Investigation of Interaction between NSAIDS and Antihypertensive Drugs using Computational Techniques

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Declaration

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

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Wishing ahead wonderful times to all wonderful people!

Zunaira Rauf

Dedication

I dedicate my work to
My Beloved Family

Abstract

In the current work, interaction studies of five Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and four Angiotensin Converting enzyme (ACE) enzyme amino acids with the antihypertensive drug, Lisinopril have been carried out on the molecular level using Quantum Mechanical Molecular Orbital Calculations with Density Functional Theory (DFT) and Hartree-Fock (HF) method using 6-31G basis set. Investigation of interactions was conducted with the aim of observing the comparative pharmacodynamics interaction studies of NSAIDs and amino acids with Lisinopril by scrutinizing the changes in geometric and electronic parameters of NSAIDs and amino acids of before and after complex formation with Lisinopril. Geometric parameter revealed increased electronic charge distribution on NSAIDs as compared to amino acids when interacted with lisinopril. Electronic parameters gave a measure of electron donating and electron accepting character of Lisinopril. Both geometric and electronic parameter revealed that electrostatic attraction and hence complex formation of NSAIDs with lisinopril is stronger as compared to that with amino acids. So in a competitive reaction NSAIDs complex formation is more favorable. According to the band gap value of ε_{HOMO} (lisinopril) - ε_{LUMO} (NSAIDs) lowest value of band gap is for aspirin which showed highest probability of electron transfer from lisinopril to aspirin as compared to other NSAIDs. This confirmed the formation of most strong charge transfer complex formation of aspirin-lisinopril and distortion of lisinopril leading to decrease in its antihypertensive effect. It was also observed that ε_{LUMO} of aspirin-lisinopril complex becomes more negative thus showing increased stability of aspirin-lisinopril complex. This investigation ultimately leads to reveal the drug interactions caused by NSAIDs in hypertension patients and suggests that aspirin should not be prescribed with Lisinopril. Decrease in antihypertensive effect of antihypertensive drugs by NSAIDs was also supported by discrete modeling, an approach of system biology.

Table of Contents

List of Abbreviations

NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
RA	Rheumatoid arthritis
PGs	Postaglandins
COX	Cyclooxygenase Enzyme
ARB	Angiotensin Receptor Blockers
ACE	Angiotensin Converting Enzyme
MM	Molecular Mechanics
QM	Quantum Mechanics
MD	Molecular Dynamics
HPLC	High Performance Liquid Chromatography
HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
UV-VIS	Ultra Violet Visible Spectrum
ΔG	Total change in Free Enegy
ΔH	Change in enthalpy
ΔS	Change in entropy

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Chapter 1

Introduction

Drug interactions are very important because they affect millions of patients every year and can cause serious threats to human life. These interactions are common in elderly as they are often at multi-drug therapy. Not all the drug interactions are perilous; some may be beneficial when side effects of one drug are minimized by some other drug. These interactions can reduce or enhance a drug effect and are a source of morbidity and mortality in patients getting multi-drug therapy [1]. Often clinicians are unaware of their adverse effects. Therefore, it is difficult for them to prescribe possibly non-interacting drug combinations [2]. When a patient is treated for multiple diseases, drug of one disease may change the absorption, distribution, metabolism, excretion and toxicity (ADMET) of other drugs [3]. Drug interaction studies have been carried out for about more than centuries. Presently in the modern era, researchers from all over the world are searching *in silico* techniques for better understanding of drug compounds and their interacting behaviors. There are a number of experimental methods being used to study drug-drug interaction. In addition to experimental methods use of different theoretical and computational approaches to study these interactions at molecular level provide insight into electronic information of both drug compounds and their complexes to elucidate their binding behavior.

1.1 Drug Interactions

Drug interactions can be beneficent and at the same time can be adverse. Adverse drug interactions are the most serious kind of drug interactions. Broadly, these drug interactions can be divided into two categories on the basis of plasma drug concentrations and effects of drugs on plasma concentrations of other drugs.

1.2 Types of Drug Interactions

Drug-drug interactions can be categorized into pharmacokinetic and pharmacodynamic drug interactions on the basis of drug metabolism and impact upon each other.

1.2.1 Pharmacokinetic Drug Interactions

Pharmacokinetic drug interactions are indirect interactions *i.e.*, one drug may affect the absorption, distribution, metabolism, excretion and toxicity of the other drug in the body. These interactions can cause alternations in plasma concentrations of both drugs. For example ciprofloxacin, which is an antibiotic interferes with the pharmacokinetics of olanzapine, drug used to treat schizophrenia, by blocking the enzyme required for breakdown of olanzapine. In result olanzapine is increased in blood which may cause severe muscle spasm [4].

These interactions can be useful or adverse. Useful drug interactions occur when one drug increases the efficacy of other drug. This can be done either by increasing effectiveness of the drug, or by minimizing the toxicity by reversing the side effects of the other drug. In both cases drug interaction are desired [5]. Adverse drug interactions are particularly most crucial type

of drug interactions. Such drug interactions can be very lethal for the patients which are on multi drug therapy. Significant percentage of the hospitalizations are due to adverse drug events, as both patients and practitioners are unaware of the consequences of these interactions [6].

1.2.2 Pharmacodynamic Drug Interactions

Pharmacodynamic drug interactions include path mechanism of drugs. This type of drug interactions can be responsible for altered drug effects at similar plasma concentrations. Pharmacodynamic drug interactions directly affect the pharmacologic effect of the drugs. These interactions occur when multiple drugs having interrelated targets are co-administered. Such interactions may result in additive, antagonistic or synergistic effects of other drugs. For instance when ciprofloxacin is co-administered with glibenclamide, which is an antidiabetic, it may increase the antidiabetic effect of glibenclamide and may cause hypoglycemia [4].

Just like pharmacokinetic drug interactions these drug interactions can also be adverse and advantageous. Pharmacodynamic drug interactions are useful when one drug has an additive effect on the efficacy of the other drug. Moreover, these drug interactions can also be adverse and harmful. Adverse, when drug of one kind suppresses the efficacy of the other drug, or when it enhances the toxicity of other drug in combination. Such interactions are very destructive and may increase the death rate [7].

Patients having life time diseases like rheumatoid arthritis (RA) and hypertension are more prone to such interactions. During their treatment multiple drugs may interact with each

other especially in the case of concurrent therapy. Such interactions may complicate the treatment thus posing serious threats to the human life [8].

1.3 Interaction between NSAIDs and Anti-hypertensive Drugs

Some adverse drug effects have been reported in hypertension patients when Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are co-prescribed due to common cold, fever or pain. NSAIDs are considered clinically suitable therapy for the treatment of arthritis. Moreover, hypertension and arthritis are diseases of elderly patients and usually found in combination. Most often these drugs are used concurrently and their use increases with the age. Thus the potential for concomitant administration of NSAIDs and antihypertensive drugs is quite noticeable.

NSAIDs have been found to affect the efficacy of (i) some other NSAIDs for example aspirin is found to interact with ibuprofen [9] (ii) antihypertensive drugs for instance indomethacin is responsible to reduce the efficacy of propranolol [10] (iii) antacids, for example diflunisal is found to interact with aluminium hydroxide [11]. The interaction between NSAIDs and anti-hypertensive drugs is quite common and has been well documented in the literature. Number of studies have reported that NSAIDs are responsible to reduce the efficacy of antihypertensive drugs and aggravates the pre-existing hypertension in the patients [12].

1.3.1 Action Mechanism of NSAIDs

NSAIDs are the most commonly prescribed drugs due to their analgesic, anti-pyretic and anti-inflammatory effects. Generally NSAIDs inhibit the conversion of arachidonic acid into

prostaglandins (PGs) by blocking the cyclooxygenase (COX) to cure pain and inflammation [13] . Inhibition of COX enzyme prevents the synthesis of PGs, which plays an important role in cardiovascular homeostasis. This decline of PGs in tissues may interrupt the circulatory control [14]. In order to alleviate pain NSAIDs inhibit the synthesis of PGs which are the inflammatory substances. This inhibition has several side effects like gastrointestinal mucosal injury [15], peptic ulcers [16] , salt water retention and high blood pressure [17, 18].

NSAIDs exert their effect by blocking the COX pathway of arachidonic acid mechanism. They inhibit the conversion of arachidonic acid to inflammatory PGs which causes fall in production of different PGs including Prostaglandin E (PGEs), Prostaglandin I (PGIs) and thromboxane. These PGs are involved in many physiological and pathological processes and are also responsible in maintaining cellular homeostasis.

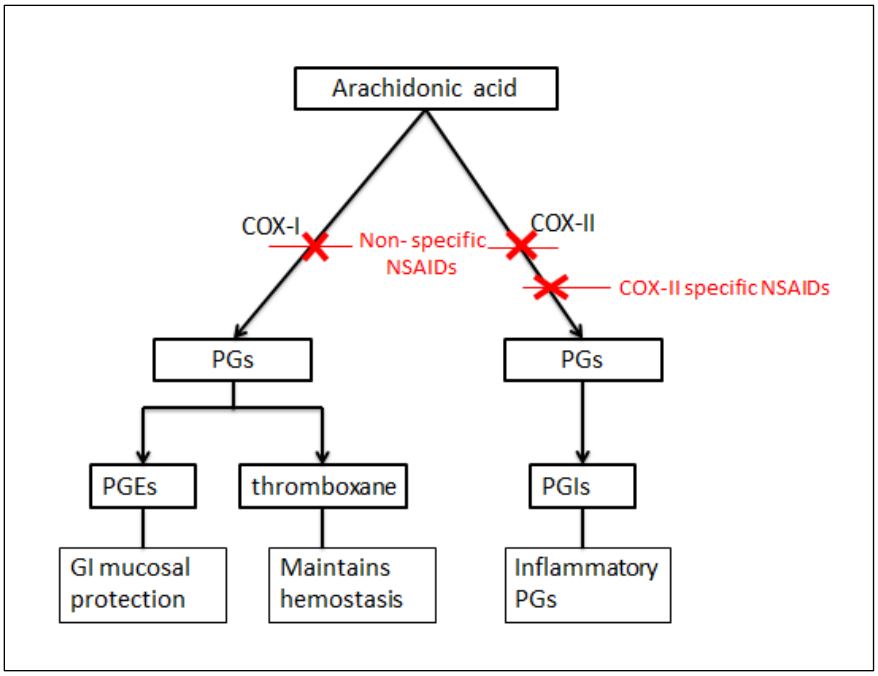


Figure 1.1 Mechanism of action of NSAIDs

These NSAIDs are either COX-II selective or non-specific, *i.e.*, inhibits both COX-I and COX-II. Most of the NSAIDs produce their anti-inflammatory action by blocking COX-II whereas, inhibition of COX-I causes side effects like gastrointestinal and renal toxicity [14] .

1.3.1.1 Selective COX-II and non-selective inhibitors

COX has its two distant isoforms COX-I and COX-II which are genetically independent and are located on different chromosomes, thus having different properties. These isoforms are involved in conversion of different PGs from arachidonic acid. COX-I is responsible in conversion of constitutional PGs which strengthens the gastrointestinal mucosa and maintains the hemostasis whereas COX-II produces inflammatory PGs. NSAIDs exert their antipyretic and analgesic

effect by inhibiting either both isoforms of COX or COX-II. COX-II specific inhibitors are considered to be safe NSAIDs, but they are also reported for cardiovascular side effects [19].

In the present study five biologically important NSAIDs were selected for interaction studies with lisinopril using computational methodology.

1.3.1.1 (a) Aspirin

Aspirin is a salicylate also known as acetylsalicylic acid. It was synthesized in 1897 and its chemical structure is shown in Figure 1.2. Mechanism of action of aspirin was discovered by Vane in 1971. It differs in mechanism of action from most other NSAIDs by inhibiting both isoforms of COX. It is used to reduce pain, fever and inflammation. It exhibits its antiplatelet effect by inhibiting thromboxane [20].

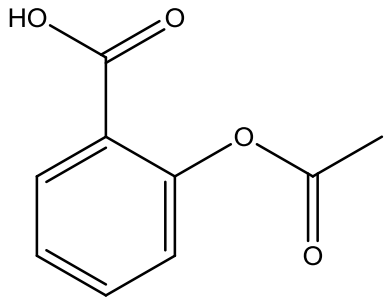


Figure 1.2 Chemical structure of Aspirin

1.3.1.1 (b) Etodolac

Etodolac is an antipyretic and anti-inflammatory drug and its chemical structure is shown in Figure 1.3. It performs its action by inhibiting COX-II thus blocking the synthesis of inflammatory PGs [21] .

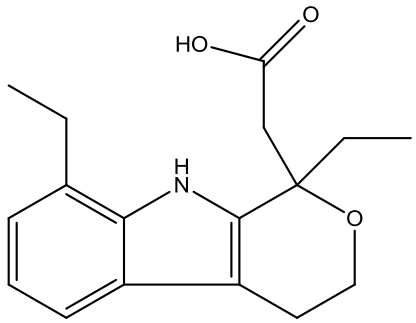


Figure 1.3 Chemical structure of Etodolac

1.3.1.1 (c) Ibuprofen

Ibuprofen is a chiral NSAID with chemical structure as shown in Figure 1.4. It is derivative of propanoic acid. It was synthesized in 1961 by Stewart Adams. Ibuprofen is used to reduce pain and to cure fever and inflammation. Like other NSAIDs ibuprofen also inhibits COX-II and blocks synthesis of PGs [22].

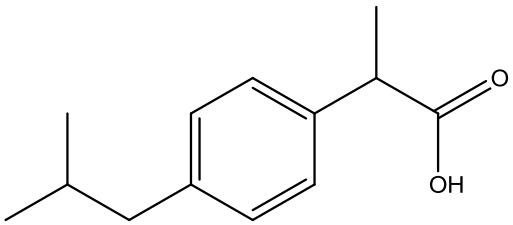


Figure 1.4 Chemical structure of Ibuprofen

1.3.1.1 (d) Mefenamic Acid

Mefenamic acid is an anthranilic acid. It is an NSAID which was discovered in 1960 by Parke Davis. It is used to treat pains and inflammations. Mefenamic acid performs its action by inhibiting COX enzyme. It is a non-specific NSAID and inhibits both isoforms of COX enzyme [23]. Its chemical structure is shown in Figure 1.5.

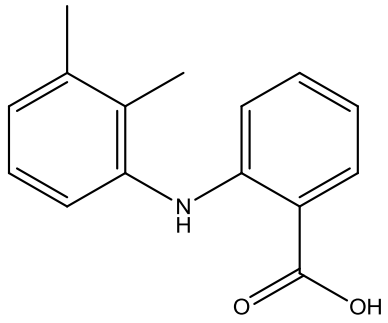


Figure 1.5 Chemical structure of Mefenamic acid

1.3.1.1 (e) Naproxen

Naproxen is an NSAID of propanoic acid class. It has anti pyretic, analgesic and anti-inflammatory effect. Naproxen works by inhibiting both COX-I and COX-II. It also prevents platelet aggregation by inhibiting thromboxane [24]. The chemical structure of naproxen is shown in Figure 1.6.

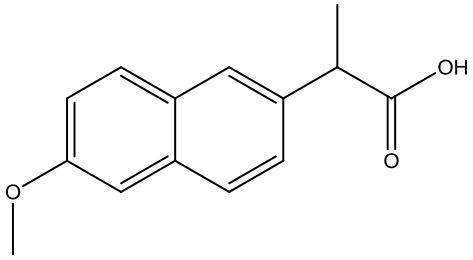


Figure 1.6 Chemical structure of Naproxen

1.3.2 Antihypertensive Drugs:

Hypertension is one of the major health problems these days. According to survey report about one billion people in the world are affected from abnormal blood pressure issues [25]. It may lead to severe cardiovascular diseases and increase the risk of stroke, heart attack and other cardiac

abnormalities [26]. Hypertension is frequently observed in the old age patients, its risk is much increased in diabetic and obese patients [27]. Elderly patients often have multiple diseases like diabetes, hypertension and RA. For hypertension, antihypertensive drugs are normally prescribed as first-line treatment. Drug interaction between NSAIDs and antihypertensive drugs can disturb the blood pressure control in patients using both medications simultaneously. The rate by which NSAIDs can affect the antihypertensive activity of antihypertensive drugs depend upon the role of PGs in mechanism of action of those antihypertensive agents [28].

NSAIDs by inhibiting PGs can limit the ability of different classes of antihypertensive agents by regulating the blood pressure. Five classes of antihypertensive drugs include beta blockers, calcium channel blockers, angiotensin receptor blockers (ARB), diuretics and angiotensin converting enzyme (ACE) inhibitors. Choice of drug varies with the condition of patient. In hypertension, controlling the blood pressure is more important than the choice of drug used for treatment [29].

1.3.2.1 Angiotensin Converting Enzyme (ACE) Inhibitors

ACE inhibitors inhibits angiotensin II which results in vasodilation and decrease in blood pressure. Renin gets activated when body faces low blood pressure. It cleaves angiotensinogen to form angiotensin-I. ACE then cleaves this angiotensin-I to form angiotensin-II which is a vasoconstrictor and increases blood pressure. Angiotensin-II also degrades bradykinin which activates PGs to maintain hemostasis. ACE inhibitors lower blood pressure by blocking the cleavage of angiotensin-I to angiotensin-II by ACE. ACE inhibition does not deactivate

bradykinin which in turn also induces vasodilation and increases the antihypertensive effect of ACE inhibitors. Bradykinin also stimulates the PGs to further increase the vasodilation [30]

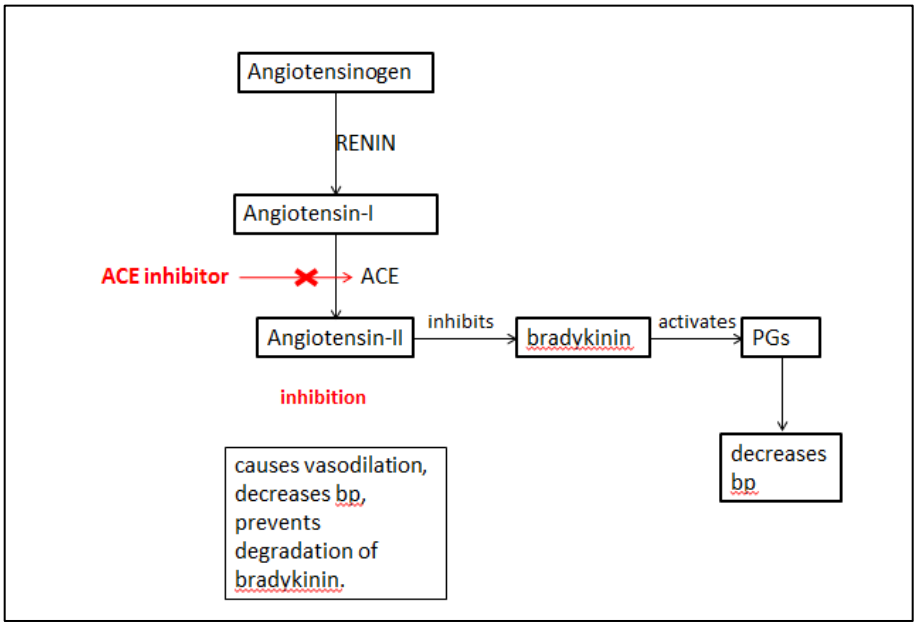


Figure 1.7 Mechanism of action of ACE inhibitors

NSAIDs are responsible to decrease the efficiency of ACE inhibitors by blocking the PGs synthesis and decreasing the vasodilation and thus increasing the blood pressure. Due to the large dependence of ACE inhibitors on PGs increases their potential interaction with NSAIDs [31]. In the present study, selected antihypertensive drug is an ACE inhibitor, Lisinopril.

1.3.2.2 Lisinopril

Lisinopril is an ACE inhibitor and is used to cure hypertension. Its chemical formula is $C_{21}H_{31}N_3O_5$. Chemical structure of Lisinopril is shown in Figure 1.8. Lisinopril was introduced in early 1990 and was discovered after captopril and enalapril, as the third ACE inhibitor.

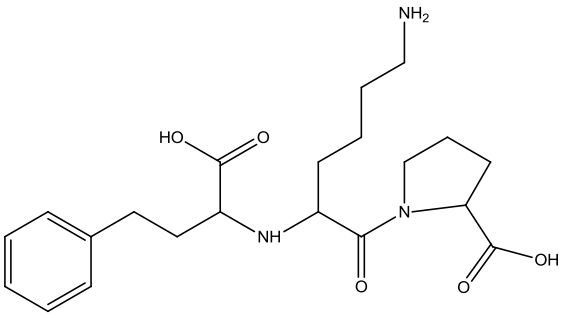


Figure 1.8 Chemical structure of antihypertensive drug Lisinopril (ACE inhibitor)

Hypertension is very common in elderly especially in those who have diabetes or obesity. Similarly, RA is also an old age disease in which NSAIDs are given as the first line treatment. Numerous studies have indicated that NSAIDs cause high blood pressure in patients with hypertension [15, 16]. The pathway through which NSAIDs perform their action inhibits prostaglandins which cause salt water retention, which in turn elevates blood pressure. It is well documented that NSAIDs interact with antihypertensive drugs and affect their efficiency. NSAIDs may partially or completely antagonize the effect of many anti-hypertensive agents [8].

