbamyl choline, N-methyl- and N,N-dimethyl carbamyl cholines and by N:N-dimethyl carbamoyl fluoride,4-6 butnot by all inhibitory carbamates . The relationship between structure and stability, activity as inhibitors of acety1 and butyryl cholinesterase's, and toxicity to house flies and mice have been studied in a series of thirty N-alkyl and N,N dialkyl Carbamates.(Hodgson and Casida, 1961)

1-4 Alzheimer's disease

Alzheimer's disease (AD) is a disorder that attacks the central nervous system through progressive degeneration of its neurons. Patients with this disease develop dementia which becomes more severe as the disease progresses. It was suggested that symptoms of AD are caused by decrease of activity of cholinergic neocortical and hippocampal neurons. Treatment of AD by ACh precursors and cholinergic agonists was ineffective or caused severe side effects. ACh hydrolysis by AChE causes termination of cholinergic neurotransmission. Therefore, compounds which inhibit AChE might significantly increase the levels of ACh depleted in AD. Indeed, it was shown that AChE inhibitors improve the cognitive abilities of AD patients at early stages of the disease development.

German psychiatrist, Dr. Alois termed this disorder as ‘preseniledementia’ first in 1906. In 1901, he observed a lady patient with a progressive loss of cognitive functions (memory loss, language problems and unpredictable behavior). After her death in 1906, Dr. Alzheimer sampled and examined her brain tissues and observed two types of abnormal deposits located inside and between the nerve cells, currently termed as amyloid plaques and neuro fibrillary tangles, respectively. Later, Dr. Emil Kraepelin, colleague of Dr. Alzheimer recommended thename ‘Alzheimer’s disease’ in place of ‘presenile dementia’. More than 100 years after description of AD, the two major pathological hallmarks still remain as the main explanation of the pathogenesis of AD. Other obvious hallmarks include brainatrophy, dementia and inflammation(.Maurer and Gerbaldo, 1997)

1-5 Literature review:

A series of carbamate derivatives were synthesized and their carbonic anhydrase I and IIisoenzymes and acetylcholinesterase enzyme (AChE) inhibitory effects were investigated. All carbamates were synthesized from the corresponding carboxylic acids via the Curtius reactions of the acids with diphenylphosphorylazide followed by addition of benzyl alcohol. The carbamates were determined to be very good inhibitors against for AChEandhCA I, and II isoenzymes.AChE inhibition was determined in the range 0.209–0.291 nM. On the other hand, tacrine, which is used in the treatment of Alzheimer’s disease possessed lower inhibition effect (Ki: 0.398 nM). Also, hCA I and II isoenzymes were effectively inhibited by the carbamates, with inhibition constants (Ki) in the range of 4.49–5.61nM for hCA I, and 4.94–7.66 nM for hCA II,respectively. Acetazolamide, which was clinically used carbonic anhydrase (CA) inhibitordemonstrated Ki values of 281.33 nMforhCAI

and 9.07nM for hCA II. The results clearly showed that AChE and both CA isoenzymes were effectively inhibited by carbamates at the low Nanomolarlevels.(Su¨leymanetal;2015).

A Quantitative Structure Activity Relationship (QSAR) study has been an attempted on a series of 88 *N*-aryl derivatives which display varied inhibitory activity towards both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), targets in Alzheimer's drug discovery. QSAR models were derived for 53 and 61 compounds for each target, respectively, with the aid of genetic function approximation (GFA) technique using topological, molecular shape, electronic and structural descriptors. The predictive ability of the QSAR model was evaluated using a test set of 26 compounds for AChE ($r^2pred = 0.857$), ($q^2 = 0.803$) and 20 compounds for BChE ($r^2pred = 0.882$), ($q^2 = 0.857$). The QSAR models point out that AlogP98, Wiener, Kappa-1-AM, Dipole-Mag, and CHI-1 are the important descriptors effectively describing the bioactivity of the compounds.(Solomon, *etal*., 2009)

In view of the nonavailability of complete X-ray structure of carbamates co crystallized with AChE enzyme, the 3D-QSAR model development based on co-crystallized conformer (CCBA) as well as docked conformer-based alignment (DCBA) is not feasible. Therefore, the only two alternatives *viz.* pharmacophore and maximum common substructure-based alignments are left for the 3D-QSAR comparative molecular field analyses (CoMFA) and comparative molecular similarity indices analyses (CoMSIA) model development. the 3D-QSAR models have been developed using both alignment methods, where CoMFA and CoMSIA models based on pharmacophore-based alignment were in good agreement with each others and demonstrated significant superiority over MCS-based alignment in terms of leave-one-out (LOO) cross-validated q^2 values of 0.573 and 0.723 and the r^2 values of 0.972 and 0.950,

respectively. The validation of the best CoMFA and CoMSIA models based on pharmacophore (Hip-Hop)-based alignment on a test set of 17 compounds provided significant predictive r^2 [r^2pred(test)] of 0.614 and 0.788 respectively. The contour map analyses revealed the relative importance of steric, electrostatic and hydrophobicity for AChEinhibition activity. However, hydrophobic factor plays major contribution to the AChE inhibitory activity modulation which is in strong agreement with the fact that the AChE is having a wide active site gorge (~20Å) occupied by a large number of hydrophobic amino acid residues.**(**Chaudhaery, etal, 2009**)**

In order to identify the essential structural features and physicochemical properties for acetylcholinesterase (AChE) inhibitory activity in some carbamate derivatives, the systematic QSAR (Quantitative Structure Activity Relationship) studies (CoMFA, advance CoMFA and CoMSIA) have been carried out on a series of (total 78 molecules) taking 52 and 26 molecules in training and test set, respectively. Statistically significant 3D-QSAR (three-dimensional Quantitative Structure Activity Relationship) models were developed on training setmolecules using CoMFA and CoMSIA and validated against test set compounds. The highly predictive models (CoMFAq^2 = 0.733, r^2 = 0.967, predictive r^2 = 0.732, CoMSIA q^2 = 0.641, r^2 = 0.936, predictive r^2 = 0.812) well explained the variance in binding affinities both for the training and the test set compounds. The generated models suggest that steric, electrostatic and hydrophobic interactions play an important role in describing the variation in binding affinity. In particular the carbamoyl nitrogen should be more electropositive; substitutions on this nitrogen should have high steric bulk and hydrophobicity while the amino nitrogen should be electronegative in order to have better activity. These studies may provide important insights into structural variations leading tothe development of

novel AChEinhibitorswhich may be useful in the development of novelmolecules for the treatment of Alzheimer's disease.(Roy, *etal*., 2008)

1-6 Research objective:

1-6-1 main objective:

The main objective of the conducted study is to use quantitative structure-activity relationships(QSAR) to calculate biological activity of N,N-dimethyl carbamate derivatives as acetyl cholinesterase inhibitor

1-6-2 specific objectives:

1- To make quantitative structure-activity relationships (QSARs)

2- To predict biological activity of synthesized compound.

