CHAPTER – II

CRYSTAL STRUCTURE ANALYSIS AND MOLECULAR DOCKING STUDIES OF PHENYLACRYLATE DERIVATIVES
2.1. Introduction

Phenyl acrylates or cinnamic esters derivatives are naturally occurring substances found in fruits, vegetables, flowers and are consumed as dietary phenolic compounds. They play a vital role in the formation of commercially important intermediate molecules which are necessary for the production of different pharmaceutical ingredients. The acrylate ion (CH$_2$=CHCOO$^-$) is the ion of acrylic acid. Acrylates are the salts and esters of acrylic acid. They are also known as propenoates. Acrylates contain vinyl groups, that is, two carbon atoms double bonded to each other, directly attached to the carbonyl carbon. Acrylates and methacrylates (the salts and esters of methacrylic acid) are common monomers in polymer plastics, forming the acrylate polymers.

Acrylates easily form polymers because the double bonds are very reactive. In biological chemistry, methyl trans phenylacrylate is a key intermediate in shikimate and phenylpropanoid pathways. Shikimic acid is a precursor of many alkaloids, aromatic amino acids, and indole derivatives. Phenyl acrylate can also be named as cinnamic ester or cinnamate. Phenylacrylate play vital role in the synthesis of other important compounds.
For example, Phenyl acrylate derivatives can be converted into immensely important compounds including styrenes and stilbenes through decarboxylation reaction.

Phenyl acrylates are also obtained from various plant sources and find their application in perfumery, cosmetic industries and in pharmaceutics. For example, methyl caffeate is found in plant like *Gaillardia pulchella*, *Gochnatrus rusbyana*, *Netopterygium incisum* and as 4glycoside in the fruits of *Linum usitatissimum*. The compound is reported to have antitumour activity against Sarcoma 180 as well as antimicrobial activity (Nam et al., 2001). Methoxy substituted phenylacrylate such as ethyl 3, 4, 5-trimethoxycinnamate is present in *Piper longum* and plays an important role in controlling inflammatory diseases [Kumar et al., 2005]. Similarly long chain cinnamic ester like methoxy substituted octylcinnamates are well known sunscreen agent and ideally suited for cosmetic applications since they are non irritating to skin and provide lubricity to prevent drying effect of wind (Alexander & Choudary, 1996).

The Phenylacrylate derivatives are extremely versatile and have featured in various drugs. There are many drugs which are used against the Mycobacterium tuberculosis, but the main disadvantages of these drugs are that they develop resistance more abruptly. Cerulenin, an inhibitor of fatty acid and trans-cinnamic acid, which was recently shown to augment the activity of various antibiotic drugs against M. avium (Rastogi et al, 1998).
Antimicrobial activity of methyl 3-phenylacrylate derivatives is due to the presence of ester and amide groups.

Narasimhan and co-workers have reported the antibacterial activity against Escherichia coli and Staphylococcus aureus, Bacillus subtilis (Gram negative and Gram positive respectively) and antifungal activity against Candida albicans and Aspergillus niger.

Methyl 3-phenylacrylate derivatives exhibit high antioxidant activity, due to the presence of vinyl fragments. This property attracts attention to the study of these compounds as potential drugs for the treatment of pathologies related to the lipid peroxidation in cellular membranes (Chen & Ho, 1997). Trans-phenylacrylate show antiviral activity against equid herpesvirus 1 (EHSV-1) (Yoon et al, 2007).

Halogenated cinnamic acid derivatives showed the highest CNS depressant activity (Yabe et al., 2010). 4-propoxycinnamic acid, a derivate of phenylacrylate resulted in a significant improvement in anti-malarial activity (Kanaani & Ginburg, 1992). m- hydroxy or p-methoxy residues of cinnamic acid were significantly important substituent as an effective insulin releasing agent(Adisakwattana et al, 2005)

Methyl cinnamate used widely and is found in flavour and fragrance compositions created for products which include soaps and cosmetics as well as beverages and baked goods. Reported applications for cinnamic acid and its derivatives also include: preparation of herbicidal compositions; as a raw
material in the synthesis of heterocyclic color complexes. When cinnamic acid and p-methoxycinnamic acid intercalated into ZnAl layered double hydroxide by co-precipitation reaction, then it show UV rays absorption property. In addition to this methoxy substituted octyl –cinnamates is having excellent property of UV absorption that is why these are used in sunscreen composition.

In view of the wide range of pharmaceutical activities of the phenylacrylate derivatives, bromophenoxy, nitrophenoxy and methoxyphenoxy containing compounds, and in order to obtain detailed information about the molecular conformations of the following three phenylacrylate derivatives in the solid state, the X-ray structure determination of compounds I to III are carried out and the results are presented here. This chapter deals with the results of the X-ray crystal structure determination and its molecular modelling studies with Mushroom Tyrosinase as anti-microbial target of the following three phenyl acrylate derivatives code-named I, II and III.