1.4 Techniques Used To Study Drug-Drug Interactions

Adverse drug interactions can lead to serious threats to life. Many researches have carried out different techniques to study the drug interactions. These interaction studies can be carried out at different levels. First level which is very basic level is molecular level which involves direct chemical interactions of the drugs. Second level of interaction is cellular and third level is clinical level.

Cellular level and clinical level are beyond the scope of present study. Our interest is limited to molecular level investigations. A number of experimental and theoretical techniques are being used over the world to study molecular level interactions. Molecular level interactions include a large number of experimental and theoretical techniques. Different computational methods to explore drug-drug interactions include molecular mechanics (MM), quantum mechanics (QM), QM/MM and molecular dynamics (MD). Some common experimental techniques include cyclic voltammetry technique, mass spectrometry, UV-vis approach, electrophoresis, fluorescence techniques, equilibrium dialysis, high performance liquid chromatography (HPLC) etc.

In the present work, in depth study on interaction studies of NSAIDs (aspirin, etodolac, ibuprofen, mefenamic acid and naproxen) with antihypertensive drug Lisinopril with respect to the structure is carried out. Electronic properties like HOMO, LUMO, ionization energy, electron affinity, and binding constant etc. of the compounds and their complexes, which are directly involved in interaction are calculated. Electron donating and accepting character of compounds give in depth knowledge about the interaction. Charge transfer character was also calculated to find out the strength of interaction. Geometric and electronic parameters of NSAIDs and antihypertensive drug were calculated before and after complex formation to determine the binding affinity of NSAIDs with antihypertensive drugs. A number of thermodynamic parameters including ΔG, ΔH and ΔS were calculated for complexes of NSAIDs with Lisinopril and Lisinopril complexes with amino acids (glutamic acid, histidine, lysine and tyrosine). Based

on these thermodynamic parameters formation constant was obtained which is a measure of binding strength of complexation.

Chapter 2

Literature Review

Concurrent use of multiple medications can increase the risk of drug interactions. Many patients are unaware of the drastic effects which potentially interacting drugs can cause. The phenomenon of polypharmacy is common in elderly, where patients are suffering from multiple diseases at a time and are having different medications. Elevated blood pressure is one the most common cause for cardiovascular failures and disorders. Almost one billion people around the world are suffering from hypertension, which is more common in elderly patients. Suitable treatment for hypertension is important especially in older patients who are at a higher risk of heart stroke and heart diseases [26].

Moreover, old age patients also experience pain due to some type of arthritics. NSAIDs are the drugs frequently used to cure the pain and inflammations in conditions like RA, osteoarthritis or gout [32]. Such type of medication is found to elevate the blood pressure in patients with hypertension. Co-administration of NSAIDs with some antihypertensive agents increase the possibility for the drug interaction in which NSAIDs may completely or partially antagonize the hypotensive effects of antihypertensive drugs [33].

2.1 Drug Interactions of NSAIDs

NSAIDs are the most common drugs prescribed for chronic arthritis called RA, as pain killers and to reduce inflammation [32] . NSAIDs perform their anti-inflammatory action by blocking COX which is involved in PGs synthesis [34]. Blocking of COX inhibits production of

PGs, the inflammatory agents, which are responsible for the vasodilatation modulation, glomerular filtration, sodium and water exudation, and the renin-angiotensin [35]. NSAIDs are prescribed in combination with other drugs to nullify its side-effects, which may lead to drug interactions. Different types of drug interactions are reported in NSAIDs by many researchers. They have been found to have such clinically adverse drug interactions also with antihypertensive drugs and anti-diabetic drugs [36].

Pharmacokinetic drug interactions were reported in NSAIDs by Verbeeck, *et al*. These interactions were not only with other NSAIDs, thus affecting their pharmacokinetics, but with certain other classes of drugs. Aspirin was found to significantly reduce the plasma concentrations of other NSAIDs when given in combination. An interaction was found between aspirin and ibuprofen when used at a time [37]. Brouwers *et al*. reported drug interaction of aspirin with methotrexate, which is used to cure RA and cancer. It also interacts with an immunosuppressant, cyclosporine, when co-administrated. They further reported the interactions of aspirin with anticoagulants, anti-hyperglycaemic and the antihypertensive agents. Indomethacin was found to effect the blood concentrations of aminoglycosides in neonates. Gastrointestinal bleeding can be very dangerous which was caused by pharmacokinetic or pharmacodynamic interactions of NSAIDs. According to the Brouwers studies any of the drug interactions can be very critical for the patients [7]. Fendrick, *et al*. reported some potential interactions of some important members of NSAIDs. It was demonstrated that NSAIDs when used in combination with some other medications like anticoagulants, corticosteroids, or antihypertensive agents can lead to serious effects. Some of the potential interactions of NSAIDs are shown in Table 2.1 [38].

Table 0.1 Potential Drug Interactions with NSAIDs [38]

Combinations of Drugs	Effects
Aspirin with NSAIDs and NSAIDs with NSAIDs	Increased risk of serious gastro-intestinal complications
NSAIDs with anticoagulants	Increased risk of intestinal bleeding
NSAIDs with corticosteroids	Increased risk of gastrointestinal ulceration and hemorrhage
Aspirin with ibuprofen	Reduced antiplatelet effect of aspirin
NSAIDs with antidiabetic agents	Increased hypoglycemic effect
NSAIDs with antihypertensive agents	Increased hypertensive effect

2.2 Drug Interactions of Antihypertensive Drugs

Interactions between antihypertensive agents and other type of drugs can either induce or suppress the effects of antihypertensive drugs. Other drugs may disrupt the metabolism of antihypertensive agents through changing renal excretion or by inhibiting or inducing the associated enzyme [39]. Wood *et al.* studied such drug interactions in hypertensive patients when they were given anti-hypertensive drugs as blood pressure lowering drugs along with some other drugs like digoxin, nifedipine and carbamazepine. It was deduced that potency of antihypertensive drugs may be changed due to the drug interactions caused by co-administration of carbamazepine. It was further recommended that physician should adjust the antihypertensive therapy of patients efficiently rather than increasing the unnecessary doses [40]. Opie *et al.* reported different classes of antihypertensive drugs having remarkable pharmacokinetic drug interactions. Thus alter the effectiveness of other drugs. Reported classes with drug interactions

are beta blockers, calcium-channel blockers, diuretics, ACE inhibitors and Alpha blockers. These interactions can be between different classes of antihypertensive drugs and with drugs of different diseases. For example amlodipine which is a calcium channel blocker, has been found to have interaction with simvastatin, which is a used to treat hyperlipidaemia. A beneficial drug interaction between diuretics and angiotensin-converting enzyme (ACE) inhibitors was also reported [41]. Bacic-Vrca *et al*. conducted a study by using Lexi-Interact software to demonstrate drug interactions which are clinically important in elderly patients with hypertension. In this study 265 patients were included. Results showed clinically noteworthy drug interactions in 240 patients and it was concluded that elderly patients with hypertension are at greater risk of drug interaction [1].

2.3 Interaction of NSAIDs with Antihypertensive Drugs

Due to availability and pain killing effect of NSAIDs they are the group of drugs used most frequently. Patients with RA and elderly have greater consumption of these drugs and are more prone to their adverse effects. Such patients are often at multiple drug therapy, due to which they can have drug interactions. Moreover, these patients may experience the drug interaction between NSAIDs and antihypertensive drugs. This interaction is quite common in clinical practice these days, so doctors need to borne in mind such interactions before prescribing analgesics to antihypertensive patients [42].

Durao *et al,* studied the effect of indomethacin in 7 hypertensive patients. It was found to have interactions with some antihypertensive drugs like thiazide, loop diuretics, β-adrenergic blockers, α-adrenergic blockers, and angiotensin-converting enzyme inhibitors, but not with

calcium channel blockers. Adverse drug interaction was found in patients who received indomethacin and beta-blockers. Antihypertensive effect of antihypertensive drugs is lowered by NSAIDs, as they elevate the blood pressure by blocking the cyclooxygenase pathway of arachidonic acid metabolism which in result inhibits the synthesis of prostaglandin [10].

Salvetti *et al.* conducted a study, to find the intensity of known drug interaction of NSAIDs with antihypertensive agents on 16 hypertensive patients. Indomethacin was given as NSAID and its effect was observed on oxprenolol, which is a non-selective β-blocker. Attenuation of 50% hypertensive effect of oxprenolol was observed when it was given in combination with indomethacin. Data showed that PG synthesis inhibition by indomethacin can be responsible for decreasing the blood pressure lowering ability of oxprenolol [43].

Webster *et al.* also proposed that flurbiprofen, an NSAID, reduced the hypotensive effect of propranolol in hypertension patients. This attenuation was not due to alternation of pharmacokinetic properties of propranolol or atenolol, but it can be due to pharmacodynamics interactions of these drugs [44].

Houston *et al.* conducted a study for three weeks in patients who were treated with a calcium channel blocker. Verapamil was given as antihypertensive drug which is a calcium channel blocker, thus NSAIDs did not attenuated the antihypertensive effect of verapamil. It was suggested that verapamil can be a good alternative in patients who are also on NSAID therapy. The effect of several NSAIDs like ibuprofen, naproxen and placebo was evaluated in these patients. This study was conducted on 162 patients whose ages were between 18-75 years. It was

concluded that the three NSAIDs, ibuprofen, naproxen and placebo were not responsible for the elevation of blood pressure in these patients [33].

Johnson *et al.* conducted a meta-analysis to study the influence of NSAIDs on blood pressure and inferred that NSAIDs are responsible for affecting the therapeutic index of β blockers more than any other class of antihypertensive drugs. They found the most striking elevations in blood pressure by piroxicam, which is an NSAID, whereas, the elevations by aspirin and sulindac were not so obvious. The study confirmed the antagonizing behavior of NSAIDs with antihypertensive drugs, which may affect the antihypertensive therapy to an extent that morbidity may increase related to hypertension [39].

Polonia *et al*. explained the mechanism of action of NSAIDs due to which they are found to antagonize the hypotensive effect of many antihypertensive drugs by increasing the blood pressure. Proposed mechanism was inhibition of PG which elevates blood pressure due to which they have adverse interactions mainly with diuretics, ACE inhibitors and β-blockers but not with calcium channel blockers. It has been claimed that sodium retention by NSAIDs is not the only clarification for drug interaction in hypertensive patients, but also the PG inhibition. It has been found that indomethacin attenuated the antihypertensive effect of enalapril by 45% and also produced sodium retention. It has been proposed in the study that calcium channel blockers can be preferable as antihypertensive drugs than diuretics, beta-blockers and ACE inhibitors, probably due to their PG independent mechanism, in hypertensive patients who are also on NSAIDs therapy [8].

A double-blind crossover study was conducted by Morgan *et al.* in hypertension patients treated with amlodiphine or enalapril which is a pain killer. The effect of indomethacin was compared in both types of patients. Indomethacin caused weight gain and plasma renin deficiency in either type of patients. It was postulated that this influence of indomethacin is because of inhibition of PG synthesis and sodium retention. Patients with amlodipine experienced fewer effect of indomethacin on blood pressure due to the better effect of sodium retention on blood pressure. Indomethacin caused plasma renin deficiency and inhibition of PG synthesis which had full effect on blood pressure, *i.e.* increase in blood pressure. It was claimed that enalapril can be a better choice in essential hypertension patients who are given ACE inhibitors as antihypertensive agents [45].

It was postulated by Morgan *et al.* that indomethacin raises blood pressure in elderly patients treated with enalapril. It did not elevate blood pressure in those who receive amlodipine or felodipine. They further proposed that most of the NSAIDs can be responsible for sodium retention and alternation of renal function. Salt sensitivity of patients also affects the blood pressure response in them. In salt sensitive patients, elevated blood pressure was reported [46].

Llorca *et al.* studied the behavior of ibuprofen in hypertensive patients at clinical level and determined that it have interactions with antihypertensive agents of different groups. Moreover, it was found to reduce the antihypertensive activity of antihypertensive drugs due to inhibition of PG synthesis, which affects the blood pressure and cause sodium and water retention [47].

An adverse drug interaction in the patients of osteoarthritis and hypertension was studied by Hamzat *et al.* using clinical approach. They studied that patients of osteoarthritis and hypertension are more prone to adverse drug interactions of NASIDs and antihypertensive drugs. They recommended that physicians should keep in view the non-pharmacological approaches, as NSAIDs are responsible in reducing the effects of antihypertensive drugs [48].

An analysis was performed by Bavry *et al.* on hypertensive patients with coronary artery diseases from the INternational VErapamil Trandolapril STudy (INVEST). Patients were categorized into chronic and nonchronic NSAIDs users on the basis of NSAIDs use. There were 882 chronic NSAID users and 21,694 nonchronic NSAID users. The study was conducted for more than 2.7 years. They reported the increased risk of adverse drug events in patients who were on NSAIDs use [18].

A retrospective study was done by Aljadhey *et al.* on adult hypertensive patients. About 2,680 patients were considered for the study, 1340 of which were NSAIDs users and 1340 were acetaminophen users. The study was conducted to examine the effect of NSAIDs on blood pressure and antihypertensive drug therapy. It was reported that elevated blood pressure was reported in case of ibuprofen, an NSAID, as compared to acetaminophen. Moreover, less blood pressure elevation was found in calcium channel blocker and ACE inhibitors than β blockers, which is contrary to the study done by Polonia, where it was claimed that ACE inhibitors show adverse drug interaction with NSAIDs with remarkable blood pressure elevations. Therefore this problem of drug interaction of NSAIDs with antihypertensive drugs need more detailed studies [17].

Fournier *et al*. conducted a cohort study on 5710 hypertensive patients to study the impact of NSAIDs on the antihypertensive drugs. They reported that the hypertensive effect was amplified due to diclofenac and piroxicam especially with ACE inhibitors and Renin-angiotensin receptor blockers (ARBs). It was recommended that prescription of ARBs should be avoided if NSAIDs are co-administered [49].

Recently, Jabeen *et al.* has investigated the binding affinity of antihypertensive drug with NSAIDs by using UV–Vis spectroscopy and cyclic-voltammetric technique. They suggested that aspirin and mefenamic acid can be prescribed in patients having NSAID drug therapy of acetazolamide because their complex showed poor binding affinity with acetazolamide [50].

The adverse drug interaction between NSAIDs and anti-hypertensive drugs has very harmful effects not only on the drug therapy but also disrupts the renal function, causing ulcers, and salt and water retention. Although, drug interaction between NSAIDs and antihypertensive drugs is well studied by many researchers and experimentalists but more in-depth studies are required for this problem.

As it is clear from literature until now that no study has been conducted on DFT and HF calculations of aspirin, etodolac, ibuprofen, Lisinopril, Mefenamic acid and naproxen using Gaussian 09 followed by complex formation of NSAIDs-lisinopril. Present study is the first attempt on the detailed theoretical and computational investigation of NSAIDs (aspirin, etolodoac, ibuprofen, Mefenamic acid and naproxen), Lisinopril and their complexes using DFT and HF studies.

Chapter 3

METHODOLOGY

The present study is focused on the theoretical and computational investigation of NSAIDs, antihypertensive drugs and their complexes using Density Functional Theory Methods and Hartree Fock method.

Computational chemistry provides a wide range of methods for researchers to carryout extensive, expensive and risky experiments at lesser cost. It uses computer simulations for chemical calculations and designing of molecules with specific properties. Some methods can not only model the stable molecules but they can also be used for modeling unstable intermediates and transition states. Moreover, computational chemistry facilitates the modeling and simulation of many chemical and biological systems to understand and predict their behavior at the molecular level [51].

On the basis of structure of molecules and their reactivity computational chemistry is categorized into two broad areas, molecular mechanics and quantum mechanics. Molecular mechanics simulations predict the structure and properties of molecules by using the laws of classical physics. Electrons in a molecular system are not explicitly included in molecular mechanics calculations. Rather, calculations are performed on the basis of interactions among the nuclei. Electronic effects are implicitly included by approximation. This approximation make these computations computationally inexpensive and can be used to for large systems but these methods cannot be used in chemical problems where electronic effects predominate [52].

Whereas, quantum mechanics describes the behavior of electrons and nuclei in terms of their motion and distribution and also elucidates the molecular interactions. It works on the principle of wave particle dual properties of electrons, so all the information about the electronic structure can be derived directly from wave function. The present study focused on interaction studies of two types of drugs using electronic structure methods.

3.1 Electronic Structure Methods

Electronic structure methods use the laws of quantum mechanics. Quantum mechanics proposed that the energy and other properties of a molecule can be attained from a wave function. This wave function is obtained by solving the time independent Schrödinger Wave equation.

$$\mathrm{H}\,\psi = \mathrm{E}\,\psi \qquad 3.1$$

Ψ is a many-electron wave-function, E is the eigen value of the operator (total energy of the system) and H is the called Hamiltonian operator, equal to:

$$\mathrm{H} = \frac{-h^2}{8\pi^2 m} V^2 + \mathrm{V} \qquad 3.2$$

The Hamiltonian is made up of kinetic and potential energy terms.

$$\mathrm{H} = \mathrm{T} + V \qquad 3.3$$

3.1.1 Born-Oppenheimer Approximation

Quantum mechanics methods are characterized by various mathematical approximations to its solution. The Born-Oppenheimer approximation is the first of the several approximations used to simplify the solution of Schrödinger wave equation. This approximation separates the nuclear

and electronic motions and considers that nuclei are at rest with respect to electrons. The vibrational motion of nuclei is quite slow in comparison to the speed of motion of electrons. Born-Oppenheimer approximation is based on the consideration that in a molecular system nuclei look fixed to the electrons. The full Hamiltonian for the molecular system can be written as,

$$H = T^{elec}(r) + T^{nucl}(R) + V^{nucl-elec}(R,r) + V^{elec}(r) + V^{nucl}(R) \quad 3.4$$

T^{elec} is the kinetic energy of electron r, T^{nucl} is kinetic energy of nuclei R, $^{Vnucl-elec}$ is nuclei electron potential energy, V^{elec} is potential energy of electron r and V^{nucl} is potential energy of nuclei R.

Electronic Hamiltonian which neglects the kinetic energy term of nuclei is given by:

$$H^{elec} = -\frac{1}{2}\sum_{i}^{electrons}\left(\frac{\partial^2}{\partial x_i^2}+\frac{\partial^2}{\partial y_i^2}+\frac{\partial^2}{\partial z_i^2}\right) - \sum_{i}\sum_{I}^{electrons-nuclei}\left(\frac{Z_i}{|R_I - r_i|}\right) + \sum_{i}\sum_{j<i}^{electrons}\left(\frac{1}{|r_i - r_j|}\right) + \sum_{I}\sum_{J<I}^{nuclei}\left(\frac{Z_I Z_J}{|R_I - R_J|}\right) \quad 3.5$$

There are three types of electronic structure methods. These include Semi-empirical methods, *ab-initio* methods and Density Functional Theory (DFT) methods.

Semi-empirical methods use parameters derived from experimental data to simplify the approximation to the Schrödinger equation. These methods include only valance electrons and do not take into account electronic correlation. Semi-empirical methods are relatively cheaper and can be used for very large molecular systems.

ab-initio methods unlike semi-empirical methods do not use experimental parameters in their computations for Schrödinger wave equation. These methods are solely based on lows of quantum mechanics and take into account values of physical constants like speed of light, masses and charges of electrons and nuclei and Plank's constant. The main drawback of *ab-initio* methods is the heavy demand on the computational power. Hartree-Fock and Quantum Monte Carlo are the wave functions based on *ab-initio* calculations.

Density functional methods are similar to *ab-initio* methods in many ways but are more effective. These methods include the fact that electrons in a molecular system react to one another and attempt to keep out of one another's way *i.e.*, electronic correlation. HF calculations take into account this effect only in an average sense, which make these methods less accurate than DFT methods.