Chapter two

Materials and methods

2- Materials and Methods:

In this work set of data used to study quantitative structure activity relationship of N,N-dimethyl carbamate derivatives were obtained from previous published article containing biological acivity of carbamat derivatives having similar skeleton the Twelve carbamate derivatives of data set divided into training set and test set .a training set composed Ten compound and obtained by partial least squares (PLS)

2-1 ACD lab program

ACD/ChemSketch Freeware is a drawing package that allows you to draw chemical structures including organics, organometallics, polymers, and Markush structures. It also includes features such as calculation of molecular properties (e.g., molecular weight, density, molar refractivity etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log*P*. The freeware version of ChemSketch does not include all of the functionality of the commercial version

2.1.1 General method of ACD/lab program

The main program page consists of the toolbar and taskbar and there were two options:1-ChemSketch and 3-3D-view .Option 1-ChemSketch have two modes, name by Structure and Draw. Structure mode was used to draw chemical molecules, while Draw mode was used to create and edit graphical objects. Upon startup, the Draw Normal mode and Carbon were automatically selected, by clicking and dragging the cursor, C-C bonds were created. The change was made by selecting a heteroatom from the element list in the left toolbar and clicking on an atom in the structure to replace it. Bond lengths and bond angle standardized by clicking on Clean Structure from option Tools in the taskbar. The properties of the drown structures were calculated by using appropriate option in the toolbar and then copied into ChemSketch window as a text field. By

selecting a structure and clicking on generate Name for structure in tools the IUPAC name was generated as a text field.This procedure was used to draw all derivatives structure and equations in this project.

2-2 Minitab

is a statistics package developed at the Pennsylvania State University by researchers Barbara F. Ryan, Thomas A. Ryan, Jr., and Brian L. Joiner in 1972. It began as a light version of OMNITAB, a statistical analysis program by NIST. Statistical analysis software such as Minitab automates calculations and the creation of graphs, allowing the user to focus more on the analysis of data and the interpretation of results. It is compatible with other Minitab, Inc. software.

2.2.1. General method of Minitab program

Minitab program was used to perform a multiple linear regression analysis to get the equation which we can use to predict the biological activity of our derivatives structure, by select from toolbar: Stat ► Regression ► Regression ►Fit Regression Model. The response and the predictor(s) variable were specified, and selection were conformed. Next, back up to the Main Menu which regression has run.

2-3 Molecular Operating Environment(MOE)

is a drug discovery software platform that integrates visualization, modeling and simulations, as well as methodology development, in one package. MOE scientific applications are used by biologists, medicinal chemists and computational chemists in pharmaceutical, biotechnology and academic research. MOE runs on Windows, Linux, Unix, and MAC OS X. Main application areas in MOE include structure-based design, fragment-based design, pharmacophore discovery, medicinal chemistry applications, biologics applications, protein and antibody modeling, molecular modeling and simulations, chemoinformatics and QSAR. The

Scientific Vector Language (SVL) is the built-in command, scripting and application development language of MOE.

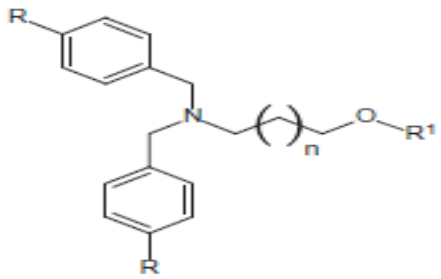
2-3 QSAR

The mathematical formula which related biological activity with structure (physicochemical parameter) was obtained by using Minitab to performing multiple linear regressions by choosing randomly three descriptors as independent variable and plotted against biological activity as dependent variable a number of equations were obtained the best of them were:

- PIC50= 5.89187-0.06775 LogP-0.01059 PEOE-VSA-1

  R2 = 0.86 RMSE = 0.12

Table (2,1): N,N-dimethyl carbamate direvativies 1-10



Com	R	R1	n	IC50	PIC50	LogP	PEOE-VSA-1	Dipole x	SMR-VSA2
1	H	CON(CH3)2	1	57	4.244	3.921	122.5490	0.0916	0.000
2	H	CON(CH3)2	2	41	4.387	4.3630	122.5490	0.1857	0.000
3	H	CON(CH3)2	3	60	4.221	4.8050	122.5490	0.0449	0.000
4	H	CON(CH3)2	4	85	4.070	5.2470	139.9517	0.1692	0.000
5	CH3	CON(CH3)2	3	26	4.585	5.4010	102.4563	0.0250	0.000
6	CH3	CON(CH3)2	4	69	4.161	5.8430	119.8589	0.1857	0.000
7	F	CON(CH3)2	2	13	4.886	4.6690	49.0196	0.5330	18.2420
8	F	CON(CH3-)2	4	12	4.921	5.5530	66.4222	0.4584	18.2420
9	CF3	CON(CH3)2	2	7	5.155	6.2325	49.0196	0.7867	15.5190
10	CF3	CON(CH3)2	4	31	4.5086	7.1165	66.4222	0.6747	15.5190

2.3.1 QSAR equations:

By using MOE procedure and by choosing randomly descriptors as independent variable and plotted against biological activity as dependent variable a number of QSAR equations were obtained the best of them were:

- PIC50= 5.89187-0.06775 LogP-0.01059 PEOE-VSA-1
  R2 = 0.86 RMSE = 0.12
- PIC50 = 4.7165-0.1121 LogP +1.246 Dipole x

R2 = 0.61 RMSE = 0.21

- PIC50 = 4.50259-0.04501 LogP +0.03709 SMR-VSA2
  R2= 0.69 RMSE = 0.19

Table no (2.2) descriptors used to perform multiple linear regression to generate QSAR equation :

- PIC50= 5.89187-0.06775 LogP-0.01059 PEOE-VSA-1
  R2 = 0.86 RMSE = 0.12

No	**LogP**	**PEOE-VSA-1**	**PIC50 pre**	**PIC50**
1	3.921	122.5490	4.3278	4.244
2	4.3630	122.5490	4.2979	4.387
3	4.8050	122.5490	4.2679	4.221
4	5.2470	139.9517	4.0536	4.070
5	5.4010	102.4563	4.4404	4.585
6	5.8430	119.8589	4.2261	4.161
7	4.6690	49.0196	5.0562	4.886
8	5.5530	66.4222	4.8119	4.921
9	6.2325	49.0196	4.9502	5.155
10	7.1165	66.4222	4.7060	4.5086

Table no (2.3) descriptors used to erform multiple linear regression to generate QSAR equation :

- PIC50 = 4.7165-0.1121 LogP +1.246 Dipole x

  R2 = 0.61 RMSE = 0.21

NO	LogP	Dipole x	PIC50 pre	PIC50
1	3.921	0.0916	4.3911	4.244
2	4.3630	0.1857	4.4589	4.387
3	4.8050	0.0449	4.2338	4.221
4	5.2470	0.1692	4.3391	4.070
5	5.4010	0.0250	4.1422	4.585
6	5.8430	0.1857	4.2929	4.161
7	4.6690	0.5330	4.8573	4.886
8	5.5530	0.4584	4.6652	4.921
9	6.2325	0.7867	4.9981	5.155
10	7.1165	0.6747	4.7595	4.5086

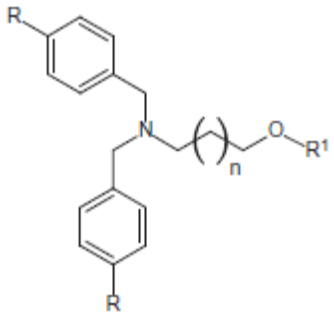
Table no (2.4) descriptors used to perform multiple linear regression to generate QSAR equation :

- PIC50 = 4.50259-0.04501 LogP +0.03709 SMR-VSA2

  R2= 0.69 RMSE = 0.19

NO	**LogP**	**SMR-VSA2**	**PIC50 per**	**PIC50**
1	3.921	0.000	4.3261	4.244
2	4.3630	0.000	4.3062	4.387
3	4.8050	0.000	4.2863	4.221
4	5.2470	0.000	4.2664	4.070
5	5.4010	0.000	4.2595	4.585
6	5.8430	0.000	4.2396	4.161
7	4.6690	18.2420	4.9691	4.886
8	5.5530	18.2420	4.9293	4.921
9	6.2325	15.5190	4.7977	5.155
10	7.1165	15.5190	4.7579	4.5086

- Table no (2.5) ACD/Lab results of some N,N-dimethyl carbamatedrevatives and their calculated activity from QSAR equation:



- PIC50= 5.89187-0.06775 LogP-0.01059 PEOE-VSA-1

  R2 = 0.86 RMSE = 0.12

No	R	R1	N	Log p	PEOE-VSA-1	PIC50
1	Cl	CON(CH3)2	1	5.5470	108.183	4.3699
2	Cl	CON(CH3)2	2	5.547	108.183	4.3699
3	Cl	CON(CH3)2	3	5.989	108.1835	4.3399
4	Cl	CON(CH3)2	4	6.431	125.5861	4.1256
5	NO2	CON(CH3)2	1	3.791	49.0196	5.1157
6	NO2	CON(CH3)2	2	4.675	49.0196	5.0857
7	NO2	CON(CH3)2	3	4.675	49.0196	5.0558
8	Br	CON(CH3)2	1	5.117	66.4222	4.8414
9	Br	CON(CH3)2	2	5.517	136.2693	4.0743
10	Br	CON(CH3)2	3	5.959	136.2693	4.0444
11	Br	CON(CH3)2	4	6.401	136.2693	4.0144
12	NH2	CON(CH3)2	1	6.843	153.6719	3.8001
13	NH2	CON(CH3)2	2	2.577	49.0196	5.1979
14	NH2	CON(CH3)2	3	3.019	49.0196	5.1680
15	NH2	CON(CH3)2	4	3.461	49.0196	5.1380
16	CH2CH3	CON(CH3)2	1	3.903	66.4222	4.9237
17	CH2CH3	CON(CH3)2	2	5.467	135.5346	4.0855
18	CH2CH3	CON(CH3)2	3	5.909	135.5346	4.0555

19	CH2CH3	CON(CH3)2	4	6.351	135.5346	4.0256
20	OH	CON(CH3)2	1	6.793	152.9372	3.8113
21	OH	CON(CH3)2	2	3.305	49.0196	5.1486
22	OH	CON(CH3)2	3	3.747	49.0196	5.1186
23	OH	CON(CH3)2	4	4.189	49.0196	5.0887
24	OH	CON(CH3)2	4	4.631	66.4222	4.8744
25	OCH3	CON(CH3)2	1	3.833	49.0196	5.1128
26	OCH3	CON(CH3)2	2	4.275	49.0196	5.0829
27	OCH3	CON(CH3)2	3	4.717	49.0196	5.0529
28	OCH3	CON(CH3)2	4	5.159	66.4222	4.8386
29	NH2CH3	CON(CH3)2	1	3.215	49.0196	5.1547
30	NH2CH3	CON(CH3)2	2	3.657	49.0196	5.1247
31	NH2CH3	CON(CH3)2	3	4.099	49.0196	5.0948
32	NH2CH3	CON(CH3)2	4	4.541	66.4222	4.8805
33	NH(CH3)2	CON(CH3)2	1	3.751	49.0196	5.1184
34	NH(CH3)2	CON(CH3)2	2	4.193	49.0196	5.0884
35	NH(CH3)2	CON(CH3)2	3	4.635	49.0196	5.0585
36	NH(CH3)2	CON(CH3)2	4	5.077	66.4222	4.8442
37	I	CON(CH3)2	1	6.301	49.0196	4.9456
38	I	CON(CH3)2	2	6.743	49.0196	4.9157
39	I	CON(CH3)2	3	7.185	49.0196	4.8857
40	I	CON(CH3)2	4	7.627	66.4222	4.6714

2.4 Molecular Operating Environment(MOE) program:

MOE program was used to detect protein – ligand binding sites and effective docking for the highest activity which calculated from QSAR equation.

2.4.1. Preparation of protein:

MOE was used to prepare the protein that was selected (code: 1EVE, resolution of 2.50A °) , first test the active site by select from toolbar : compute► simulation ►dock► site►?. Then similar chain and water molecule were removed by select from right option: SEQ► detected it ►delete selected chain . Then protein was isolated and become ready for docking.

2.4.2. Preparation of ligand(compound):

MOE was used to prepare the designed derivatives by select from toolbar: file ►open to get compound, then select: compute ►conformation ►conformational search ►Edit; to minimize energy of compound to become ready for docking.

2.4.3. Docking:

MOE was used for docking process by opening protein which prepared firstly then select from the toolbar : compute ► simulation ► Dock ;window which opened were select : ligand ► MDP file ► Browse ; to get compound, and refinement ► forcefield ► Run .The result of running were export and saved as JPG.