The present study focuses on the use of electronic structure methods for the selected five NSAIDs (Aspirin, Etodolac, Ibuprofen, Mefenamic acid and Naproxen) and antihypertensive drug namely Lisinopril. Molecular structures of NSAIDs and antihypertensive drug were drawn, minimized and optimized using GAUSSIAN 03 package. In order to make complexes of these drugs, their structures were imported to HYPERCHEM 8.0 for merging. NSAIDs, antihypertensive drug and their complexes were evaluated by calculating their electronic and geometric parameters. In the current study, calculated geometric parameters were bond length, bond angle and dihedral angles which provided details about the most stable and optimized configurations of the individual drugs and their complexes. Calculated electronic parameters were Highest Occupied Molecular Orbitals (HOMO), Lowest Unoccupied Molecular Orbital

(LUMO), dipole moments, ionization energies, electron affinities, chemical potential, electrophilicity index, chemical hardness and molecular electrostatic potential (MEP). HOMO and LUMO gave an estimate about the electron donating and electron accepting characteristic of drugs while other parameters evaluated the selected compounds on the basis of chemical reactivity. These parameters supported the classification of compounds on the basis of interactions and potency. Based on the observations from theoretical data NSAIDs were evaluated for their interaction with lisinopril.

3.2 GAUSSIAN 03

Gaussian is an electronic structure computational chemistry program designed by John Pople and his research group at Carnegie-Mellon University and released in 1970 as Gaussian 70. The name was derived from Gaussian orbitals which were used by Pople instead of Slater-type orbitals to speed up the calculations. The recent version of the program is Gaussian 09 which offer standard capabilities for SCF methods, Molecular mechanics, Semi-empirical methods, DFT methods, exchange functionals, correlation functionals, complete active state (CAS), hybrid functionals and QM/MM method etc.[53]. Gaussian is capable of predicting many properties of molecules and reactions including:

- molecular structures and energies
- structures and energies of transition states
- molecular orbitals
- atomic charges and electrostatic potentials
- vibrational frequencies

- NMR properties
- IR and Raman spectra
- thermochemical properties
- polarizabilities and hyper polarizabilities
- reaction pathways [52]

Gaussian 03 with Gauss View 5.0.8 along with HyperChem is the software package for this study.

3.2.1 Gauss View 5.0.8

Gauss View is a graphical interface for Gaussian which intended to generate input files for submission to Gaussian. It allows building of molecules which are submitted to Gaussian as input files. Output files generated by Gaussian can be visualized graphically using Gauss View. It is not assimilated in Gaussian's computational mode, however it is a back end processor which supports Gaussian.

Gauss View provides three major benefits to Gaussian users which includes:

1. It allows speedy drawing even for bulky molecules, by using simple mouse operations one can rotate, translate, zoom in and zoom out the molecule. It also provide advanced visualization facility to user by importing molecule files with different file formats like PDB.
2. It also facilitates the user by simplifying the customization of Gaussian calculations. It makes the complex input file commands for Gaussian calculations easy and simple for both routine jobs and advanced methods.

3. Gauss View provides a variety of graphical techniques for visualization of Gaussian output files. Gaussian results which can be visualized graphically includes:
    - dispersion potential surfaces
    - optimized potential surfaces
    - surfaces for magnetic properties
    - molecular orbitals
    - electron density surfaces
    - normal modes animations due to vibrational frequencies
    - surfaces may also be viewed as contours
    - atomic charges and dipole moments
    - molecular stereochemistry information
    - IR, Raman, NMR, VCD and other spectra

It also offers a range of selection for different type of basis set and treatment of electron correlation by using different theories [54].

3.2.2 Gaussian and Gauss View Windows

Gaussian 03 with Gauss View provides many utilities to perform theoretical and computational investigations.

3.2.2.1 Building the Molecule

Gauss View facilitates the building of molecule simply by clicking and dragging through its graphical user interface. The selected group of five NSAIDs and lisinopril were constructed using this specific service. Aspirin molecule is constructed using Gauss View in Figure 3.1.

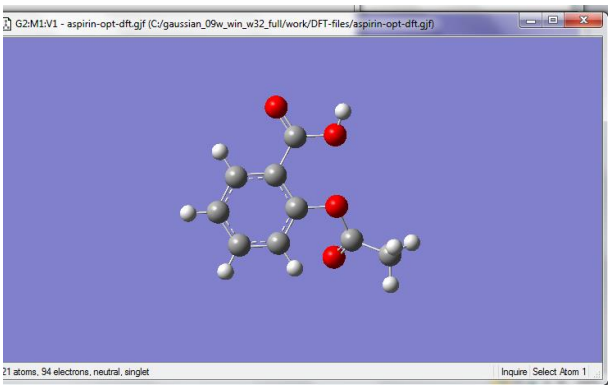


Figure 3.1 Building of the molecule Aspirin using Guass View

3.2.2.2 Job Type

Once input files are prepared they are submitted to Gaussian for further calculations. Gauss View also provide this facility to the user through graphical user interface. One can simply select the type of job from the Calculate menu as shown in Figure 3.2. Gaussian offers multiple features which include energy calculation, frequency calculation, geometry optimization, NMR, thermodynamic parameters etc. In this study Gaussian was used for energy calculation and geometry optimization.

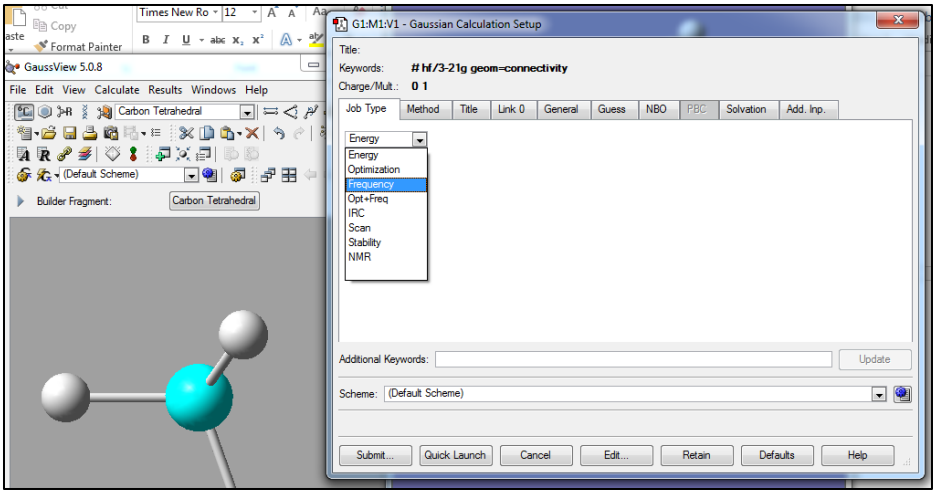


Figure 3.2 Job types offered by Gaussian using Gauss View

3.2.2.2 (a) Energy Calculation

This method evaluates the energy and other features at a fixed confirmation of the provided input structure. These calculations are performed for many purposes including

- To obtain basic information about the molecule
- On a molecular geometry to be used as a starting point for optimization as a stability test.
- To calculate precise values for energy and various features for geometry optimization at a lower basis set.
- When it the only affordable calculations for the large systems.
- With any basis set, single point energy calculations can be performed.

3.2.2.2 (b) Geometry Optimization

Geometry optimization attempts to estimate the confirmation of the molecule with lowest energy. The procedure computes the energy and wave function at a starting geometry and then iteratively searches a new geometry with lower energy until the lowest energy configuration is achieved. The technique evaluates the force on each atom by calculating the first derivative of energy with respect to atomic positions. Most optimization algorithms also evaluate the second derivative of energy with respect to the molecular coordinates and update the force constants. An optimization is complete when it is converged i.e. force on each atom is zero. The procedures perform successive searches to find the lowest energy structure which can be a saddle point [55].

Geometry optimization calculations will provide the information about the atomic coordinates of optimized molecule, its optimized parameters i.e., atomic distances and angles, HOMO/LUMO values, Mullikan atomic charges and dipole moments [52].

3.2.2.3 Methods

GAUSSIAN offers a variety of quantum mechanical methods as shown in Figure 3.3. The method menu includes the state of the system (ground, excited), choice of electronic structure method; spin state, selection of basis set, charge and multiplicity. These electronic structure methods use specific sets of approximations which are combined with specific algorithms to calculate molecular orbitals and energy. Generally these methods can be divided into 4 main types: semi-empirical, ab initio, density functional and molecular mechanics.

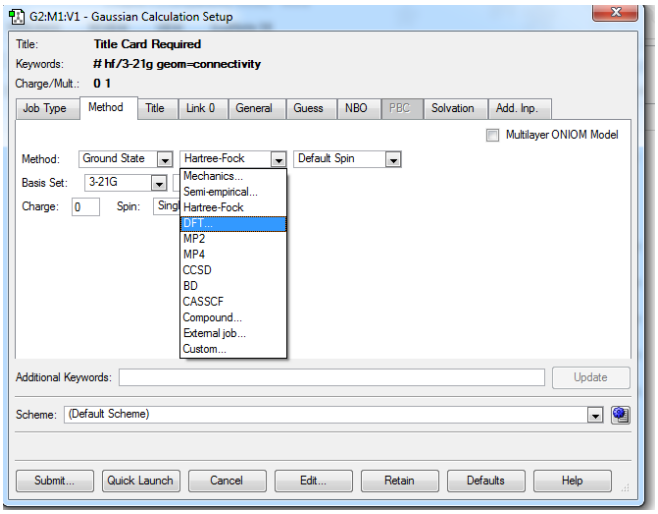


Figure 3.3 List of methods offered by Gaussian

Among these electronic structure methods, the present study focuses on the Hartree Fock and Density Functional Theory (DFT) methods.

3.2.2.3 (a) ab-initio

Ab-initio computations don't take into account experimental data but are based only on theoretical principles. Numbers of different ab-initio methods have the same basic approach but

they differ in mathematical approximations. Ab-initio methods are computationally expensive but offer sophisticated quantitative predictions for a wide range of systems.

Hartree-Fock Method

Hartree Fock (HF) method offers a rational model for a broad range of queries and molecular systems. It also has potential to calculate the vibrational frequencies and structures of transition states and stable molecules. It considers the approximation that Columbic electron-electron repulsion can be averaged, instead of taking into account the explicit repulsion interactions. However HF has also some restrictions due to the fact that HF theory does not take into account the effects of electron correlation. For those systems where electron correlation is important HF method is not satisfactory. For example, it is deficient for precise modeling of the energetics of reactions and bond dissociation.

3.2.2.3 (b) Density Functional Theory (DFT)

DFT is among the most famous and accurate quantum mechanical methods. Strategy of modeling electron correlation through general functionals (function of a function) of electron density underlies DFT method. The method was invented in early 1960s and it overcomes the limitations of HF. These methods have their origins to Hohenberg-Kohn theorem, published in 1964. The theorem elucidated the presence of a unique functional which exactly defines the ground state energy and density.

The current DFT methods are based on Kohn and Sham theorem according to which the approximate functionals partition the electronic energy into several terms:

$$E = E^T + E^V + E^J + E^{XC} \quad 3.6$$

E^T is the kinetic energy term due to motion of electrons, E^V describes the potential energy of nuclear-electron attraction and repulsion between pairs of nuclei, E^J is the electron-electron repulsion term and E^{XC} is the exchange correlation which includes the remaining interactions of electrons. All terms except the nuclear-nuclear repulsion are functions of ρ electron density. E^J is given by the following expression:

$$E^J = \frac{1}{2}\iint \rho(r_1)(\Delta r_{12})^{-1}\rho(r_2)dr_1dr_2 \quad 3.7$$

Hohenberg and Kohn demonstrated that E^{XC} is determined entirely by the electron density. E^{XC} is approximated as integral involving only spin densities and their gradients:

$$E^{XC}(\rho) = \int f(\rho_\alpha(r), \rho_\beta(r), V\rho_\alpha(r), V\rho_\beta(r))d^3r \quad 3.8$$

ρ_α refers to α spin density, ρ_β refers to β spin density and ρ shows the total electron density ($\rho_\alpha+\rho_\beta$).

E^{XC} is usually divided into exchange and correlation parts corresponding to same-spin and mixed-spin interactions.

$$E^{XC}(\rho) = E^X(\rho) + E^C(\rho) \quad 3.9$$

3.2.2.3 (c) Hybrid Functionals

DFT methods incorporate several approaches in order to calculate the exchange and correlation energy. Kohn-Sham DFT calculations are performed in an iterative manner that is analogous to Self-Consistent Field (SCF) method. Kohn and Sham pointed out the similar behavior in HF

theory. HF also includes an exchange term as part of its formulation. Hybrid functional utilizes DFT and HF exchange energies. Becke has formulated functionals defining E^{XC} as:

$$E_{hybrid}^{XC} = c_{HF} E_{HF}^{X} + c_{DFT} E_{DFT}^{XC} \quad 3.10$$

where the c's are constants.

3.2.2.3 (d) Molecular Mechanics (MM)

MM methods deal atoms as spheres and bonds as springs. These methods use an algebraic equation for the energy calculation, not a wave function or electron density. The constants in the equation are calculated from experimental data. The combination of constants and equations is called a force field. MM calculations don't explicitly include the electrons in a molecular system. Instead electronic effects are implicitly included in force fields through parametrization. This approximation makes MM computations computationally inexpensive and can be used for large systems.

3.3 Basis Set

Basis set is a set of functions combined in linear combinations to form molecular orbitals. These basis functions are centered on the atomic nuclei and resemble to atomic orbitals. An individual molecular orbital is defined as:

$$\phi_i = \sum_{\mu=1}^{N} c_{\mu i} x_{\mu} \quad 3.11$$

$c_{\mu I}$ is molecular orbital expansion coefficients, χ_μ refers to an arbitrary basis function and ϕ shows arbitrary molecular orbital

Gaussian functions have the general form:

$$g(\alpha, r) = cx^n y^m z^l e^{-\alpha r^2} \quad 3.12$$

Where, α is a constant determining the size of function. In a Gaussian function $e^{-\alpha r2}$ is multiplied by powers of x, y, z and a constant for normalization.

3.3.1 Pople Split Valance Basis Set

Split valence basis sets which are introduced by John Pople are represented as "*X-YZg*", where X shows the primitive Gaussian number consisting of basis function from every core atomic orbital. The two numbers after hyphen shows that this is *split-valence double-zeta* basis set. Some of frequently used basis sets of this category are; 3-21G, 3-21G(d), 6-31G, 6-31G(d) etc. the smaller the basis set the faster the computation will run. Bigger basis set perform the computation more extensively thus requires heavy computational power and are more accurate.

3.4 Calculated Parameters

Running energy minimization and optimization resulted in output files with a number of geometric and electronic parameters. These parameters provide an insight to the interacting behavior of the drugs.

3.4.1 Geometric Parameters

Molecular geometry is the three dimensional arrangement of the atoms that creates a molecule. It determines number of properties of a material which includes chemical reactivity, color, polarity, biological activity, magnetism and matter phase. Molecular geometries can be illustrated in terms of bond lengths, bond angles and dihedral angles. Bond length in a molecule is the average distance between the centers of two atoms bonded together. A bond angle is the angle between three atoms with at least two bonds, dihedral angle is the angle between four atoms bonded together.

3.4.2 Electronic Parameters

Electronic parameters can be calculated from the output files generated by Gaussian. These electronic parameters give information about the reactivity of the compounds, their donor acceptor character and charge distributions. The calculated parameters are HOMO and LUMO energies, dipole moment, ionization energies, electron affinities, chemical potential, chemical hardness, electrophilicity index and molecular electrostatic potential.

3.4.2.1 HOMO and LUMO energies

These parameters are very important for the analysis of interacting behavior as they represent the character of molecular orbital. The energy of a molecular structure depends on energy of its electrons in occupied molecular orbitals. Highest Occupied Molecular Orbital (HOMO) represents weakly held electrons for bonding which are available for donation and is a character for nucleophilic component. Lowest Un-occupied Molecular Orbital (LUMO) receives the electrons after they get excited. The energy difference between the HOMO and LUMO is

referred as the HOMO–LUMO gap. The measure of this gap would help in predicting the strength and stability of molecules and complexes. This will give an idea about the interaction between the molecules and the tendency of the two molecules to stick together and produce a stable complex with lower energy.

3.4.2.2 Dipole Moments

Dipole Moment (α) is the measure of the overall polarity of the system. It measures the separation of positive and negative charges in a system. Dipole moment is defined as:

$$\alpha = Q \times r$$

where Q is the magnitude of the charges and r is the distance between the charges.

3.4.2.3 Ionization Energy and Electron Affinity

Ionization energy (*I*) is the minimum amount of energy required to remove an electron from the neutral atom or molecule in gaseous state, where as Electron affinity (*A*) is the energy change when an electron is added to an atom or molecule in a gaseous state to form an anion. According to the Koopman's theorem, the HOMO and LUMO orbital energies are related to gas phase ionization energy (*I*) and electron affinity (*A*).

$$A = -\varepsilon_{LUMO}$$

$$I = -\varepsilon_{HOMO}$$

3.4.2.4 Chemical Potential

Chemical potential (μ) is the negative of electronegativity of a molecule and can be calculated using the following equation:

$$\mu = (\varepsilon_{HOMO} + \varepsilon_{LUMO}) /2$$

Where, μ defines the escapes tendency of electrons from a system under equilibrium. The larger the chemical potential, the less stable and more reactive the molecule is.

3.4.2.5 Chemical Hardness

Chemical hardness (η) measures the stability and chemical reactivity of a system. It refers to the HOMO–LUMO gap and measures the resistance of a molecule to change in the electron distribution in a collection of electron and nuclei. Chemical hardness can be estimated using following relation:

$$\eta = (\varepsilon_{LUMO} - \varepsilon_{HOMO}) /2$$

The greater the HOMO-LUMO gap the molecule is harder, less reactive and more stable.

3.4.2.6 Electrophilicity index

Electrophilicity index (ω) was introduced by Parr and it is the measure of a system's stability after accepting additional electrons from the surroundings. Electrophilicity index can be estimated using chemical potential and chemical hardness as shown:

$$\omega = \mu^2 /2\eta$$

3.4.2.7 Molecular Electrostatic Potential (MEP)

The molecular electrostatic potential (MEP) of a molecule is the force acting on a proton through the electrical charge cloud generated by the electrons and nuclei. Electrostatic potential of a molecule provide the information about the molecules reactivity towards positively or negatively

charged reactants. MEP can be visualized by mapping its values onto the surface of the molecule.

3.5 HYPERCHEM 8.0.6

HyperChem is a sophisticated molecular modeling software which employ semi-empirical quantum mechanical methods to study the structure and energetic of molecules. It allows quantum mechanical, molecular mechanics and molecular dynamics calculations with 3D visualization and animations [56]. It provide user with multiple options with a user friendly graphical user interface as shown in Figure 3.4 .In the present work HyperChem 8.0.6 was used for merging of drugs. Structures were imported from gauss view and were merged using Hyperchem.

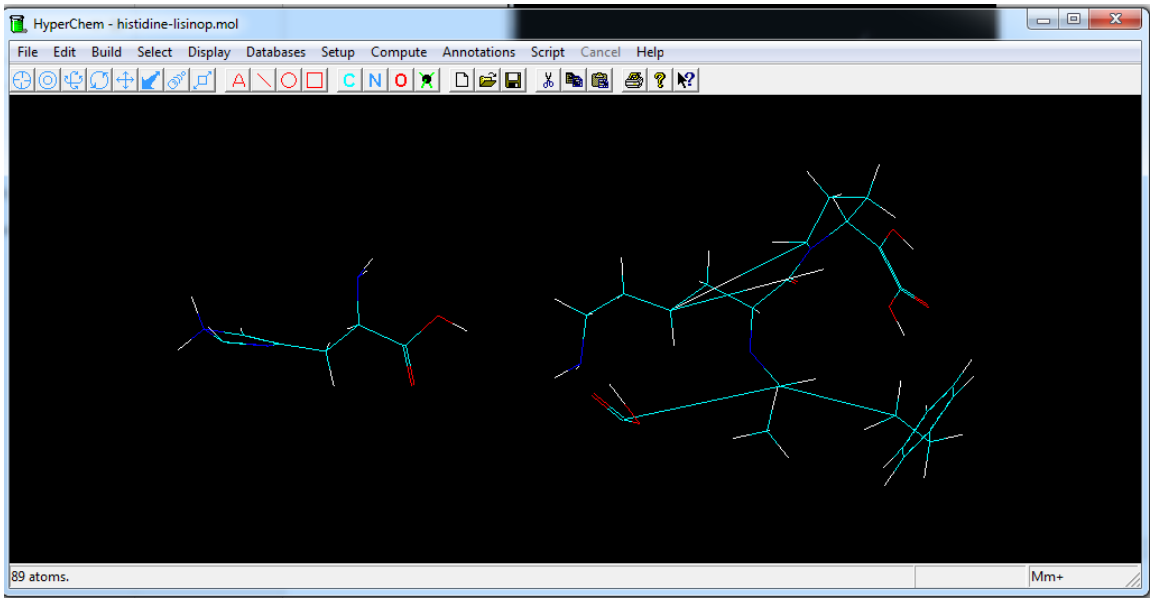


Figure 3.4 Strucutre merged using HyperChem 8.0.6

3.6 Molecular Operating Environment (MOE)

MOE is a comprehensive molecular modeling software system designed by Chemical Computing Group Inc. This platform integrates visualization, simulations and multiple applications. It supports drug design through molecular simulations, protein structure analysis, data processing of small molecules, docking and many more. MOE is used for molecular modeling and simulations, protein modeling, drug designing, pharmacophore designing and high throughput discoveries [57]. In the present work MOE was used to find the ligand interaction. Number of amino acids directly interacting with lisinopril was identified using Amber force field method. Protein structure of ACE co-crystallized with Lisinopril was obtained from PDB with PDB-ID 1O81 [58].