Chapter three

Results and discussion

3. Result

From the MOE the QSAR equation were obtained and made the docking for designed compound and correlation between predicted PIC50 and cross validation

3.1 docking result

Docking is used to predict the binding orientation for the (3) compounds which have high biological activity:

Figure (3.1):Inter action between compound 5 and receptor (1EVE):

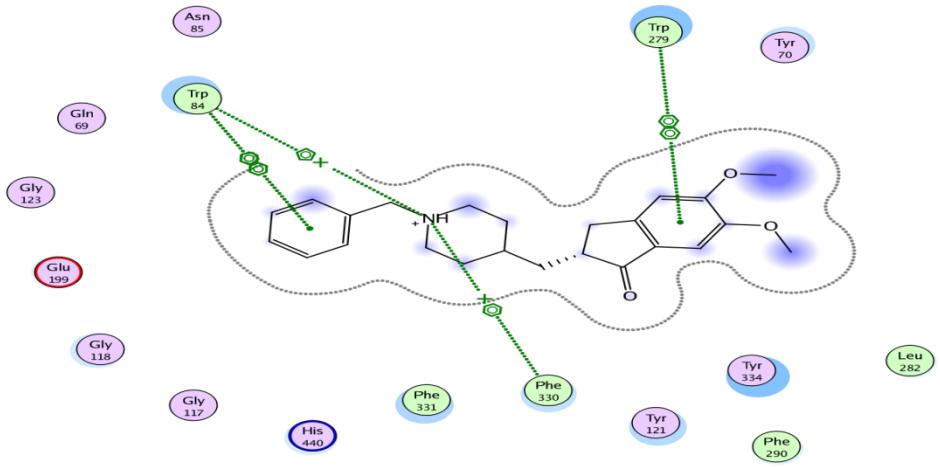


Figure (3.2):The inter action between compound 8 and receptor (1EVE):

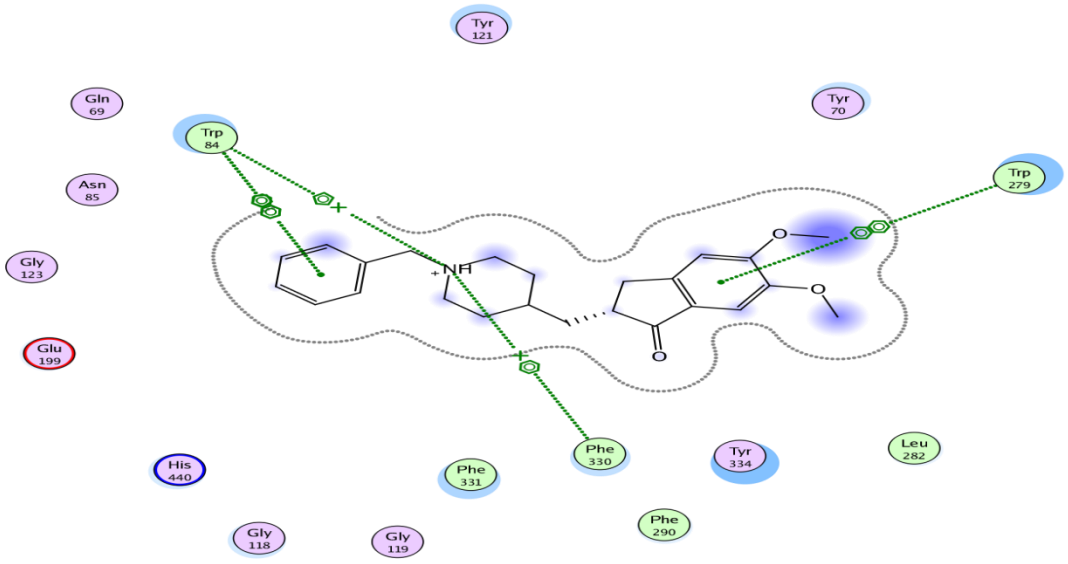


Figure (3.3):The inter action between compound 1 and receptor (1EVE):

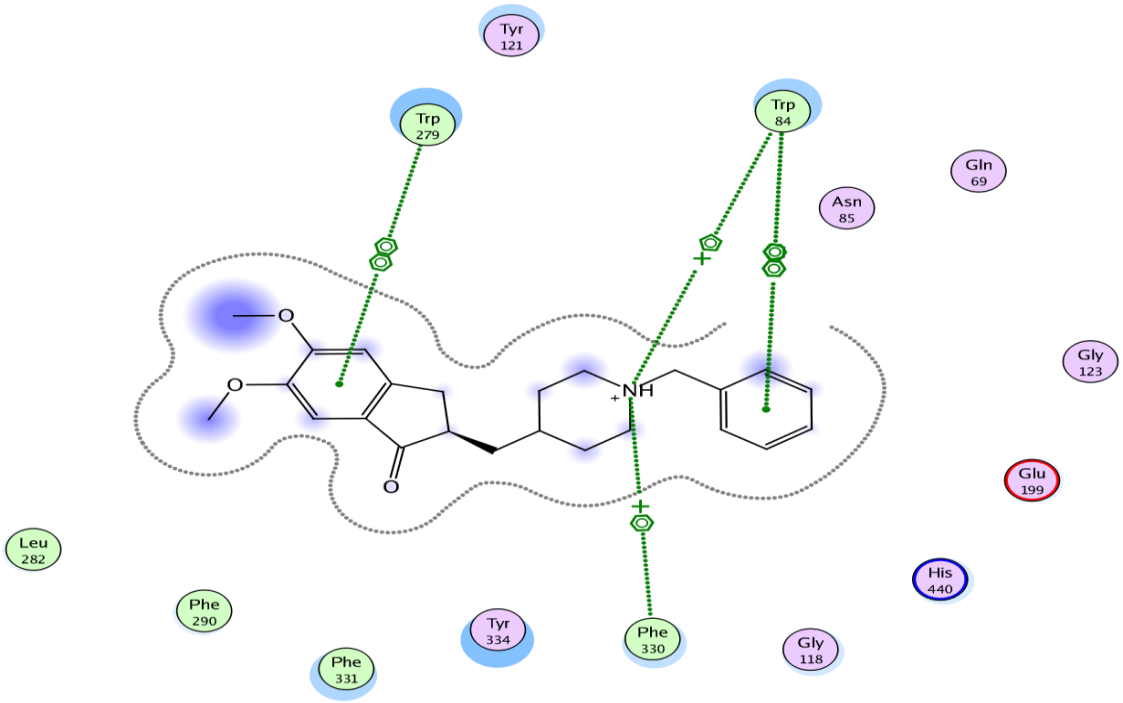


Table (3.1)The inter action data and energy scores of investigated ligand with receptor (1EVE) :

Compound	Free binding energy ,s	Tybe of bond interacted	Amino acid interacted
Donepezil	-33.8859	Four π-π interaction	Phe330, Tyr334, His 440
compound 1	-28.3630	Ten π-π interaction	Phe330, Trp 279, Trp 84
compound 5	-29.4420	Twelve π-π interaction	Phe330, Trp 279, Trp 84
compound 8	-29.5630	Ten π-π interaction	Phe330, Trp 279, Trp 84

Docking is used to predict the binding orientation for the (6) compounds which have high predicted biological activity to their protein targets (1EVE) and the results were illustrate in figures bellow.