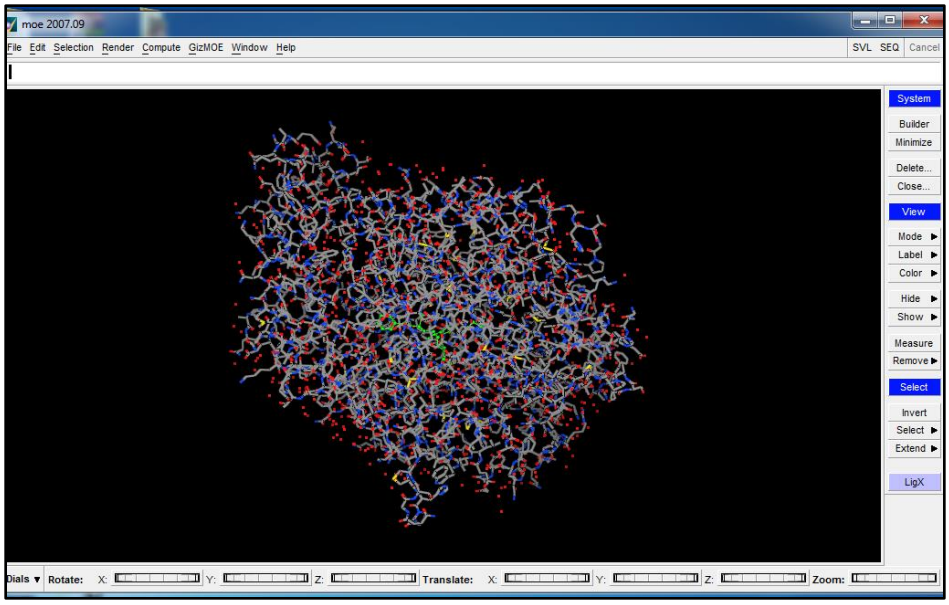


Figure 3.5 Angiotensin Converting Enzyme co-crystallize with Lisinopril

Many computational techniques in system biology have their origin in computational chemistry particularly in classical chemical kinetics. These techniques can be broadly categorized into continuous modeling approaches that employ linear and partial differential

equations (PDEs) to employ changing behavior, and discrete Boolean and multivalued approaches. The estimation of model parameters is a fundamental problem in continuous modeling techniques because these parameters cannot be measured exactly in most of the cases. The introduction of Boolean modeling provides necessary abstraction to focus only on the qualitative behaviors of a network.

In this work, we use Boolean modeling to study cause and effect relationship of NSAID on salt water retention and blood pressure in hypertension patients. We model different components in the form of a directed graph in which the nodes or vertices represent entities; whereas the edges represent activation and inhibition. In this model, directed arrows represent activation and blunt arrows represent inhibition.

Activation

If an entity (for example a drug) e1 positively regulates the concentration level of another entity (for example an enzyme or substrate) e2, then e1 is called the activator of e2. In this scenario, the introduction of e1 or increase in its concentration level will elevate the expression of e2.

Inhibition

If an entity (for example a drug) e1 negatively regulates the expression of another entity (for example an enzyme or substrate) e2, then e1 is called the inhibitor of e2. In this scenario, the introduction of e1 or increase in its concentration level will degrade the production of e2.

Implementation in Jimena

The network has been modeled in Jimena software [reference] which employs Boolean trees to model the function of a Boolean network. In a Boolean tree, the leave nodes (x1, x2 and x3) provide inputs to the function, whereas non-leaf nodes serve as unary or binary Boolean operations (AND, OR, NOT).

Each gate performs logical operation by combining the inputs received in accordance with Boolean operations. The value of the function is determined by root node.

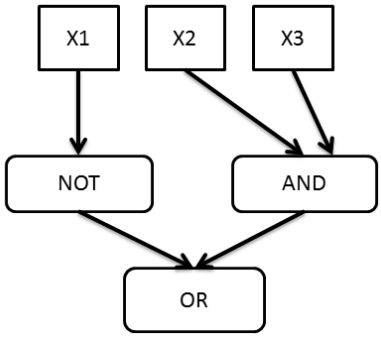


Figure 3.6 Structure of a logical tree

Chapter 4

Results and Discussion

In the present work, theoretical and computational studies of five NSAIDs with antihypertensive drug were carried out at molecular level using DFT-B3LYP and HF methods with 6-31G basis set to investigate the interaction of NSAIDs and amino acids of ACE with lisinopril. A number of geometric and electronic parameters were calculated before and after complex formation of NSAIDs and amino acids of ACE with lisinopril. The parameters include bond length, bond angle, dihedral angle, ε_{HOMO}, ε_{LUMO}, electron affinity, dipole moments, ionization energy, chemical potential, chemical hardness and electrophilicity index gave an estimate of strength of interaction of NSAIDs with lisinopril.

4.1 NSAIDs Characteristics

The selected five NSAIDs, mentioned in section 1.3.1 have been investigated using the Gaussian 09 package. The calculated electronic and geometric parameters from output files were used to determine interaction of selected NSAIDs with lisinopril.

4.1.1 Aspirin

The energy calculation and geometric optimization of aspirin was performed using the DFT/B3LYP and HF method with 6-31G basis set, and the significant calculated parameters are listed in Table A-1. The table contains geometrical values including bond lengths, bond angles and dihedral angles for Aspirin before and after complex formation with lisinopril.

The optimized structure and atomic charges generated by Gaussian 09 are shown in Figure 4.1. Aspirin is an NSAID with a planar structure possessing localized charge distribution.

It is evident from the Figure 4.1 that the most electronegative atoms in aspirin are 13O, 15O and 12O with partial charge distribution values -0.568, -0.552 and -0.409 respectively. The highly electropositive atoms 16C and 11C with charges 0.533 and 0.437 are also represented in figure. The presence of electronegative atoms in aspirin facilitates its electrostatic interaction with electropositive moieties of lisinopril.

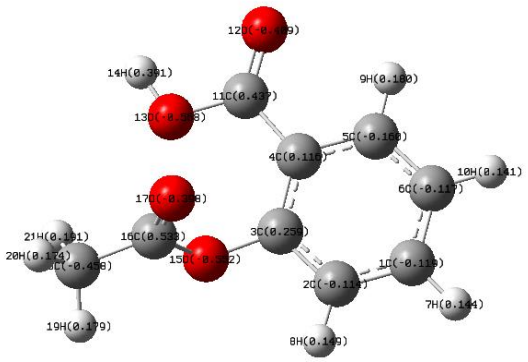


Figure 4.1 Optimized structure and atomic charges of Aspirin using DFT/B3LYP method

The structure and charges optimized by HF method are given in Figure 4.2. Here again the highly electronegative atoms are 15O and 13O with atomic charge -0.788 and -0.753 respectively. 12O and 17O are comparatively less electronegative and the most electropositive atoms are 11C and 15C with atomic charges 0.807 and 0.781 respectively. Difference in the results of DFT and HF may be attributed to the electronic correlation effect which is significantly present in DFT method and absent in HF method.

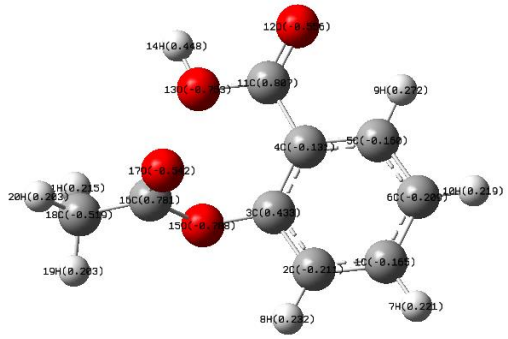


Figure 4.2 Optimized structure and atomic charges of Aspirin using HF method

4.1.2 Etodolac

The computed geometrical parameters of etodolac are mentioned in Table A-2. The table includes the values for bond length, bond angle and dihedral angle for etodolac before and after complex formation with Lisinopril.

The optimized structure and atomic charge distribution calculated by DFT/B3LYP are represented in Figure 4.3. As compared to 34O, 39O and 40O atoms, the atom 17N is the most electronegative atom with partial charge distribution of -0.831 attached to the two electropositive atoms 19C and 6C. Increased electropositivity of 19C and 6C may be due to electron withdrawing effect of highly electronegative 17N atom. The structure contains 38C as the most electropositive atom attached to two electronegative oxygen atoms 39O and 40O.

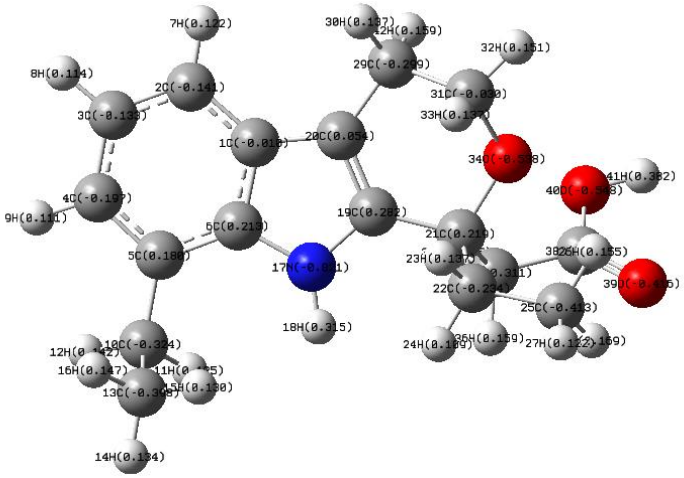


Figure 4.3 Optimized structure and atomic charges of Etodolac using DFT/B3LYP method

HF calculations showed that 17N is the most electronegative atom with atomic charge distribution of -1.060 as compared to 34O, 39O and 40O. 17N is attached to two electropositive carbon atoms, 6C and 19C. The highest electropositive atom is 38C with atomic charge of 0.805 bonded to two comparatively low electronegative atoms 39O and 40O. The optimized structure and atomic charges are represented in the Figure 4.4.

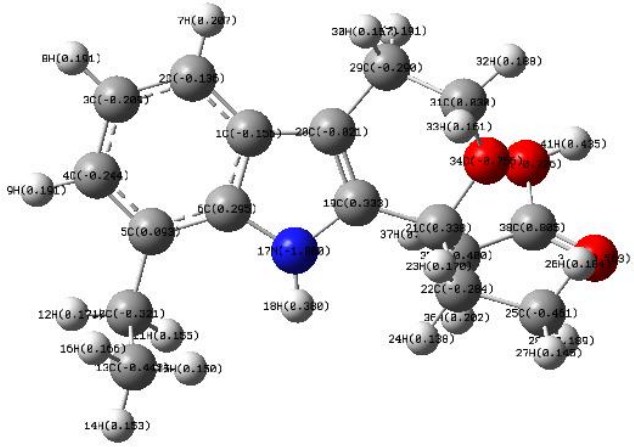


Figure 4.4 Optimized structure and atomic charges of Etodolac using HF method

4.1.3 Ibuprofen

Ibuprofen is an NSAID with planer geometry. The important computed electronic parameters of ibuprofen using DFT/B3LYP are represented in Table A-3. The optimized structure and atomic charges generated by Gaussian 03 using DFT method are shown in Figure 4.5. According to the structure in figure 15O possess the highest electronegative character with the atomic charge distribution -0.554 as compared to 14O with the atomic charge -0.424. 13C has the highest electropositivity with a charge distribution of 0.502 because of the two electrons with drawing atoms in its neighborhood.

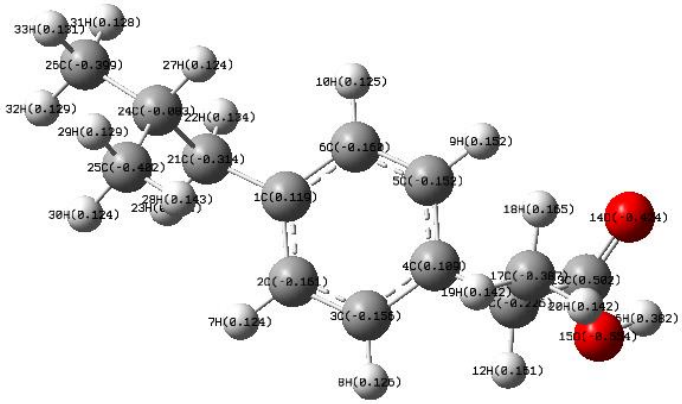


Figure 4.5 Optimized structure and atomic charges of Ibuprofen using DFT/B3LYP method

The optimized structure and atomic charges of ibuprofen calculated by HF method is shown in Figure 4.6. 32O and 31O (same atoms as 15O and 14O in DFT) are the highest electronegative atoms with the atomic charge distribution -0.733 and -0.564. 30C is attached to the two highest electronegative atoms 32O and 31O with the highest electropositive character and partial charge of 0.783.

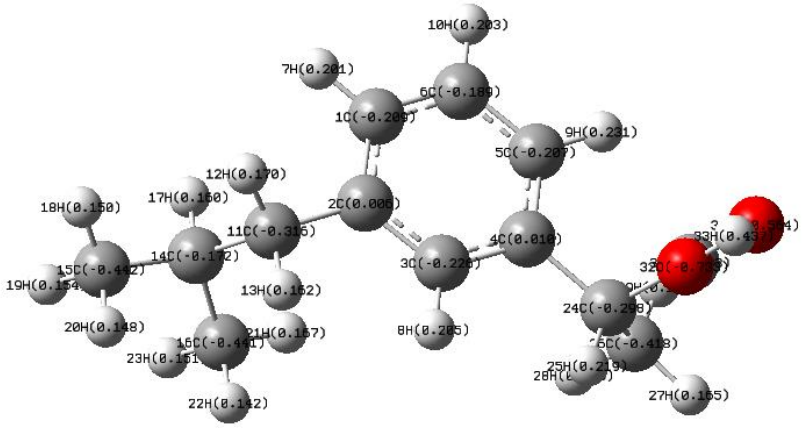


Figure 4.6 Optimized structure and atomic charges of Ibuprofen using HF method

4.1.4 Mefenamic acid

Mefenamic acid was assessed by Gaussian 09 using DFT and HF method and the important computed geometrical parameters are listed in Table A-4. The optimized structure and atomic charges generated by DFT-B3LYP are shown in Figure 4.7. The structure reveals that 18N is the most electronegative with atomic charge distribution of -0.831 as compared to 32O and 31O with atomic charges of -0.549 and -0.421 respectively. 18N is bonded to two comparatively less electropositive carbon atoms, 3C and 20C. 30C with an atomic charge of 0.406 is found to be the most electropositive atom in Mefenamic acid.

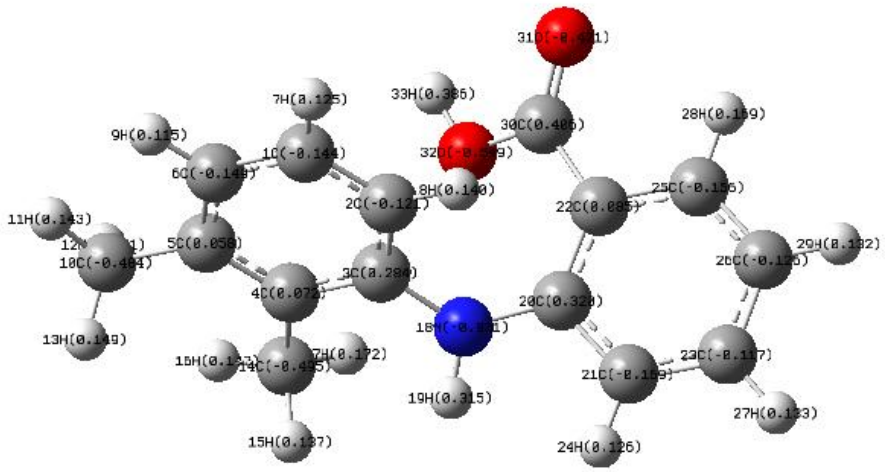


Figure 4.7 Optimized structure and atomic charges of M.A calculated by DFT/B3LYP method

HF calculations also confirmed that 18N is the most electronegative atom attached to two electropositive atoms 22C and 5C (same as 20C and 3C in DFT). It is clear from the Figure 4.8 that 30C is the most electropositive atom with an atomic charge of 0.793 bonded to two less electronegative atoms 31O and 32O with partial charge distribution of -0.575 and -0.791 respectively.

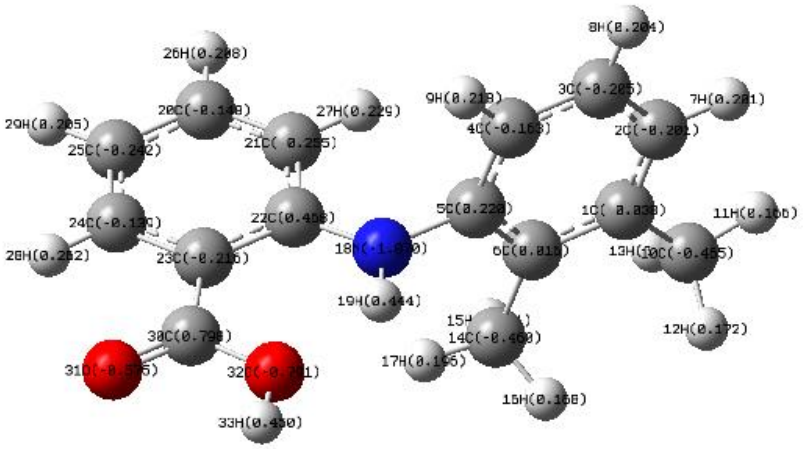


Figure 4.8 Optimized structure and atomic charges of M.A calculated by HF method

4.1.5 Naproxen

Naproxen is an NSAID used to cure pain and fever. The optimized structure and atomic charges of naproxen are shown in Figure 4.9. The computed geometrical parameters by DFT method are listed is Table A-5. It is clear from the figure that 17O is more electronegative than 29O and 30O with the atomic charge -0.566. 28C is attached to 29O and 30O and has the highest electropositive character with atomic charge 0.503.

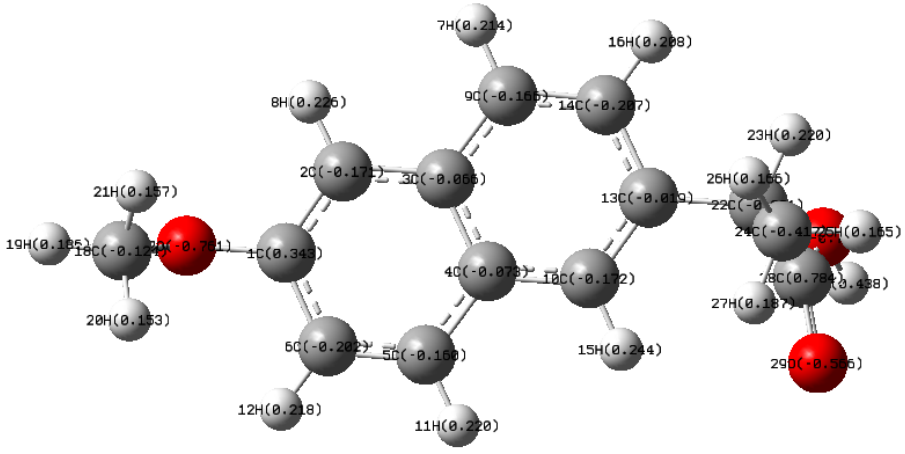


Figure 4.9 Optimized structure and atomic charges of Naproxen calculated by DFT\B3LYP method

The optimized structure and atomic charges generated by HF are shown in Figure 4.10. The analysis showed that there is an increase in the charge difference. 17O with an atomic charge -0.761 is the most electronegative atom in naproxen and is attached to 18C and 1C. 30O and 29O are the most electropositive atom in the molecule is 28C with partial charge distribution 0.784.

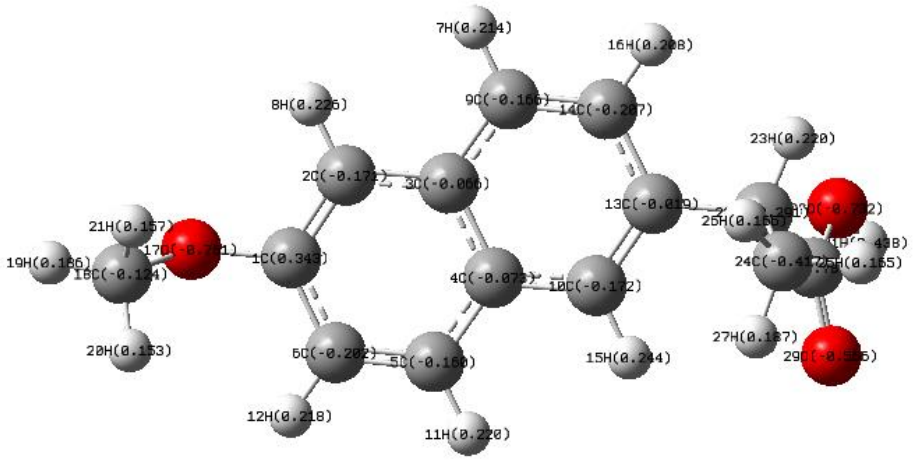


Figure 4.10 Optimized structure and atomic charges of Naproxen calculated by HF method

4.2 Lisinopril Characteristics

The optimized structure, atom numbering and respective atomic charges of the selected antihypertensive drug, Lisinopril, generated by Gaussian 09 using DFT method and HF method are listed in Figures 4.11 and 4.12. The significant computed geometric parameters of Lisinopril using DFT/B3LYP and HF with 6-31G basis set, before and after complex formation are listed in Tables A-1 to A-5.

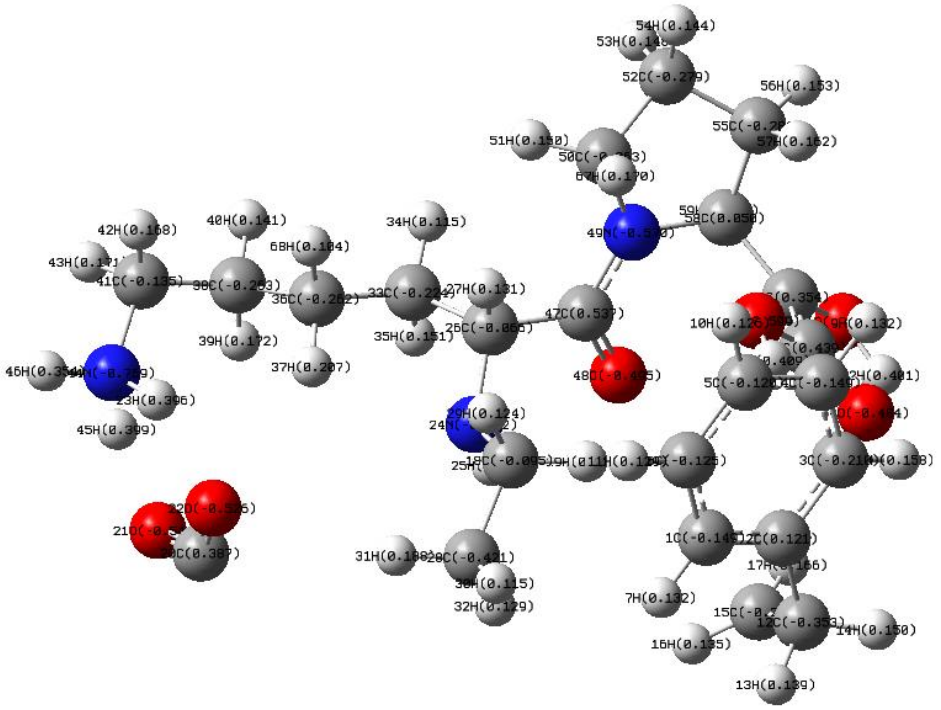


Figure 4.11 Optimized structure and atomic charges of Lisinopril calculated by DFT method

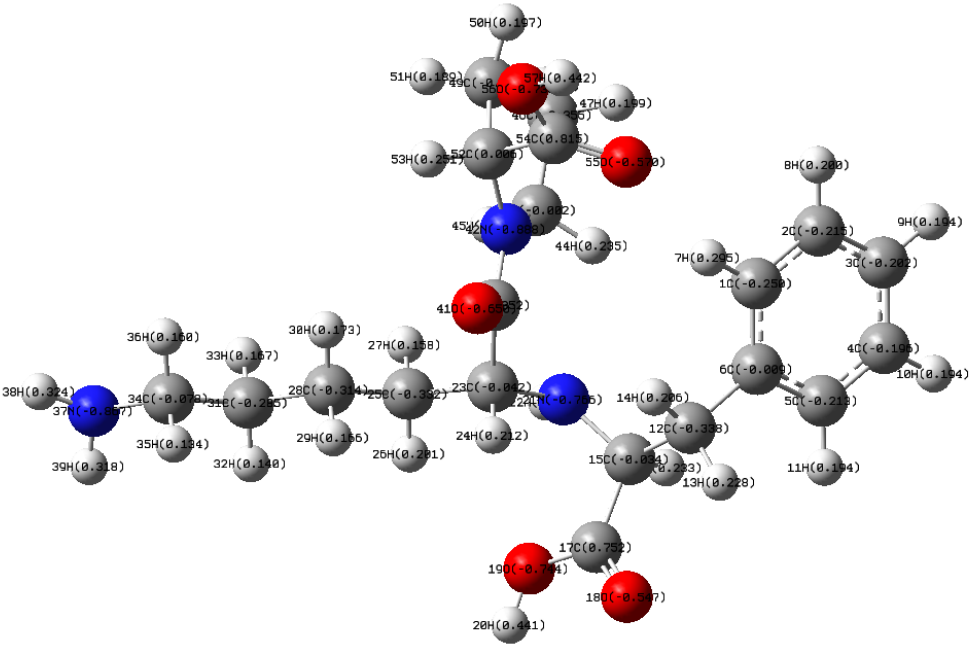


Figure 4.12 Optimized structure and atomic charges of Lisinopril calculated by HF method

4.3 NSAIDS-Lisinopril Complexes Characteristics

The selected group of five NSAIDs has been analyzed for their complexation with lisinopril in order to evaluate the presence of existing interaction.

4.3.1 Aspirin Complex with Lisinopril

Optimized structure of aspirin was merged with Lisinopril using HyperChem 8 and was evaluated by Gaussian 09 using DFT and HF studies to identify the existence of interaction between them. The summary of the geometrical optimization parameters for Asp-Lisin complex evaluated by DFT/B3LYP and HF are shown in Table A-1. The comparison of charge distribution in aspirin before and after complex formation with Lisinopril illustrated the differences in the marked region.

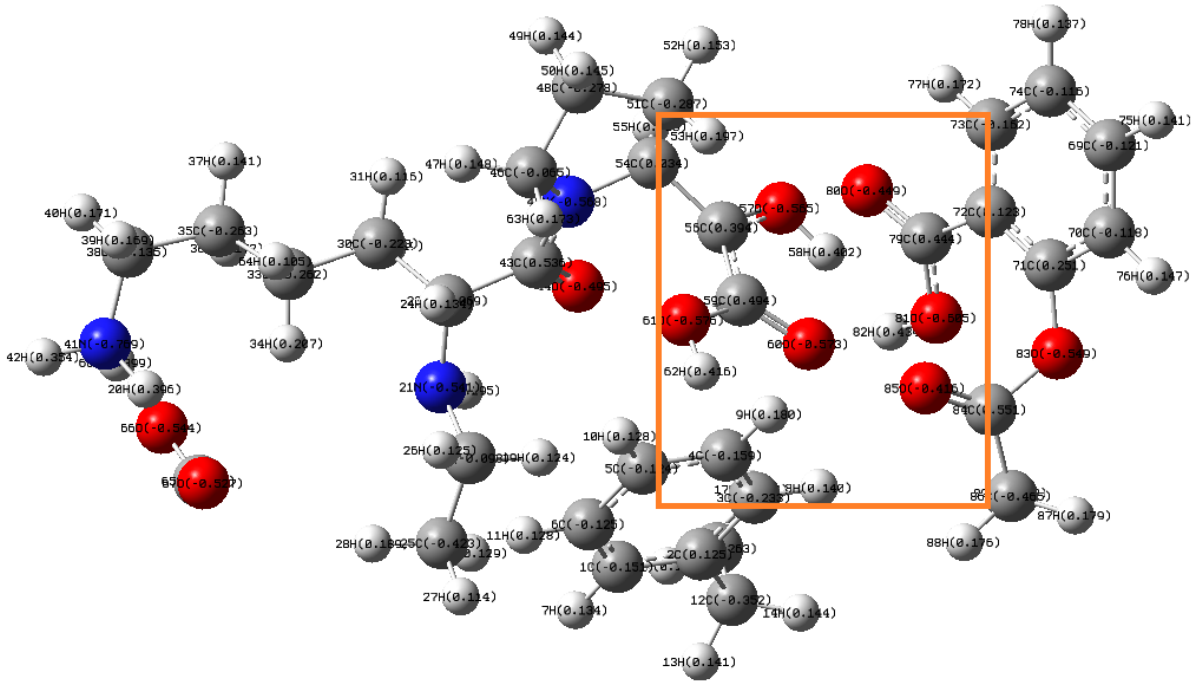


Figure 4.13 Optimized complex of Aspirin and Lisinopril calculated using DFT/B3LYP

4.3.2 Etodolac Complex with Lisinopril

Using HyperChem 8.0 optimized structure of etodolac was merged with the optimized lisinopril to form etodolac-lisinopril complex. This complex was then studied using Gaussian 09 for DFT and HF calculations. Results were analyzed to determine the strength of interaction between these drugs. The summary of the calculated parameters are shown in Table A-2. The comparison of charge distribution in etodolac before and after complex formation with lisinopril has shown remarkable differences in the marked regions. The values of 3C, 4C, 9H and 12H in etodolac in Figure 4.3 are changed from -0.133, -0.197, 0.111 and 0.142 to -0.156, -0.224, 0.155 and 0.150 (71C, 72C, 77H and 80H) in Figure 4.14 respectively. Similarly 57O, 60O, 61O, 56C, 59C, 4C, 3C and 9H in lisinopril in figure 4.11 have changed their values from -0.572, -0.487, -0.600, 0.360, 0.450, -0.154, -0.221 and 0.147 to -0.574, -0.484, -0.599, 0.354, 0.439, -0.149, -0.210 and 0.132 (61O, 64O, 65O, 60C, 63C, 4C, 3C and 9H), shown in Figure 4.14. These differences ensured the existence of interaction between etodolac and lisinopril. Partial charge distribution on etodolac is increased and on lisinopril it is decreased. This shows that ability of etodolac to attract electrons is more than that of lisinopril i.e. electronegativity of etodolac is greater than lisinopril. Partial charge of lisinopril flows to etodolac which indicates high polarizability of lisinopril as compared to etodolac.

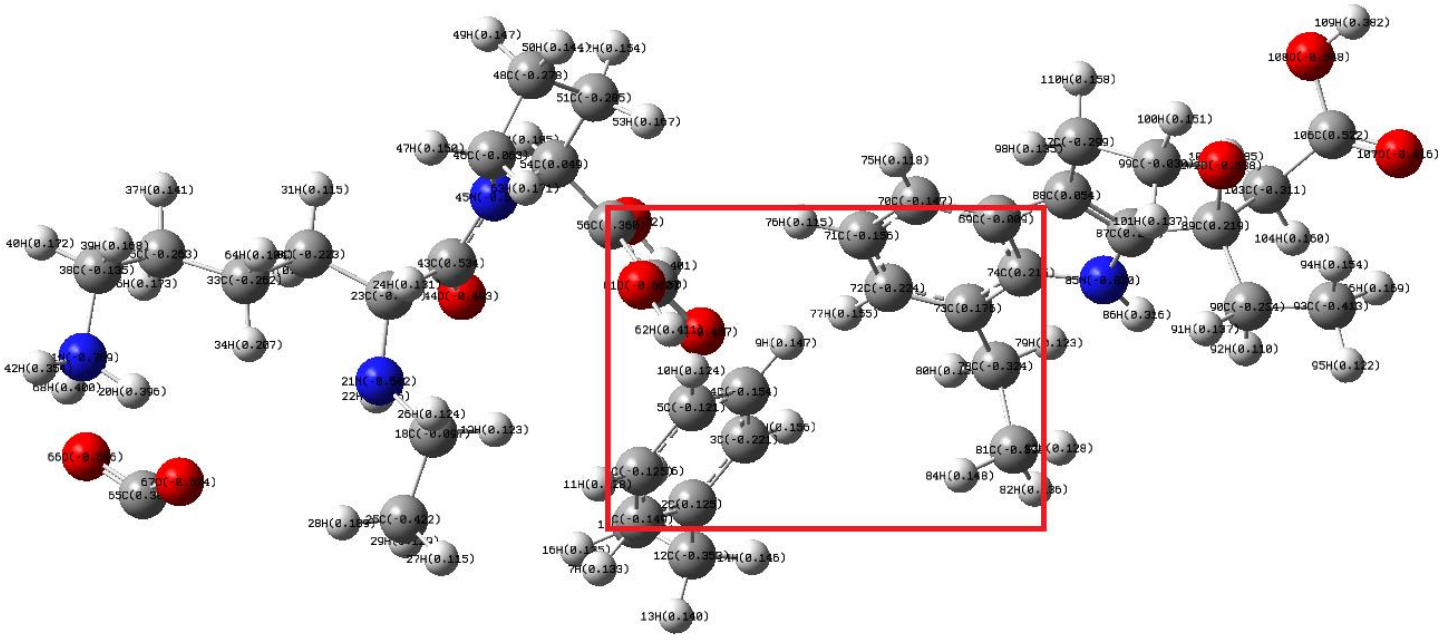


Figure 4.14 Optimized complex of etodolac and lisinopril calculated using DFT/B3LYP

4.3.3 Ibuprofen Complex with Lisinopril

Ibuprofen and lisinopril were optimized by Gaussian 09 using DFT and HF methods. These optimized structures were imported to HyperChem 8.0 for merging. Ibuprofen-lisinopril complex was further investigated for the detection of interaction between these drugs. Comparison of the computed parameters revealed changes in the highlighted region. 4C, 3C, 2C, 9H, 8H and 14H have changed their values in lisinopril (Figure 4.11) from -0.149, -0.210, 0.121, 0.132, 0.158 and 0.150 to -0.151, -0.215, 0.122, 0.131, 0.168 and 0.149 (Figure 4.15). Ibuprofen has also shown differences in 26C, 29H, 30H, 31H, 32H and 33H in Figure 4.5 from -0.399, 0.129, 0.124, 0.128, 0.120 and 0.131 to -0.404, 0.132, 0.121, 0.130, 0.126 and 0.141 (94C, 97H, 98H, 99H, 100H and 101H) in Figure 4.15. These changes depict the existence of interaction between ibuprofen and lisinopril. Partial charge distribution in both lisinopril and ibuprofen has increased accordingly which may suggest presence of ionic interaction between these drugs.

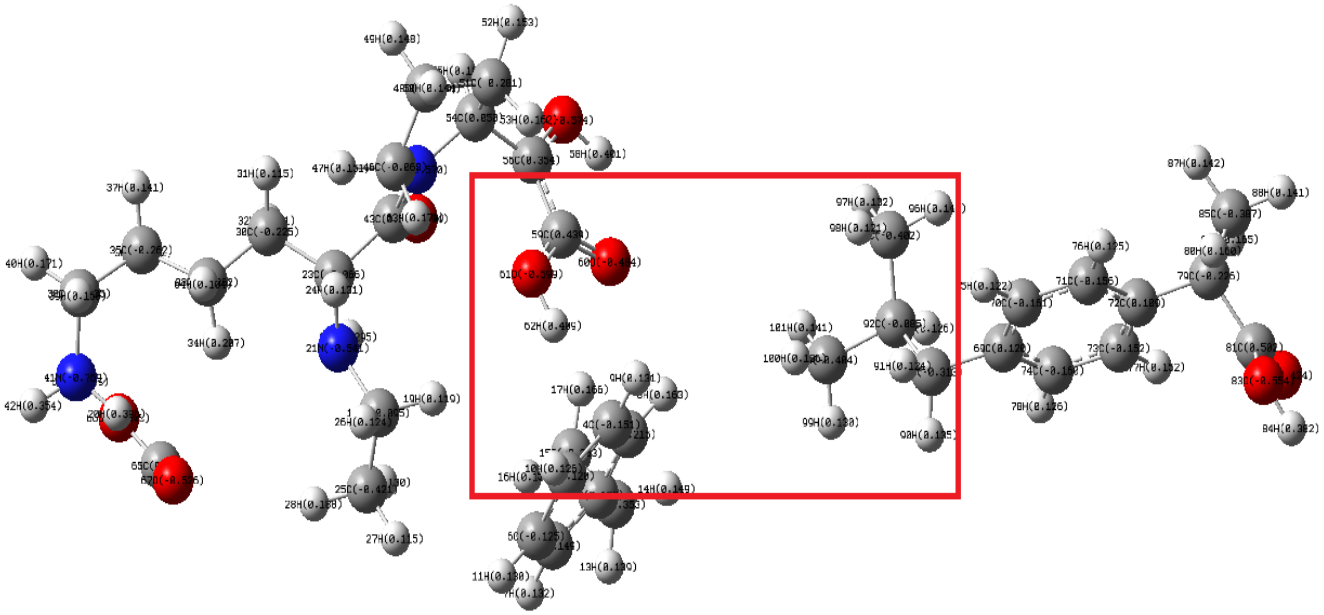


Figure 4.15 Optimized complex of Ibuprofen and lisinopril calculated using DFT/B3LYP

4.3.4 Mefenamic Acid Complex with Lisinopril

Optimized structures of mefenamic acid and lisinopril were merged to form mefenamic-lisinopril complex. This complex was then optimized using DFT and HF methods to determine their interacting behavior. Computed parameters for this complex are shown in Table A-3. The comparison of the calculated parameters of mefenamic acid before and after complex formation with lisinopril showed relative differences. Charge distribution values of 6C, 10C, 9H, 13H and 12H in mefenamic acid in Figure 4.7 are changed from -0.149, -0.484, 0.115, 0.151 and 0.149 to -0.148, -0.487, 0.111, 0.155 and 0.152 after complex formation, shown in Figure 4.16. 8H, 14H, 12C, 3C and 64O in lisinopril has also shown different values after the complex formation. Their values were changed from 0.158, 0.150, -0.353, -0.210 and -0.484, shown in Figure 4.11 to 0.160, 0.147, -0.352, -0.215 and -0.485, shown in Figure 4.16. These differences show existence of interaction between these drugs. Partial charge distribution in both drugs varied accordingly, for some atoms partial charge increased and for some atoms it is decreased.

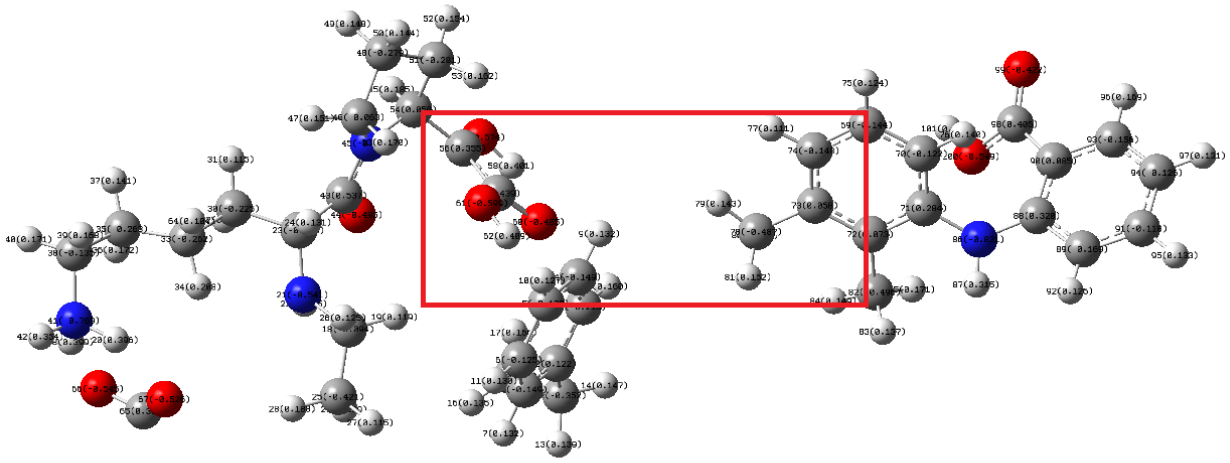


Figure 4.16 Optimized complex of Mefenamic acid with lisinopril calculated using DFT/B3LYP

4.3.5 Naproxen Complex with Lisinopril

Naproxen-lisinopril complex was generated using HyperChem and was evaluated by DFT and HF methods. Comparison of partial charge distribution of naproxen before and after complex formation showed some differences. Values of 9H, 8H, 4C and 64O in Figure 4.11 changed from 0.132, 0.158, -0.149 and -0.484 to 0.134, 0.160, -0.150 and -0.485 (Figure 4.17) in lisinopril after complex formation. Similarly, in naproxen 8H, 13H and 61C have changed their values from 0.126, 0.127 and -0.129 (Figure 4.9) to 0.133, 0.130 and -0.136 in Figure 4.17. These differences illustrated the presence of interaction between these drugs. Here again the partial charge distribution has changed accordingly in both drugs.