Figure (3.4) : the interaction between compound (10) and receptor (1EVE) :

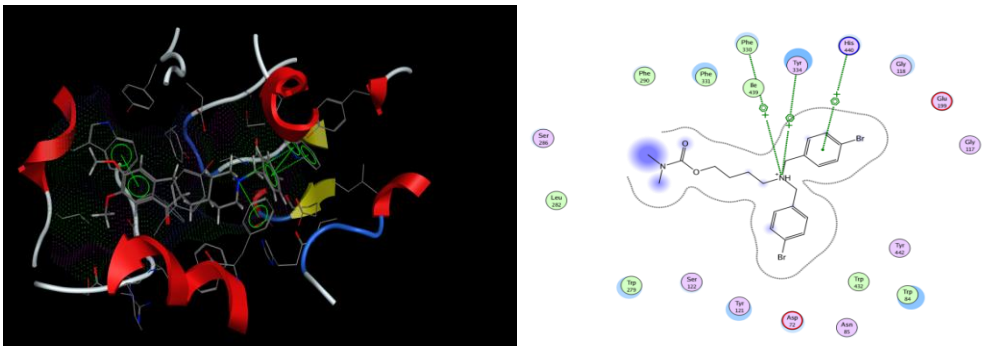


Figure (3.5) : the interaction between compound (11) and receptor (1EVE) :

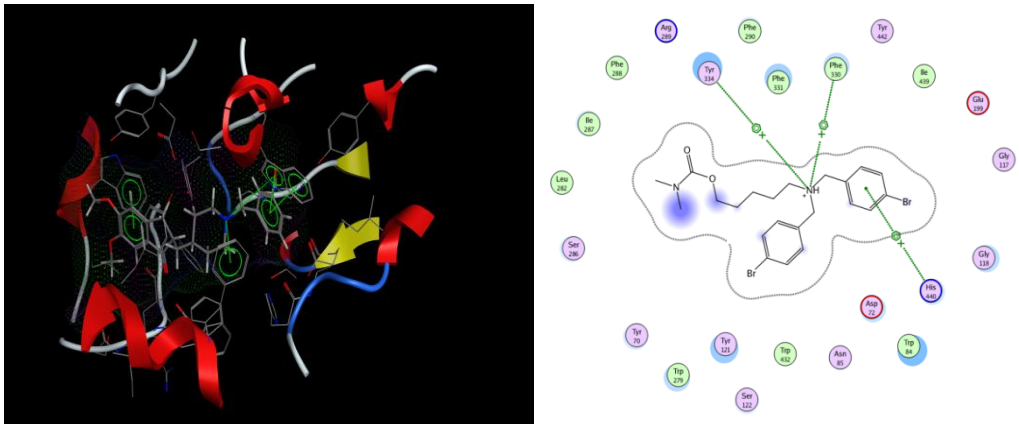


Figure (3.6) : the interaction between compound (12) and receptor (1EVE) :

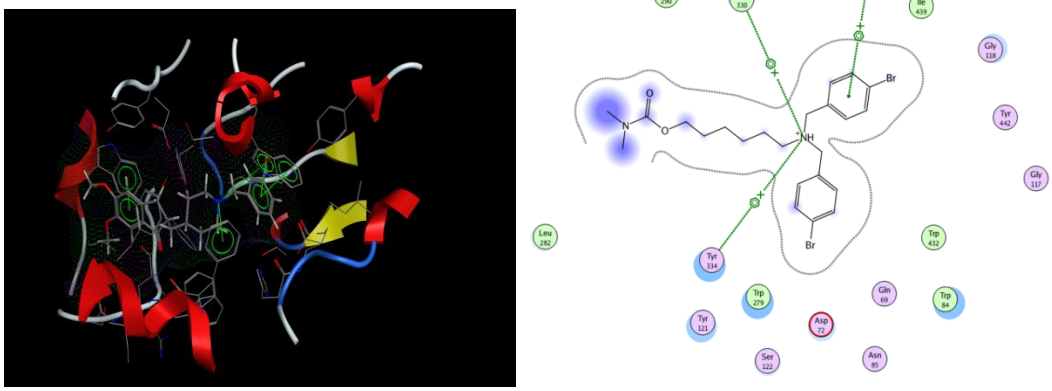


Figure (3.7) : the interaction between compound (18) and receptor (1EVE) :

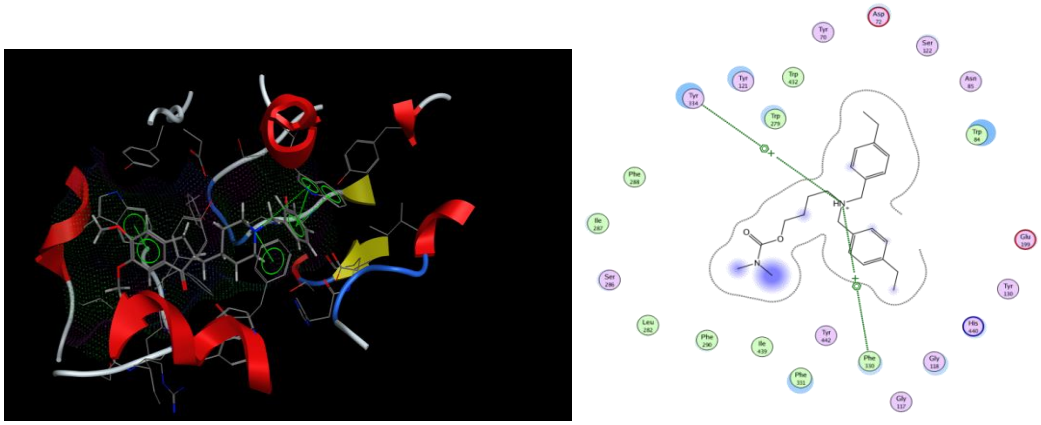


Figure (3.8) : the interaction between compound (19) and receptor (1EVE)