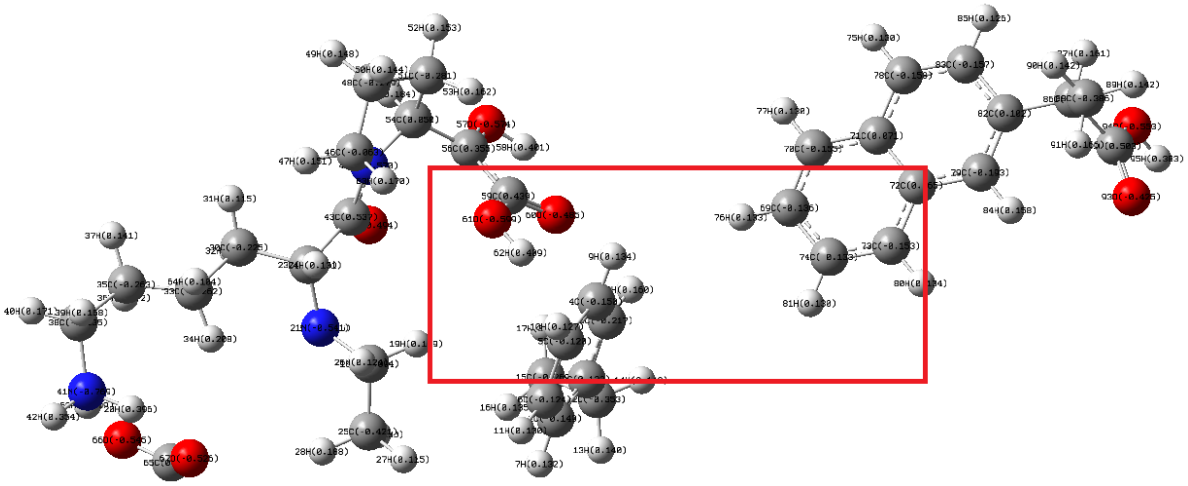


Figure 4.17 Optimized complex of Naproxen and lisinopril calculated using DFT/B3LYP

4.4 Amino Acids-Lisinopril Complexes

Co-crystallized structure of ACE with lisinopril was selected to study the interaction pattern of lisinopril with the amino acids of the ACE. Ligand interactions for ACE were evaluated using MOE. According to the obtained results four amino acids (glutamic acid, histine, lysine and tyrosine) were having direct interaction with lisinopril as shown in Figure 4.18. These amino acids were again optimized and merged with the optimized linisopril to form their complexes for electronic and geometric investigations. Amino acids-lisinopril complexes were studied using DFT method, each of them is discussed below.

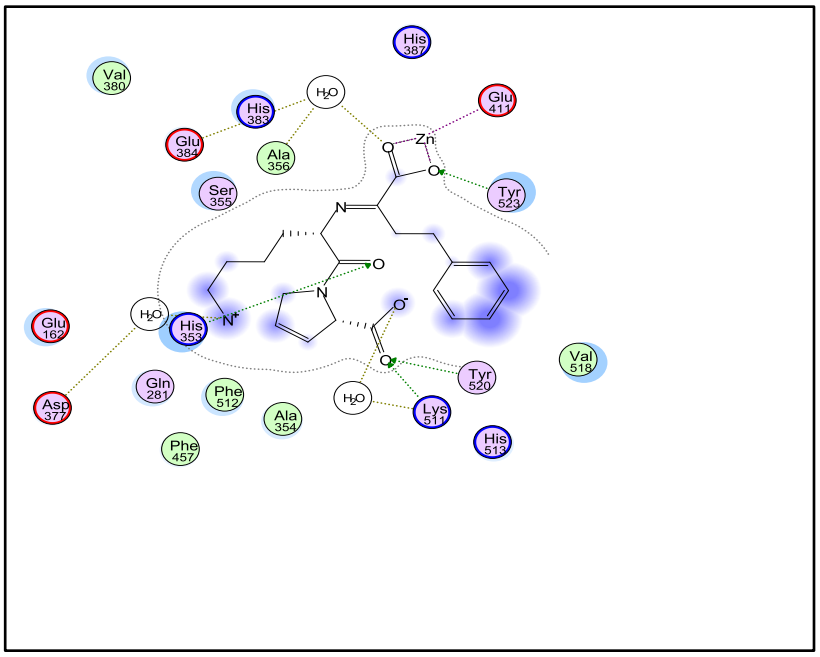


Figure 4.18 Ligand interaction of lisinopril with angiotensin converting enzyme(ACE)

4.4.1 Glutamic acid Complex with Lisinopril

Optimized structures of glutamic acid and lisinopril were merged to form glutamic0lisinopril complex. This complex was optimized using DFT/B3LYP method to investigate the binding propensity of both drugs. Geometric and electronic parameters were calculated from the output files. These parameters are shown in Table A-I. The comparison of the calculated parameters of glutamic acid and lisinopril before and after complex formation has shown differences in the marked region. 17O, 16C, 18O, 19H, 15H and 14H of glutamic acid have changed their partial charge distribution from -0.520, 0.524, -0.567, 0.447, 0.291 and 0.287 to -0.411, 0.470, -0.555, 0.388, 0.297 and 0.293. Similarly in lisinopril 44N, 45H, 46H, 20C, 21O and 22O have shown difference in their partial charge distribution. Their values changed from 0.769, 0.399, 0.354, 0.387, -0.544 and -0.526 to -0.787, 0.358, 0.425, 0.406, -0.593 and -0.505 (58N, 59H, 60H, 39C, 40O and 41O) as shown in Figure 4.19.

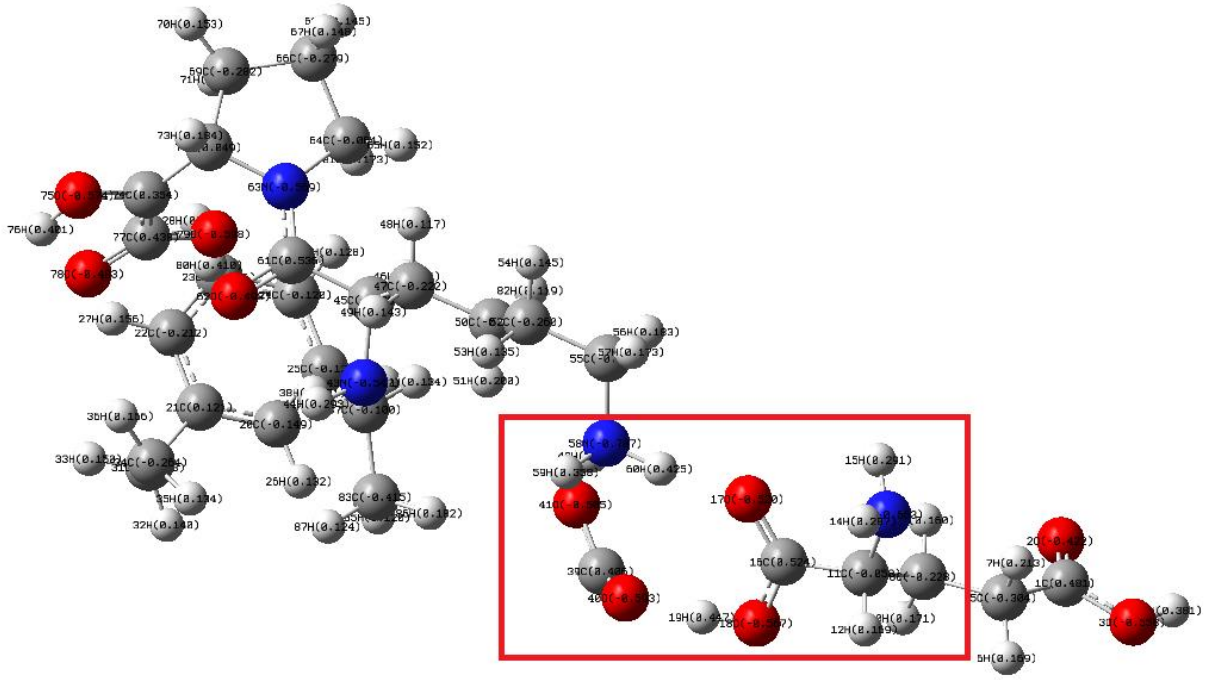


Figure 4.19 Optimized complex of Glutamic acid with lisinopril calculated by DFT/B3LYP

4.4.2 Histidine Complex with Lisinopril

Structures of histidine and lisinopril were optimized using Gaussian and their complexes were formed through hyperChem. This histidine-lisinopril complex was studies using DFT method to identify the existence and strength of the interaction. The optimized complex and its charges are represented in Figure 4.20. Partial charge distribution in histidine has been changed before and after complex formation from 0.381, -0.564, 0.477 and -0.419 to 0.426, -0.572, 0.530 and -0.515 in 21H, 20O, 13C and 19O. Similarly lisinopril has also shown difference in partial charge distribution. Here charge distribution changed from -4.11, 0.525, -0.557 and 0.389 to -0.506, 0.581, -0.570 and 0.432 in 76O, 75C, 77O and 78H as shown in Figure 4.20.

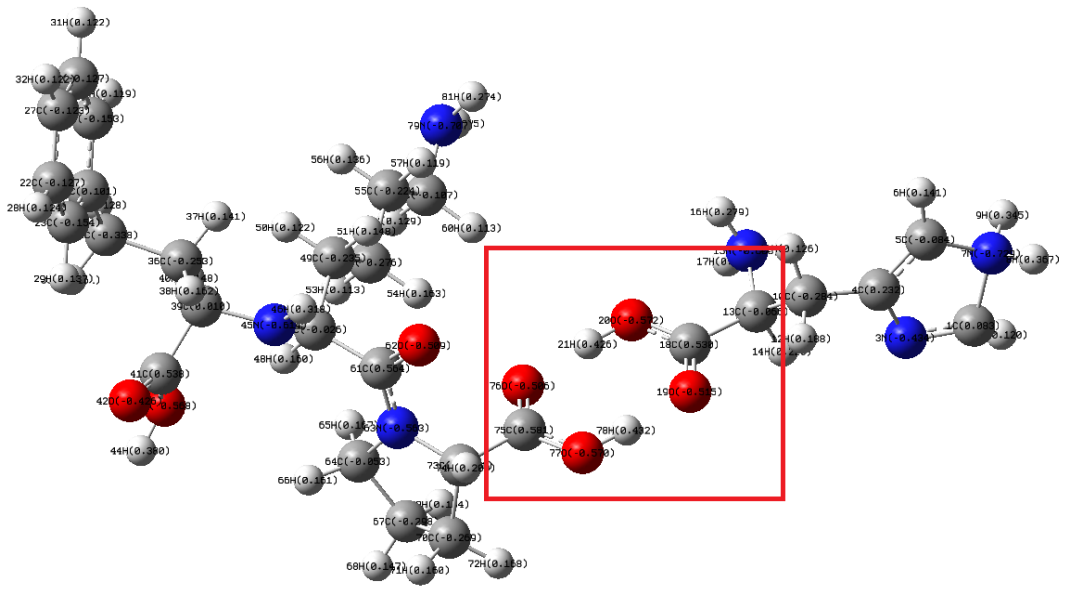


Figure 4.20 Optimized structure Histidine with lisinopril calculated by DFT/B3LYP

4.4.3 Lysine Complex with Lisinopril

To investigate the strength of interaction this complex was studied by Gaussian using DFT studies. Comparison of structures before and after complex formation has shown some differences which ensured the existence of interaction between them. After complex formation lisinopril has shown differences in its partial charge distribution. Charge distributions of 55O, 54C, 56O and 57H have changed from -0.411, 0.525, -0.577 and 0.389 to -0.477, 0.559, -0.578 and 0.442. Lysine has shown differences in its partial charge distribution in 16H, 14N, 15H, 13C, 17C and 18O, where values changed from 0.324, -0.746, 0.351, 0.333, 0.394 and -0.569 to 0.328, -0.768, 0.396, 0.341, 0.451 and -0.480. These differences are shown in Figure 4.21. it is evident that charge distribution of lisinopril is increased and that of lysine is also increased after complex formation.

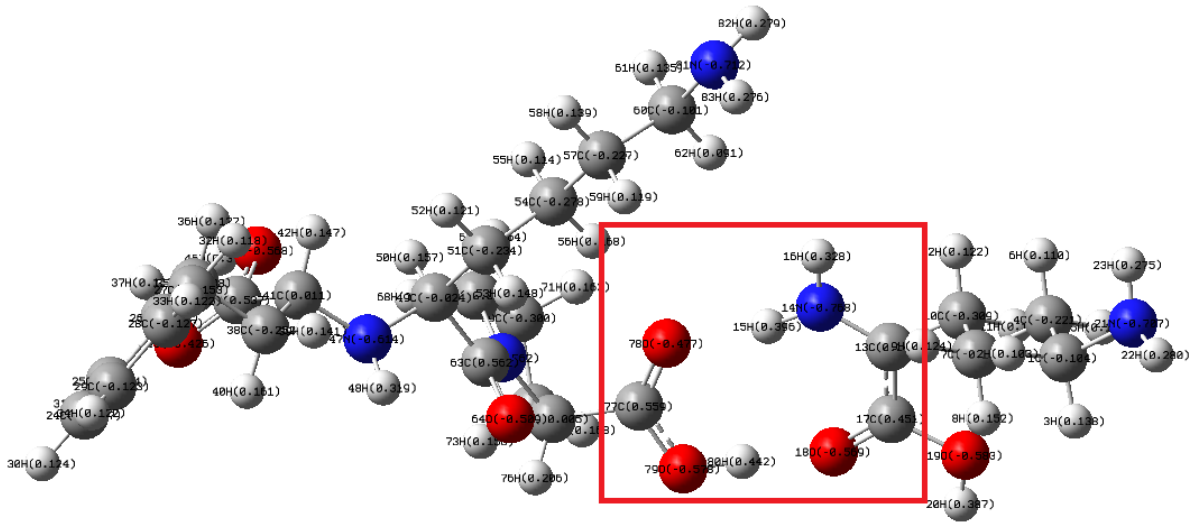


Figure 4.21 Optimized complex of lysine and lisinopril calculated by DFT/B3LYP

4.4.4 Tyrosine Complex with Lisinopril

Comparison of optimized structure of tyrosine and lisinopril before and after complex formation revealed differences in geometric parameters which are represented in Table A-. Selected region with differences in partial charge distribution is shown in Figure 4.22. 44N, 45H, 46H, 20C, 21CO and 22O of lisinopril have changed its partial charge distribution from -0.769, 0.399, 0.354, 0.387, -0.544 and -0.526 to -0.786, 0.426, 0.362, 0.407, -0.597 and -0.504 (63N, 64H, 65H, 44C, 45O and 46O) as shown in Figure 4.21. in tyrosine 21C, 22O, 23O, 24H 20H and 18N have shown difference in their partial charge distribution from 0.549, -0.524, -0.571, 0.447, 0.304 and -0.727 to 0.480, -0.412, -0.501, 0.386, 0.283 and -0.660. It is clear that charge distribution has increased in tyrosine and lisinopril during the complex formation.

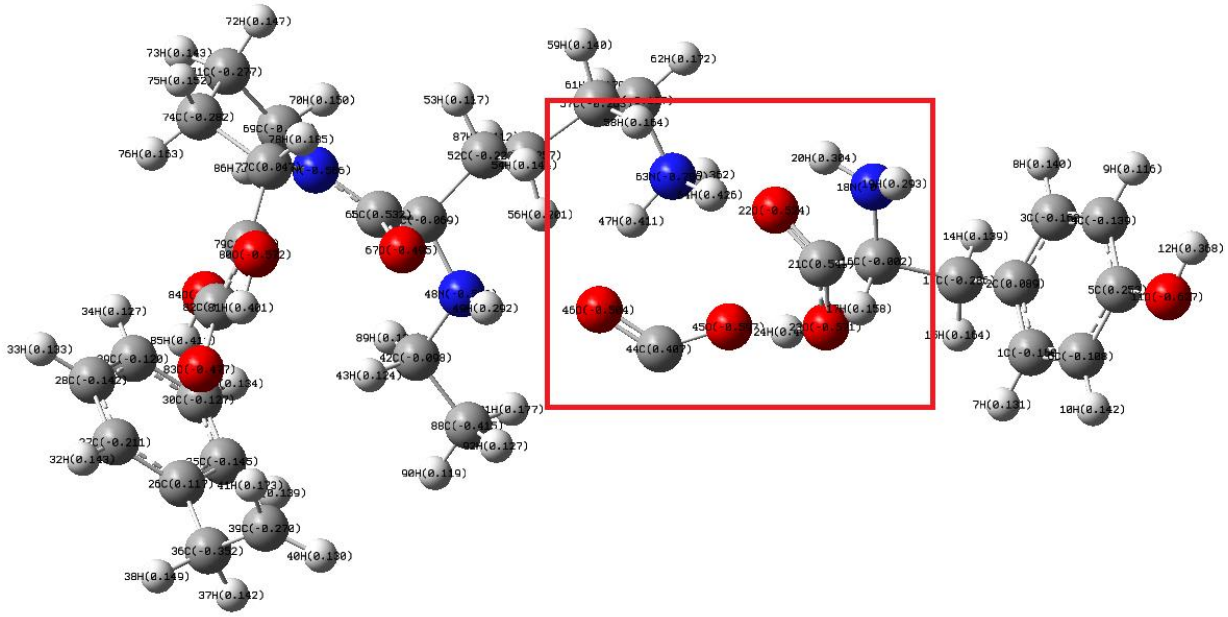


Figure 4.22 Optimized complex of tyrosine and lisinopril calculated by DFT/B3LYP

4.5 Electronic Parameters

The electronic parameters of NSAIDs, amino acids and antihypertensive drug are calculated by DFT and are listed in Table 4.1 and Table 4.2. Calculated electronic parameters comprise of the values for dipole moments (α), ε_{HOMO}, ε_{LUMO} energies, ionization energies (I), electron affinities (A), chemical potentials (μ), chemical hardness (η) and electrophilicity indexes (ω). These electronic parameters are measure of the binding interaction of selected NSAIDs and amino acids with lisinopril.

4.5.1 Energies of Frontier Orbitals

Whenever two molecules interact to form a charge transfer complex by following reaction:

$$A^+ + B^- \rightleftharpoons A^+B^-$$

electron jumps from the HOMO of the nucleophile (A) to the LUMO of the electrophile (B). Difference of these orbitals gives an estimate of band gap which shows the strength of complex formed. Energies of frontier orbitals are the measure of strength and stability of complexes. They are also referred as ε_{HOMO} and ε_{LUMO}. These values give an estimate of electron donating and electron accepting character of a given compound.

According to the FMO Theory-II we can predict reactivity between NSAIDs and lisinopril and amino acids and lisinopril. Reduction of band gap between ε_{HOMO} and ε_{LUMO} will strengthen the bond interactions. Based on this theory one can measure the strength and stability of the complexes. For NSAIDs, amino acids and lisinopril these parameters were calculated for better understanding of their complexation and are shown in Table 4.1 and Table 4.2. According to the calculated band gap (ε_{HOMO} of NSAIDs – ε_{LUMO} of lisinopril) aspirin has the minimum value. This indicates that aspirin will tend to form the most stable complex with lisinopril as compared to other NSAIDs. Similarly, according to the band gap values of amino acids lysine has the least value thus showing that it will form relatively stable complex with lisinopril.

4.5.2.1 ε_{HOMO}

ε_{HOMO} is the energy of highest occupied molecular orbital. An electron is considered to be more electron donating if its ε_{HOMO} increases. Based on this observation while interacting with aspirin, etodolac and ibuprofen lisinopril acts as a donor and these NSAIDs acts as acceptor. Whereas, mefenamic acid and naproxen have lower ε_{HOMO} values which indicates that lisinopril act as an acceptor while interacting with these two drugs. Figure 4.23 depicts the regions of NSAIDs and amino acids which will act as electron accepting and electron donating regions. As it is clear from the values that while interacting with amino acids lisinopril acts as acceptor.

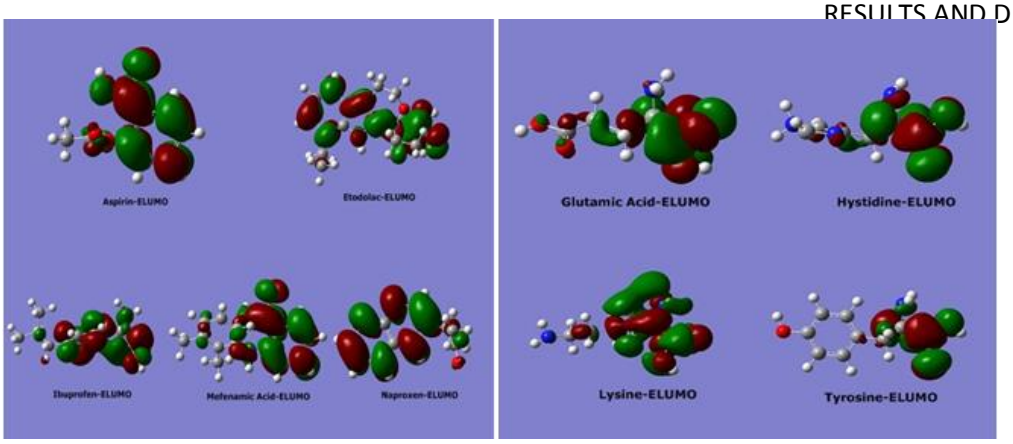


Figure 4.23 Molecular orbitals of NSAIDs and amino acids

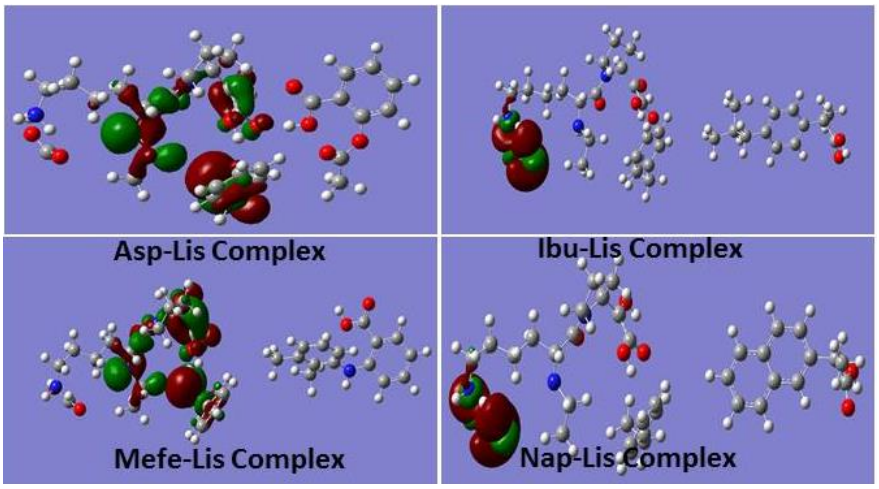


Figure 4.24 E_{HOMO} orbitals of NSAIDs-Lisinopril complexes

4.5.2.2 ε_{LUMO}

A compound acts as more electron accepting if its ε_{LUMO} decreases. According to the above calculated values shown in Table 4.1, lisinopril has low LUMO energy values for most of the NSAIDs except mefenamic acid and naproxen, as discussed earlier. While interacting with amino acids, amino acids act as donor and lisinopril as acceptor. ε_{LUMO} of lisinopril is less than NSAIDs which specifies that accepting power of lisinopril is far less than NSAIDs and NSAIDs are good acceptors as compared to lisinopril. So in presence of NSAIDs lisinopril will more feasibly interact with NSAIDs rather than amino acids of ACE enzyme thus affecting the antihypertensive ability of lisinopril.

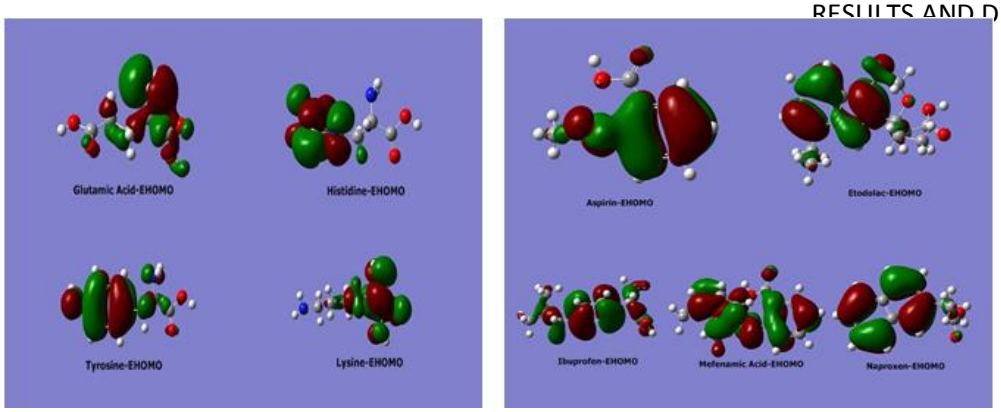


Figure 4.25 E_{LUMO} Molecular orbitals of NSAIDs and amino acids

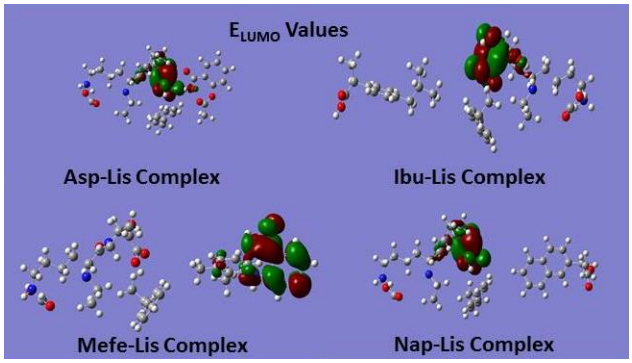


Figure 4.26 E_{LUMO} Molecular orbitals of NSAIDs-Lisinopril complexes

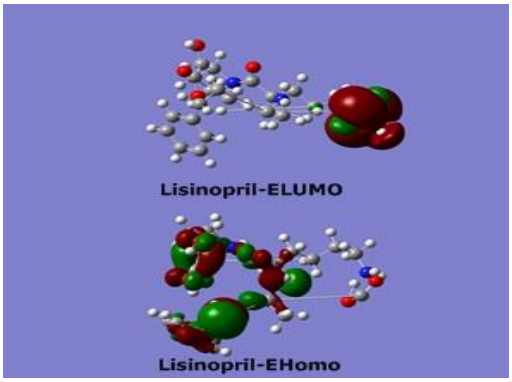


Figure 4.27 Molecular orbitals of lisinopril

Table 3.1 Significant computed Dipole Moment(α), ε_{HOMO} and ε_{LUMO} of NSAIDs and Lisinopril

Drugs	ε_{HOMO}/eV	ε_{LUMO}/eV	Complexes		Band Gap/eV
			ε_{HOMO}/eV	ε_{LUMO}/eV	
Aspirin	-0.26069	-0.06448	-0.18907	-0.10144	-0.1396
Etodolac	-0.23657	-0.01773			-0.18635
Ibuprofen	-0.21639	-0.04024	-0.14980	-0.09190	-0.16384
Mefenamic Acid	-0.19469	-0.04670	-0.18842	-0.04579	-0.15738
Naproxen	-0.20315	-0.03900	-0.15002	-0.09245	-0.16508
lisinopril	-0.20408	-0.01745			-0.18663

Table 3.2 Significant computed Dipole Moment(α), ε_{HOMO} and ε_{LUMO} of Amino acids and Lisinopril

AminoAcids	ε_{HOMO}/eV	ε_{LUMO}/eV	Complexes		Band Gap/eV
			ε_{HOMO}/eV	ε_{LUMO}/eV	
Glutamic Acid	-0.24286	-0.01768	-0.16434	-0.09328	-0.1864
Hystidine	-0.11557	-0.00314	-0.10868	-0.08481	-0.20094
Lysine	-0.16229	0.05530	-0.17270	-0.00808	-0.25938
Tyrosine	-0.21345	-0.01828	-0.16367	-0.0922	-0.1858
lisinopril	-0.20408	-0.01745			-0.18663

4.5.2 Different Parameters from DFT-MO Calculations

Different parameters are calculated from DFT-MO calculations by using the equations mentioned in section 3.4.2 and are given in Table 4.3 and 4.4.

Electron affinity is the amount of energy released when an electron is added to a neutral atom or molecule to form negative ion. According to the computed values, all NSAIDs except etodolac have high electron affinity as compared to lisinopril. These compounds will attract the electrons of lisinopril and bind more strongly as evident from FMO theory II. In case of amino acids, E.A of all amino acids is lower than lisinopril. So lisinopril can be an acceptor but it cannot be as good as NSAIDs. So, binding of NSAIDs with lisinopril is more favorable as compared to complexation of lisinopril with amino acid.

Chemical hardness which is associated to the stability and reactivity of a chemical system has been accessed by examining the selected NSAIDs, amino acids of ACE enzyme and lisinopril. According to the calculated values ibuprofen and aspirin have highest values among the NSAIDs. Hence are least polarizable and least reactive as compared to other drugs. Among the amino acids histidine is the most reactive and most polarizable due to the lowest value for chemical hardness.

Electrophilicity index is the measure of the propensity of the molecule to accept electrons. Greater the electrophilicity index, more capable the compound is of accepting the electrons. According to the calculated parameters shown in Table 3.3 and 3.4, aspirin has the highest electrophilicity index, whereas, among amino acids lysine has the highest electrophilicity index.

Table 3.3 Chemical reactivity parameters for NSAIDs and Lisinopril

Drugs	E.A(A)/eV	Chem pot (μ) eV	Chem hard(η) /eV	E.I (ω)
Aspirin	0.06448	-0.162585	0.098105	0.13472
Etodolac	0.01409	-0.106345	0.092255	0.06129
Ibuprofen	0.01773	-0.12715	0.10942	0.07388
Mefe acid	0.04670	-0.120695	0.073995	0.09843
naproxen	0.03900	-0.121075	0.082075	0.08930
lisinopril	0.01745	-0.110765	0.093315	0.06574

Table 3.4 Chemical reactivity parameters for Amino acids and Lisinopril

Drugs	E.A(A)/eV	Chem pot (μ) /eV	Chem hard(η) /eV	E.I (ω)
Glutamic Acid	0.01768	-0.13027	0.11259	0.07536
Histidine	0.00314	-0.05936	0.05622	0.03134
Lysine	-0.05530	-0.21759	0.10880	0.21758
Tyrosine	0.01828	-0.11587	0.09759	0.06879
lisinopril	0.01745	-0.110765	0.093315	0.06574

4.6 Discrete Modeling

Discrete Modeling was performed in the present work to explore the effect of NSAIDs on hypertension patients which is caused due to the interaction between two kinds of drugs, NSAIDs and antihypertensive drugs. Jimena simulation framework was used for discrete modeling in this case. Different scenarios regarding the concentrations of both drugs were focused. Each of them is discussed below.

Case 1: Introduction of NSAIDs in hypertension patients

When hypertension patients acquire NSAIDs, they may interfere with the effect of antihypertensive drugs. This interference may cause an increase in blood pressure, thus affecting the efficacy of blood pressure lowering drug. In this case both drugs were included and their effect was studied over time. The input table shown in Figure 4.28 indicates values of entities which were given to the software as input file. Each entity has its own initial value which shows its initial concentration. Figure 4.29 shows the output graphs obtained by simulation framework performed on the given input table. This graph indicates that PGs inhibition causes an increase in blood pressure which causes hindrance with the antihypertensive effect of antihypertensive drugs. At start due to introduction of antihypertensive drug blood pressure is lowered but due to the effect of NSAID blood pressure elevates, thus reduces the effect of antihypertensive drug.

Nodes Table - Ctrl+V: load state from clipboard

Node	Value	Hill normalized	Odefy decay	SQUAD decay	SQUAD steepness
ACE	0	☑	1	1	10
Angiotensin	0	☑	1	1	10
Angiotensin I	0	☑	1	1	10
Angiotensin II	0	☑	1	1	10
Antihypertensive drug	0.4	☑	1	0.001	10
Arachidonic Acid	0	☑	1	1	10
Blood Pressure	0	☑	1	1	10
Bradykinin	0	☑	1	1	10
COX (I-II)	0	☑	1	1	10
NSAIDS	0.8	☑	1	0.001	10
Prostaglandins(PGs)	0	☑	1	1	10
Renin	0	☐	1	1	10
Salt Water Retention	0	☑	1	1	10

Figure 4.28 Input Nodes Table for Case-1

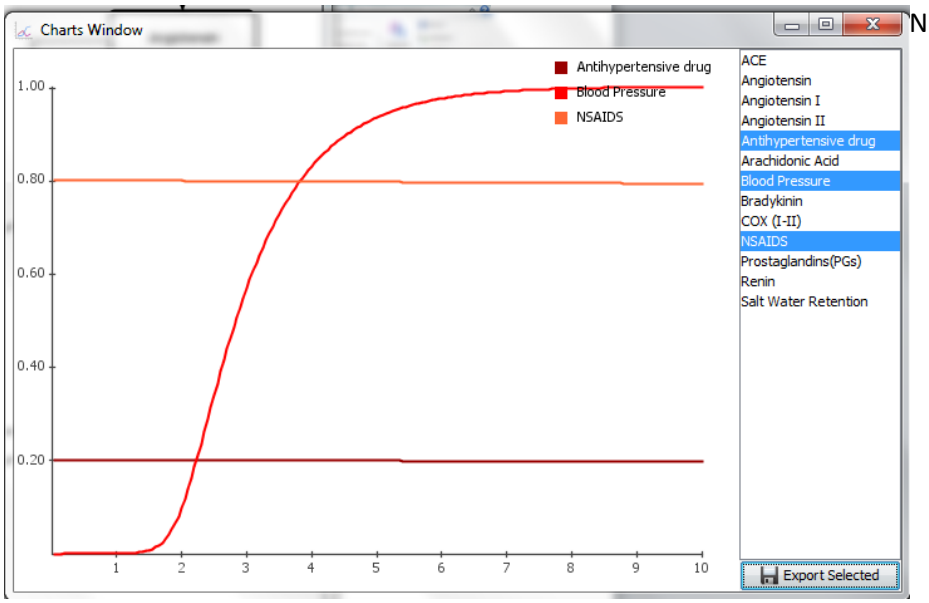


Figure 4.29 Relation of entities (NSAIDs and antihypertensive drug) in Case I

Case2: Effect on antihypertensive drug with prolonged dosage of NSAIDs

In this case effect in hypertensive patients was studied when NSAIDs were introduced to them for a long period. Figure 4.30 shows the input nodes table which depicts the initial concentration of the entities. After Jimena had perfomed simulations resulting graph was obtained, shown in Figure 4. 31. According to the results in the graph NSAIDs introduction completely antagonizes the effect of antihypertensive drug and elevates blood pressure to its maximum.

Nodes Table - Ctrl+V: load state from clipboard

Node	Value	Hill normalized	Odefy decay	SQUAD decay	SQUAD steepness
ACE	0	☑	1	1	10
Angiotensin	0	☑	1	1	10
Angiotensin I	0	☑	1	1	10
Angiotensin II	0	☑	1	1	10
Antihypertensive drug	0.05	☑	1	0.001	10
Arachidonic Acid	0	☑	1	1	10
Blood Pressure	0	☑	1	1	10
Bradykinin	0	☑	1	1	10
COX (I-II)	0	☑	1	1	10
NSAIDS	1	☑	1	0	10
Prostaglandins(PGs)	0	☑	1	1	10
Renin	0	☐	1	1	10
Salt Water Retention	0	☑	1	1	10

Figure 4.30 Input Nodes Table for CASE-II

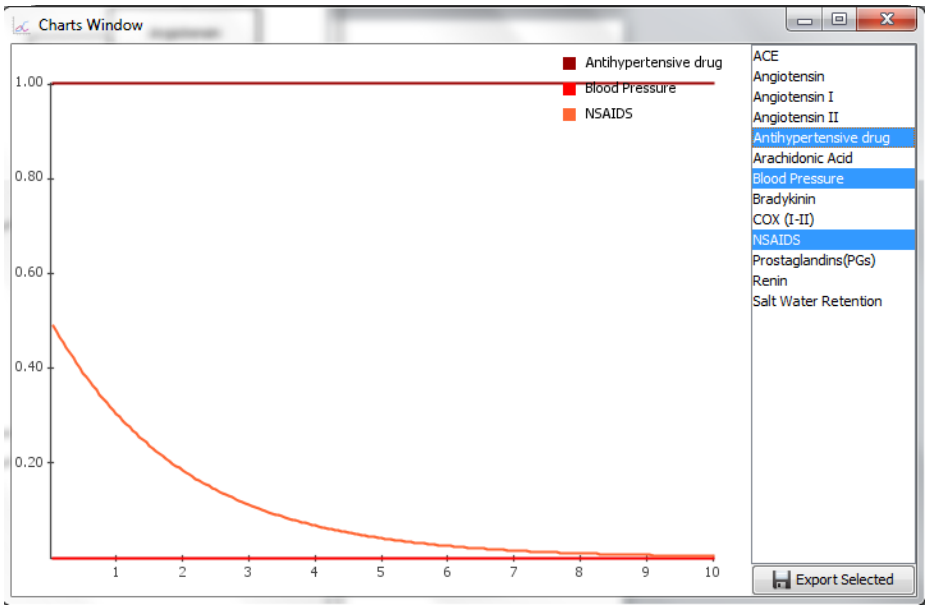
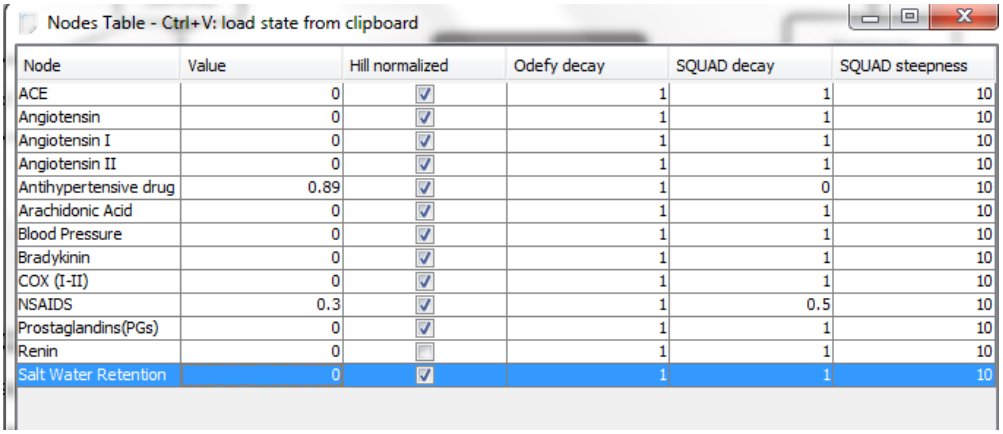


Figure 4.31 Relation of entities (NSAIDs, Antihypertensive drug) in Case II

Case 3: NSAIDs in acute hypertension

This case mimics a situation in which a patient with acute hypertension, who is on a heavy antihypertensive therapy, was given NSAIDs for small period of time. NSAIDs in this case also tend to lower the blood pressure but its effect was soon overcome by the antihypertensive effect of antihypertensive drug.