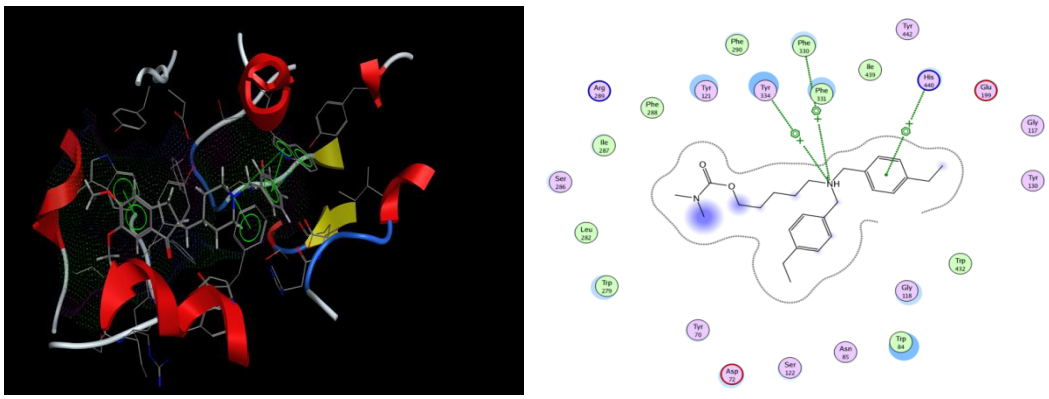


Figure (3.9) : the interaction between compound (20) and receptor (1EVE) :

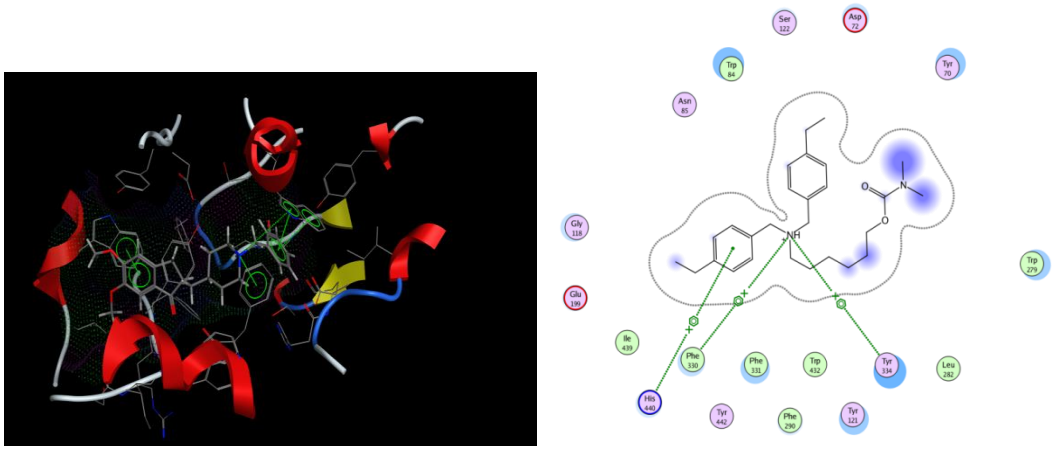
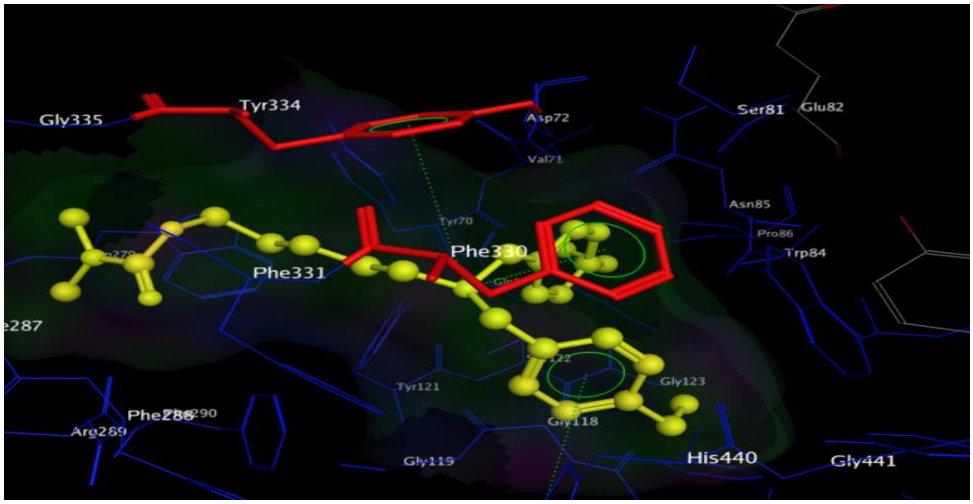
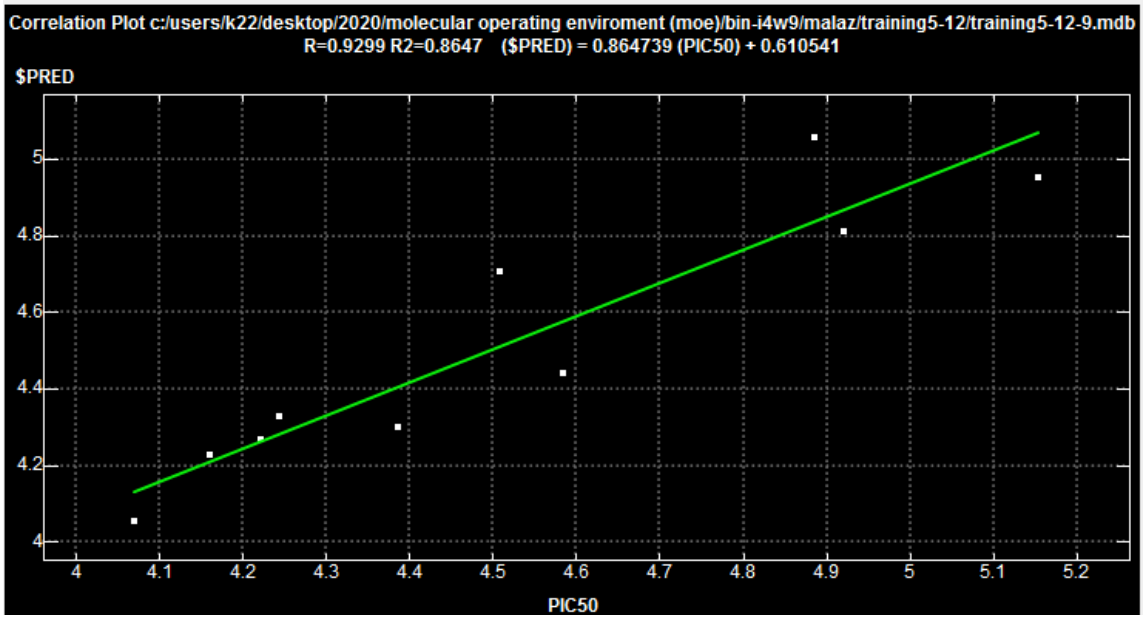