Node	Value	Hill normalized	Odefy decay	SQUAD decay	SQUAD steepness
ACE	0	☑	1	1	10
Angiotensin	0	☑	1	1	10
Angiotensin I	0	☑	1	1	10
Angiotensin II	0	☑	1	1	10
Antihypertensive drug	0.89	☑	1	0	10
Arachidonic Acid	0	☑	1	1	10
Blood Pressure	0	☑	1	1	10
Bradykinin	0	☑	1	1	10
COX (I-II)	0	☑	1	1	10
NSAIDS	0.3	☑	1	0.5	10
Prostaglandins(PGs)	0	☑	1	1	10
Renin	0	☐	1	1	10
Salt Water Retention	0	☑	1	1	10

Figure 4.32 Input Nodes Table for CASE-III

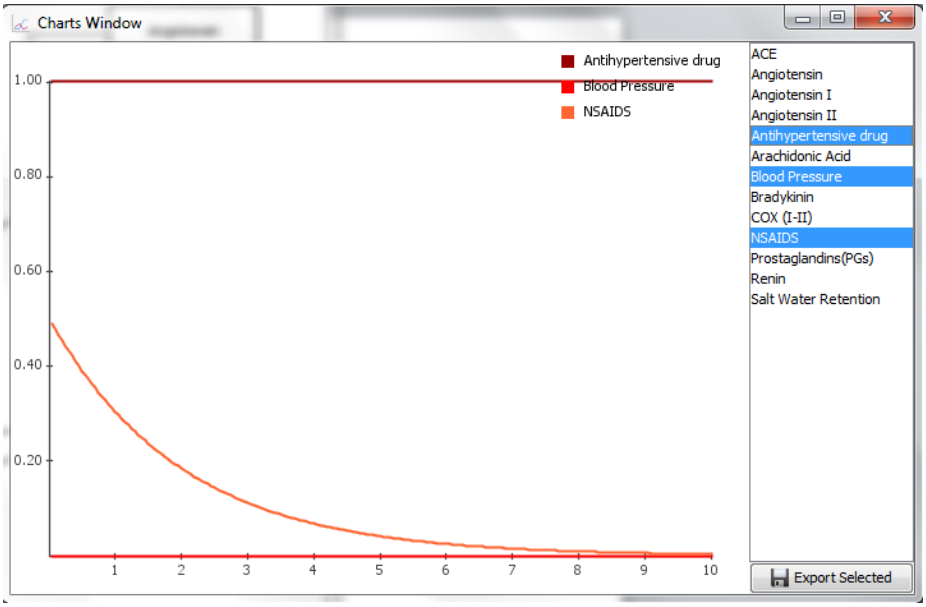


Figure 4.33 Relation of entities (NSAIDs, Antihypertensive drug) in Case II

Chapter 5

Conclusion:

Presently, comparative binding study of an antihypertensive drug lisinopril with five standard NSAIDs (aspirin, etodolac, ibuprofen, mefenamic acid and naproxen) and four selected amino acids of ACE enzyme (glutamic acid, histidine, lysine and tyrosine) were carried out using Quantum Mechanical Molecular Orbital Calculations with DFT/B3LYP and HF method with 6-31G basis set.

A number of electronic and geometric parameters were calculated for NSAIDs and amino acids before and after complex formation with antihypertensive drug lisinopril. Geometric parameters included Bond Length, Bond Angles and Dihedral angle. It was observed that after complexation electronic charge density of aspirin, etodolac and ibuprofen is increased and that of lisinopril is decreased. This shows that electronic charge is transferred from lisinopril to these three NSAIDs developing strong electrostatic interactions. In case of mefenamic acid and naproxen electronic charge distribution is slightly decreased and that of lisinopril is slightly increased, which reveals existence of Vander walls forces of attractions. Four amino acids showed a decrease in electronic distribution while interacting with lisinopril, indicating electron with drawing effect of lisinopril in case of amino acids.

Electronic parameters included energies of frontier orbital i.e. E_{HOMO}, E_{LUMO}, Electron Affinity, Chemical potential, Chemical hardness and Electrophilicity index of compounds. Comparing the E_{HOMO} and E_{LUMO} it was observed that E_{HOMO} of aspirin etodolac and ibuprofen are more negative and that of lisinopril is less negative. This shows that lisinopril has donor character and these three NSAIDs have acceptor character. Whereas, E_{HOMO} of mefenamic acid

and naproxen are less negative than lisinopril, showing acceptor character of lisinopril. Similar observations were obtained for E_{HOMO}, E_{LUMO} values of amino acids. Amino acids showed donor character while interacting with lisinopril and lisinopril showed acceptor character but the acceptor character of lisinopril is weak as compared to that of NSAIDs, which show favourable complex formation of lisinopril with NSAIDs as compared to amino acids of ACE enzyme.

Different parameters calculated from E_{HOMO} and E_{LUMO} also supported the complex formation of lisinopril with NSAIDs as compared to amino acids in comparative studies. As far as band gap is concerned according to FMO theory of E_{HOMO} (nucleophile) - E_{LUMO} (electrophile) lowest value of band gap is for aspirin and highest value is for etodolac, indicating the highest probability of charge transfer complex formation for aspirin and lowest for etodolac. E_{LUMO} of aspirin-lisinopril complex is more negative as compared to E_{LUMO} of aspirin. This confirmed binding propensity of aspirin with NSAIDs as compared to other NSAIDs and amino acids. Thus approving the drug interaction caused by NSAIDs with antihypertensive drugs.

APPENDIX A

GEOMETRIC PARAMETERS OF NSAIDS AMINO ACIDS AND THEIR COMPLEXES WITH LISINOPRIL

Table A-1 Significant computed geometric parameters of Aspirin before and after complex formation with lisinopril

Bond Lengths/ A^0			**Bond Angles/^{0}**					
Bond Length	**Aspirin**	**Asp-Lis**	**Bond Angle**	**Aspirin**	**Asp-Lis**	**Dihedral Angle**	**Aspirin**	**Asp-Lis**
R(11,12)	1.2396	1.2478	**A(3,4,11)**	126.3919	126.2743	**D(11,4,5,6)**	-178.899	-178.403
R(11,13)	1.3803	1.358	**A(5,4,11)**	115.7167	116.0916	**D(11,4,5,9)**	0.8963	1.2292
R(13,14)	0.9819	1.01	**A(4,11,12)**	124.1578	122.3456	**D(3,4,11,12)**	177.5033	168.6791
R(15,16)	1.4064	1.3976	**A(4,11,13)**	115.5724	115.7837	**D(3,4,11,13)**	-3.4265	-13.0057
R(16,17)	1.225	1.2283	**A(12,11,13)**	120.2633	121.8485	**D(5,4,11,12)**	-3.113	-11.7013
R(16,18)	1.4968	1.4968	**A(11,13,14)**	108.5541	111.2987	**D(5,4,11,13)**	175.9572	166.6139
			A(3,15,16)	120.7919	121.4873	**D(4,11,13,14)**	179.3134	179.6991
			A(15,16,17)	122.7925	122.9388	**D(12,11,13,14)**	-1.5774	-1.9765
			A(15,16,18)	109.5909	109.744	**D(3,15,16,17)**	-7.7633	-8.304
			A(17,16,18)	127.615	127.3148	**D(3,15,16,18)**	172.652	172.2164
			A(16,18,19)	110.6316	110.632	**D(15,16,18,19)**	49.7114	48.8935
			A(16,18,20)	109.7194	109.8288	**D(15,16,18,20)**	171.5854	170.872
			A(16,18,21)	108.8101	108.6603	**D(15,16,18,21)**	-68.2598	-68.946
						D(17,16,18,19)	-129.848	-130.557
						D(17,16,18,20)	-7.9739	-8.5788

Table A-2 Significant computed geometric parameters of Etodolac before and after complex formation with lisinopril

Bond lengths /A⁰			**Bond Angles/⁰**					
Bond Length	**Etodolac**	**Eto-Lis**	**Bond Angle**	**Etodolac**	**Eto-Lis**	**Dihedral Angle**	**Etodolac**	**Eto-Lis**
R(3,4)	1.4121	1.4141	**A(2,3,4)**	121.1256	121.3112	**D(1,2,3,4)**	0.0571	0.1126
R(3,8)	1.0855	1.0859	**A(2,3,8)**	119.7784	119.8434	**D(1,2,3,8)**	-179.667	-179.608
R(4,5)	1.3991	1.3994	**A(4,3,8)**	119.0954	118.8449	**D(7,2,3,4)**	179.6711	179.4839
R(4,9)	1.0866	1.0866	**A(3,4,5)**	122.1735	121.8869	**D(7,2,3,8)**	-0.0533	-0.2365
R(10,12)	1.0967	1.0962	**A(3,4,9)**	119.1701	118.8572	**D(2,3,4,5)**	0.2017	0.1906
			A(5,4,9)	118.6561	119.2544	**D(2,3,4,9)**	-179.617	-179.354
			A(5,10,12)	109.0447	109.02	**D(8,3,4,5)**	179.9279	179.9137
						D(8,3,4,9)	0.1098	0.3696
						D(4,5,10,12)	20.3691	19.945

Table A-3 Significant computed geometric parameters of Ibuprofen before and after complex formation with lisinopril

Bond Lengths /A⁰			**Bond Angles/⁰**					
Bond Length	**Ibuprofen**	**Ibu-Lis**	**Bond Angle**	**Ibuprofen**	**Ibu-Lis**	**Dihedral Angle**	**Ibuprofen**	**Ibu-Lis**
R(24,26)	1.5407	1.5409	**A(24,25,29)**	110.8182	110.8088	**D(27,24,25,30)**	179.6038	179.582
R(25,29)	1.0966	1.0965	**A(24,25,30)**	110.6238	110.6075	**D(21,24,26,31)**	-57.9739	-58.1643
R(25,30)	1.0987	1.0989	**A(28,25,29)**	108.0358	108.0556	**D(21,24,26,32)**	62.024	61.6719
R(26,31)	1.0969	1.0968	**A(28,25,30)**	107.9497	107.9452	**D(21,24,26,33)**	-178.351	-178.637
R(26,32)	1.0983	1.0985	**A(29,25,30)**	107.7007	107.7121	**D(25,24,26,31)**	177.4599	177.2789
R(26,33)	1.0964	1.0964	**A(24,26,31)**	111.3634	111.3533	**D(25,24,26,32)**	-62.5422	-62.8849
			A(24,26,32)	110.7727	110.7098	**D(25,24,26,33)**	57.0832	56.8061
			A(24,26,33)	111.0414	111.0651	**D(27,24,26,31)**	59.1289	58.9515
			A(31,26,32)	107.8274	107.7379	**D(27,24,26,32)**	179.1268	178.7877
			A(31,26,33)	107.9835	108.0519	**D(27,24,26,33)**	-61.2478	-61.5213
			A(32,26,33)	107.7002	107.7731			

Table A-4 Significant computed geometric parameters of Mefenamic Acid before and after complex formation with lisinopril

Bond Lengths/ A^0			**Bond Angles/^{0}**					
Bond Length	**Mefenamic acid**	**Mefe-Lis**	**Bond Angle**	**Mefenamic acid**	**Mefe-Lis**	**Dihedral Angle**	**Mefenamic acid**	**Mefe-Lis**
R(5,6)	1.4045	1.4046	**A(6,5,10)**	119.1391	119.1444	**D(3,4,5,6)**	1.5134	1.4858
R(6,9)	1.0858	1.0858	**A(1,6,5)**	120.8178	120.8479	**D(3,4,5,10)**	-179.152	1.4858
R(10,11)	1.0941	1.0942	**A(1,6,9)**	119.9529	119.957	**D(14,4,5,6)**	-177.567	-177.5841
R(10,12)	1.098	1.0977	**A(5,6,9)**	119.2291	119.195	**D(14,4,5,10)**	1.767	1.7428
R(10,13)	1.0972	1.0973	**A(5,10,11)**	110.5613	110.5606	**D(4,5,6,1)**	-0.6891	-0.6884
			A(5,10,12)	112.0798	112.1516	**D(4,5,6,9)**	179.4386	179.3843
			A(5,10,13)	111.9951	112.0102	**D(10,5,6,1)**	179.9621	179.9701
			A(11,10,12)	107.3866	107.2403	**D(10,5,6,9)**	0.0898	0.0427
			A(11,10,13)	107.5804	107.6044	**D(4,5,10,11)**	178.3064	178.8157
			A(12,10,13)	106.9929	107.0214	**D(4,5,10,12)**	-61.9204	-61.5493
						D(4,5,10,13)	58.344	58.8133
						D(6,5,10,11)	-2.3565	-1.8548
						D(6,5,10,12)	117.4166	117.7802
						D(6,5,10,13)	-122.319	-121.8573

Table A-5 Significant computed geometric parameters of Naproxen before and after complex formation with lisinopril

Bond Lengths/ A⁰			**Bond Angles/⁰**					
Bond Length	**Naproxen**	**Nap-Lis**	**Bond Angle**	**Naproxen**	**Nap-Lis**	**Dihedral Angle**	**Naproxen**	**Nap-Lis**
R(1,2)	1.3841	1.3804	**A(2,1,6)**	120.5288	120.2911	**D(6,1,2,3)**	-0.0258	0.0402
R(1,6)	1.4205	1.4204	**A(2,1,17)**	116.0743	120.1458	**D(6,1,2,9)**	179.95	179.8692
R(1,17)	1.3929	1.0855	**A(6,1,17)**	123.3969	119.5628	**D(8,1,2,3)**	-180.002	-179.742
R(2,8)	1.0843	1.0866	**A(1,2,3)**	120.5894	120.7621	**D(8,1,2,9)**	-0.0261	0.0873
R(10,13)	1.3841	1.385	**A(1,2,8)**	118.8874	120.462	**D(2,1,6,5)**	0.0212	-0.0706
			A(3,2,8)	120.5232	118.7758	**D(2,1,6,13)**	-179.967	179.9964
			A(4,10,13)	121.2853	121.3131	**D(8,1,6,5)**	179.9975	179.7126
			A(10,13,14)	119.0985	119.234	**D(8,1,6,13)**	0.0092	-0.2205
						D(1,2,3,4)	-0.0161	0.001
						D(1,2,3,10)	179.8188	179.7676

Table A-6 Significant computed geometric parameters of Glutamic acid before and after complex formation with lisinopril

Bond Lengths/ A^0			**Bond Angles/^{0}**					
Bond Length	**Glutamic Acid**	**Glut-Lis**	**Bond Angle**	**Glutamic acid**	**Glut-LIs**	**Dihedral Angle**	**Glutamic acid**	**Glut-Lis**
R(13,14)	1.0138	1.0138	**A(11,13,14)**	114.4882	114.4171	**D(8,11,13,14)**	-161.074	-159.867
R(13,15)	1.0158	1.0156	**A(11,13,15)**	113.3052	113.0694	**D(8,11,13,15)**	70.4014	71.774
R(16,17)	1.2334	1.2597	**A(14,13,15)**	110.8906	110.9719	**D(12,11,13,14)**	-42.4088	-41.2232
R(16,18)	1.379	1.3279	**A(11,16,17)**	125.784	121.7074	**D(12,11,13,15)**	-170.933	-169.582
R(18,19)	0.9824	1.0411	**A(11,16,18)**	111.8634	113.4676	**D(16,11,13,14)**	75.7448	77.1497
			A(17,16,18)	122.3488	124.8118	**D(16,11,13,15)**	-52.7797	-51.2092
			A(16,18,19)	110.6208	116.0386	**D(8,11,16,17)**	-111.853	-108.653
						D(8,11,16,18)	67.4463	70.0893
						D(12,11,16,17)	130.9968	134.6627
						D(12,11,16,18)	-49.7039	-46.5954
						D(13,11,16,17)	11.9989	15.2359
						D(13,11,16,18)	-168.702	-166.022

Table A-7 Significant computed geometric parameters of Lysine before and after complex formation with lisinopril

Bond Lengths/A^0			**Bond Angles/^{0}**					
Bond Length	**Lysine**	**Lys-Lis**	**Bond Angle**	**Lysine**	**Lys-Lis**	**Dihedral Angle**	**Lysine**	**Lys-Lis**
R(13,14)	1.3701	1.3586	**A(11,10,13)**	108.9971	109.3398	**D(7,10,13,14)**	89.9072	95.1055
R(13,17)	1.4258	1.4198	**A(12,10,13)**	109.3949	108.9769	**D(7,10,13,17)**	-86.7182	-82.3359
R(14,15)	1.0106	1.0264	**A(10,13,14)**	120.1337	119.2621	**D(11,10,13,14)**	-148.922	-143.52
R(14,16)	1.0055	1.0085	**A(10,13,17)**	124.4232	122.0469	**D(11,10,13,17)**	34.453	39.0384
R(17,18)	1.2619	1.2743	**A(14,13,17)**	115.3647	118.6429	**D(12,10,13,14)**	-32.1169	-26.7469
			A(13,14,15)	118.1795	123.4747	**D(12,10,13,17)**	151.2577	155.8118
			A(13,14,16)	121.8604	119.6323	**D(10,13,14,15)**	-177.677	-177.343
			A(15,14,16)	119.9068	116.8921	**D(10,13,14,16)**	4.9827	2.3107
			A(13,17,18)	124.776	127.2886	**D(17,13,14,15)**	-0.7569	0.1861
			A(13,17,19)	113.9975	114.0429	**D(17,13,14,16)**	-178.098	179.8396
			A(18,17,19)	121.2265	118.6674	**D(10,13,17,18)**	178.2175	178.2216
						D(10,13,17,19)	-1.7103	-2.1716
						D(14,13,17,18)	1.4473	0.765
						D(14,13,17,19)	-178.481	-179.628

Table A-8 Significant computed geometric parameters of Histidine before and after complex formation with lisinopril

Bond Lengths/A^0			**Bond Angles/^{0}**					
Bond Length	**Histidine**	**Hist-Lis**	**Bond Angle**	**Histidine**	**His-Lis**	**Dihedral Angle**	**Histidine**	**His-Lis**
R(10,13)	1.5435	1.5488	**A(10,13,14)**	105.9841	105.3649	**D(10,13,15,17)**	165.8818	162.6641
R(13,14)	1.1001	1.0997	**A(10,13,15)**	111.3763	111.4668	**D(14,13,15,16)**	179.9361	176.4751
R(13,15)	1.4578	1.4539	**A(10,13,18)**	110.2922	110.2233	**D(14,13,15,17)**	50.1931	47.3861
R(13,18)	1.529	1.5267	**A(14,13,15)**	107.5004	107.986	**D(18,13,15,16)**	61.9819	58.1037
R(15,16)	1.0148	1.0148	**A(14,13,18)**	106.1727	106.2284	**D(18,13,15,17)**	-67.7611	-70.9854
R(15,17)	1.0128	1.013	**A(15,13,18)**	114.9455	114.9578	**D(10,13,18,19)**	-28.5842	-44.138
R(18,19)	1.2354	1.2593	**A(13,15,16)**	114.1984	114.0565	**D(10,13,18,20)**	153.4969	137.1449
R(18,20)	1.3847	1.3403	**A(13,15,17)**	114.2275	114.2568	**D(14,13,18,19)**	85.8044	69.5225
R(20,21)	0.9823	1.0195	**A(16,15,17)**	111.3213	110.9444	**D(14,13,18,20)**	-92.1145	-109.195
			A(13,18,19)	126.5454	122.1327	**D(15,13,18,19)**	-155.496	-171.125
			A(13,18,20)	112.2965	115.1171	**D(15,13,18,20)**	26.585	10.1584
			A(19,18,20)	121.1253	122.7371	**D(13,18,20,21)**	177.0358	178.1198
			A(18,20,21)	109.4808	113.4542	**D(19,18,20,21)**	-1.0111	-0.5887

Table A-9 Significant computed geometric parameters of Tyrosine before and after complex formation with lisinopril

Bond Lengths/A^0			**Bond Angles/^{0}**					
Bond Length	**Tyrosine**	**Tyr-Lis**	**Bond Angle**	**Tyrosine**	**Tyr-Lis**	**Dihedral Angle**	**Tyrosine**	**Tyr-Lis**
R(16,17)	1.0999	1.1078	**A(17,16,18)**	107.5128	113.3281	**D(17,16,18,19)**	-52.3208	45.331
R(16,18)	1.4593	1.4505	**A(17,16,21)**	105.1441	104.9743	**D(17,16,18,20)**	178.9764	161.996
R(16,21)	1.5348	1.5189	**A(18,16,21)**	114.6449	109.2619	**D(21,16,18,19)**	64.1772	50.6828
R(18,19)	1.0138	1.0085	**A(16,18,19)**	114.1967	116.0425	**D(21,16,18,20)**	-64.5256	-94.6291
R(18,20)	1.0156	1.0101	**A(16,18,20)**	113.599	115.188	**D(13,16,21,22)**	32.8874	22.036
R(21,22)	1.2343	1.2615	**A(19,18,20)**	111.012	115.8241	**D(13,16,21,23)**	-149.233	-130.628
R(21,23)	1.3823	1.3269	**A(16,21,22)**	126.0903	121.7999	**D(17,16,21,22)**	-82.8205	114.6867
R(23,24)	0.9823	1.0412	**A(16,21,23)**	112.3375	113.9	**D(17,16,21,23)**	95.0589	64.0029
			A(22,21,23)	121.5378	124.286	**D(18,16,21,22)**	159.3296	174.1477
			A(21,23,24)	109.8659	115.8179	**D(18,16,21,23)**	-22.791	-7.1628
						D(16,21,23,24)	-177.386	-177.740
						D(22,21,23,24)	0.6038	0.9121

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