Table (3.2)The inter action data and energy scores of investigated ligand with receptor (1EVE) :

Compound	Free binding energy ,s	Tybe of bond interacted	Amino acid interacted
Donepezil	-33.8859	Four $\pi -$ □ interaction	Phe330, Tyr334, His 440
compound 10	-28.8886	three $\pi -$ □ interaction	Phe330, Tyr334, His 440
compound 11	-30,3522	three $\pi -$ □ interaction	Phe330, Tyr334, His 440
compound 12	-30.1585	two $\pi -$ □ interaction	Phe330, Tyr334.
compound18	-29.9563	three $\pi -$ □ interaction	Phe330, Tyr334, His 440
Compound 19	-35.0481	three $\pi -$ □ interaction	Phe330, Tyr334, His 440
Compound 20	-34.9494	three $\pi -$ □ interaction	Phe330, Tyr334, His 440



Figure(3.10) : correlation plot between PIC50 and predicted PIC50



Figure(3.11): correlation plot between PIC50 and XPRED

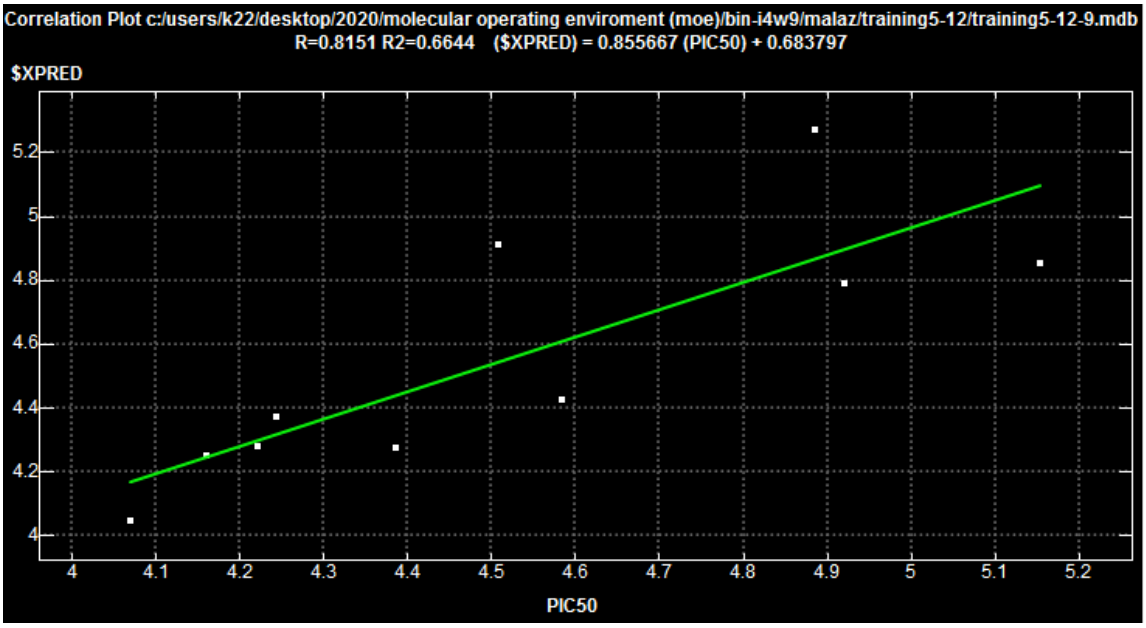
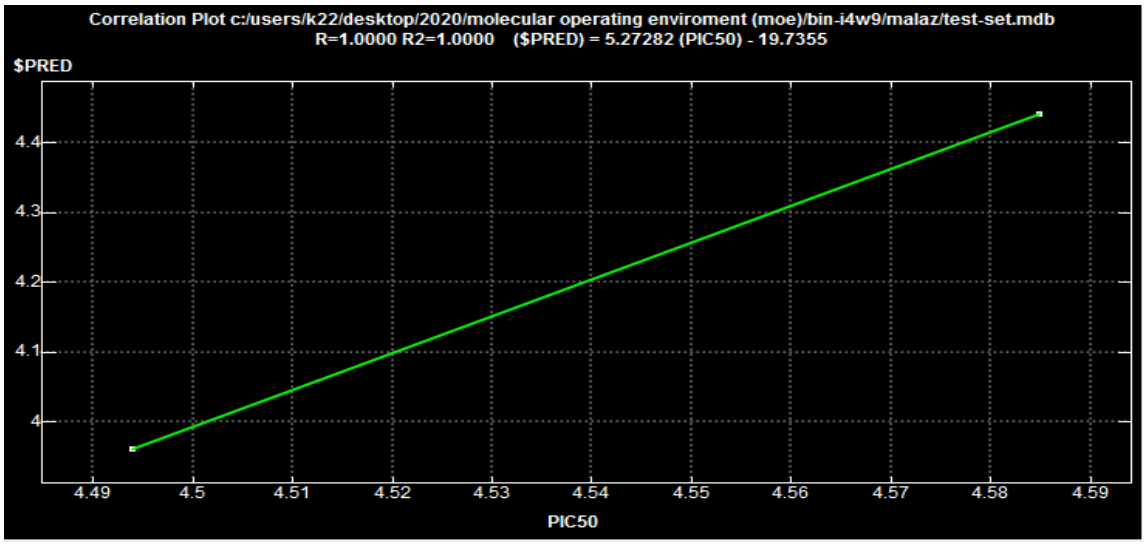


Figure (3.12) :coreelation plot between predicted PIC50and cross validation



3.3 Discussion:

The reported data clearly indicate that two main features influence the enzyme inhibition:

(i) the nature of the R substituent at the aromatic ring, which plays a key role in the inhibitory potency.

(ii) the length of the alkyl spacer, which also significantly affects the activity.

the most active compounds are 19 (R=$CHCH_3$; n=3) and 20 (R= $CH-CH_3$; n=4). These compounds are characterized by substituted aromatic rings or with a Para ethylgroup; their electron density is suitable to allow interactions with the aromatic residues in the active enzymatic site. When the aromatic ring is substituted by electron withdrawing groups

3.3.1Regression analysis:

Regression analysis is a powerful statistical method that allows to examine the relationship between two or more variable of interest. While there are many types of regression analysis

In this study a regression was performed by using MOE procedure and by choosing randomly descriptors as independent variable and plotted against biological activity as dependent variable by using SPSS a number of QSAR equations were obtained allows to determine that factors can be ignored and how these factors influence each others, and the best equation were :

PIC50 = 5.89187-0.06775 Log P - 0.01059 PEOE-VSA-1

R^2= 0.86 , RMSE = 0.12

F = 22.38 , P = 0.001, St = 0.153

As a result from this equation anew chemical structure were design which have a good inhibitory effects on specific targets .

3.3.2 Docking :

Molecular docking is the process that involves placing molecules in appropriate configurations to interact with a receptor to form stable complex.

In this study we use molecular docking to achieve an optimized conformation for our designed compounds (ligand) and proteins: (1EVE) and the free energy of the overall system is minimized ,as illustrate in

figure (3.1-3.6) there are more binding site particularly in compound 19, and 20 than 4(dibenzyl amino-) butyl di methyl carbamate.

3.4 Conclusion:

It was found that the substitution in N-N dimethyl carbamate affect directly in biological activity , electron donating substituent in Para position increase the biological activity .

QSAR is important in chemistry and drug design It saves effort and time and reduces cost.

3.5 Recommendation:

-Carbamate have high activity as acetyl cholinesterase inhibitor and it may be used as chemical weapon and QSAR studies very help full to predict druges from carbamte derivatives

- To predict accurate equation take at least twenty compound as training set .
- The R2(correlation coefficient of QSAR model) value must be less than one

References :

- Ambure, P. and Roy, K., 2014. Advances in quantitative structure–activity relationship models of anti-Alzheimer's agents. *Expert opinion on drug discovery*, 9(6),697-723.
- An, E.F.M.C., 2010. *18th European Symposium on Quantitative Structure–Activity Relationships*.
- Chaudhaery, S.S., Roy, K.K. and Saxena, A.K., 2009. Consensus superiority of the pharmacophore-based alignment, over maximum common substructure (MCS): 3D-QSAR studies on carbamates as acetylcholinesteraseinhibitors.*Journal of chemical information and modeling*, 49(6), 1590-1601
- Colovic, M.B., Krstic, D.Z., Lazarevic-Pasti, T.D., Bondzic, A.M. and Vasic, V.M., 2013. Acetylcholinesterase inhibitors: *pharmacology and toxicology*. *Current neuropharmacology*,11(3), 315-335.
- Colovic, M.B., Krstic, D.Z., Lazarevic-Pasti, T.D., Bondzic, A.M. and Vasic, V.M., 2013. Acetylcholinesterase inhibitors: *pharmacology and toxicology*. *Current neuropharmacology*,11(3), 315-335.
- De Vita, D., Pandolfi, F., Ornano, L., Feroci, M., Chiarotto, I., Sileno, I., Pepi, F., Costi, R., Di Santo, R. and Scipione, L., 2016. New N, N-dimethylcarbamate inhibitors of acetylcholinesterase: design synthesis and biological evaluation. *Journal of enzyme inhibition and medicinal chemistry*, 31(sup4), 106-113.
- Dori, A., Ifergane, G., Saar-Levy, T., Bersudsky, M., Mor, I., Soreq, H. and Wirguin, I., 2007. Read through acetyl cholinesterase in inflammation-associated neuro pathies.*Life sciences*, 80(24-25), 2369-2374.

- Gupta, R.C. ed.,. *Handbook of toxicology of chemical warfare agents*, 2015. *Academic Press*.

-Harel, M., Schalk, I., Ehret-Sabatier, L., Bouet, F., Goeldner, M., Hirth, C., Axelsen, P.H., Silman, I. and Sussman, J.L., Quaternary ligand binding to aromatic residues in the active-site gorge of acetylcholinesterase. *Proceedings of the National Academy of Sciences*, 90(19), 9031-9035 1993.

- Heinz, H. and Ramezani-Dakhel, H., 2016. Simulations of inorganic–bioorganic interfaces to discover new materials: insights, comparisons to experiment, challenges, andopportunities. *Chemical Society Reviews*, *45*(2), pp.412-448
- Hodgson, E. and Casida, J.E., 1961. Metabolism of N: N-dialkyl carbamates and related compounds by rat liver. *Biochemical pharmacology*, 8(2), 179-191.
- Kitchen, D.B., Decornez, H., Furr, J.R. and Bajorath, J., 2004. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature reviews Drug discovery*,3(11),.935-949.
- Maronpot, R.R., Montgomery Jr, C.A., Boorman, G.A. and McConnell, E.E., 1986. National Toxicology Program nomenclature for hepat oproliferative lesions of rats. *Toxicologic pathology*, 14(2),.263-273.
- Maurer, K., Volk, S. and Gerbaldo, H., 1997. Auguste D and Alzheimer's disease. *The Lancet*, 349(9064), 1546-1549.
- Metcalf, R.L., 2000. Insect control. Ullmann's Encyclopedia of *Industrial Chemistry*.
- Moore, E.A. ed., 2002. Molecular modelling and bonding (Vol. 6). *Royal Society of Chemistry*.
- Ouyang, D. and Smith, S.C., 2015. Introduction to Computational Pharmaceutics. *Computational Pharmaceutics, 1*.
- Roy, K.K., Dixit, A. and Saxena, A.K., 2008. An investigation of structurally diverse carbamates for acetylcholinesterase (AChE)

inhibition using 3D-QSAR analysis. *Journal of Molecular Graphics and Modelling*, 27(2), 197-208.

- Smith, G.J., 1987. Pesticide use and toxicology in relation to wildlife: organophosphorus and carbamate, compounds. *US Government Documents (Utah Regional Depository)*,.510.
- Smith, G.J., 1987. Pesticide use and toxicology in relation to wildlife: organophosphorous and carbamatecompounds.*Resource publication/United States Department of the Interior, Fish and Wildlife Service (USA)*
- Smythies, J., 2009. Philosophy, perception, and neuroscience. *Perception*, 38(5),638-651.
- Solomon, K.A., Sundararajan, S. and Abirami, V., 2009. QSAR studies on N-aryl derivative activity towards Alzheimer's disease. *Molecules*, 14(4), 1448-1455.
- Sussman, J.L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L. and Silman, I., 1991. Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. *Science*, 253(5022), 872-879.
- Tiwari, P., Dwivedi, S., Singh, M.P., Mishra, R. and Chandy, A., 2013. *Basic and modern concepts on cholinergic receptor*: A
- Triggiani, D., 2011. Composti di origine naturale e xenobiotici, caratterizzazionechimica,valutazionedell'attivitàbiologicasulineecellulari e poten zialiutilizzichemioterapici.