I) \((E)\) - Methyl – 2 - [(4-bromo-2-formylphenoxy)methyl] – 3 - phenylacrylate

II) \((E)\) - Methyl – 2 - [(2-formyl-6-methoxyphenoxy)methyl] – 3 - phenylacrylate

III) \((E)\) - Methyl – 2 - [(2-nitrophenoxy)methyl] – 3 - phenylacrylate
Figure 2.1 Schematic representation of Phenylacrylate Derivatives
2.2 Experimental Procedures

The phenyl acrylate derivatives presented in this thesis were synthesised as follows:

**Compound I**

A solution of 5-bromo-2-hydroxybenzaldehyde (1.0 mmol, 0.201 g) and potassium carbonate (2.0 mmol, 0.2293 g) in acetonitrile solvent (5 ml) was stirred for 15 minute at room temperature. To this solution, (Z)-methyl-2-(bromomethyl)-3-phenylacrylate (1.2 mmol, 0.25 g) was added dropwise. After the completion of the reaction, as indicated by TLC, acetonitrile was evaporated. EtOAc (15 ml) and water (15 ml) were added to the crude mass. The organic layer was dried over anhydrous sodium sulfate. Removal of solvent led to the crude product, which was purified through pad of silica gel (100–200 mesh) using ethylacetate and hexanes (1:9) as solvents. The pure title compound was obtained as a colourless solid (0.31 g, 83% yield). Recrystallization was carried out using ethylacetate as solvent.

**Compound II**

A solution of 2-hydroxy-3-methoxybenzaldehyde (1.0 mmol, 0.152 g) and potassium carbonate (2.0 mmol, 0.2293 g) in acetonitrile solvent (5 mL) was stirred for 15 min at room temperature. To this solution, (Z)-methyl-2-(bromomethyl)-3-phenylacrylate (1.2 mmol, 0.25 g) was added drop wise. After the completion of the reaction, as indicated by TLC, acetonitrile was evaporated. EtOAc (15 mL) and water (15 mL) were added to the crude
mass. The organic layer was dried over anhydrous sodium sulfate. Removal of solvent led to the crude product, which was purified through pad of silica gel (100–200 mesh) using ethylacetate and hexanes (1:9) as solvents. The pure title compound was obtained as a colourless solid (0.31 g, 95% yield). Recrystallization was carried out using ethylacetate as a solvent.

**Compound III**

To a stirred solution of 2-nitrophenol (0.14 g, 1 mmol) in acetonitrile (7 ml), potassium carbonate (0.35 g, 2.5 mmol) was added and stirred well for five minutes. To this solution, (Z)-methyl 2-(bromomethyl)-3-phenylacrylate (0.26 g, 1 mmol) in acetonitrile (0.5 ml) was added and allowed to stir well for 6 h. After the completion of the reaction, the reaction mixture was poured into water and extracted using ethyl acetate. The organic layer thus obtained was concentrated under reduced pressure and the residual mass thus obtained was purified by column chromatography on silica gel (Acme 100–200) using EtOAc-hexanes (1:9) to afford the title compound in 90% yield. The crystals suitable for X-ray crystallographic analysis were grown from a solution of ethylacetate by slow evaporation at room temperature.

**Intensity Data Collection**

X-ray diffraction intensity data were collected for compounds I, II and III on Bruker AXS SMART APEX-II single crystal X-ray diffractometer equipped with graphite mono-chromated MoKα (λ=0.7103 Å) radiation and
CCD detector. Crystals were cut to suitable size and mounted on a glass fibre using cyanoacrylate adhesive. The unit cell parameters were determined from 36 frames measured (0.5° phi-scan) from three different crystallographic zones and using the method of difference vectors. The intensity data were collected with an average four-fold redundancy per reflection and optimum resolution (0.75 Å). The intensity data collection, frames integration, Lorentz and polarization correction and decay correction were done using SAINT (Bruker, 2008) software. Empirical absorption correction multi-scan) was performed using SADABS (Bruker, 2008) program.

Compounds I, II and III had R_{int} values of 0.033, 0.032 and 0.031, respectively, indicating good quality of crystals. Compounds I and III belong to monoclinic system with equivalent intensities showed the space group to be P2_1/n and C2/c and compound II belong to Triclinic system space group P\overline{1}. Out of 16035, 30114 and 32853 reflections collected, 4185, 8303 and 3695 were the unique reflections of which 2619, 5258 and 2356 had their I > 2\sigma(I) for compounds I, II and III, respectively.

Structure Solution and Refinement

Compounds I, II and III were solved by direct methods procedure as implemented in direct methods using SHELXS-97 (Sheldrick, 2008). A total of 1076 (E>1.2), 2069(E>1.2), 918(E>1.2) reflections were available for phase refinement procedure. A total of 256 (for all ) phase sets have been refined of which the best phase set with the best combined figure of merit
(0.049, 0.045 and 0.043) revealed the position of all the non-hydrogen atoms for compounds I, II and III. The E-map estimated for the best phase set generated by the program revealed the positions of all the non-hydrogen atoms of the compounds I, II and III. The residual factors ($R_\text{E}$) calculated based on the point atom model are 0.272, 0.250 and 0.242, respectively for compounds I, II and III. The positions of all the non-hydrogen atoms were included in full-matrix least-squares using SHELXL-97 (Sheldrick, 2008) program. In the initial stages of refinement, the thermal parameters were assigned a value of 0.05 (U’s) for each atom and the refinement was followed. The scale factor was fixed as 1.0 and isotropic refinement was carried out for all the non-hydrogen atoms until convergence was reached and this was subsequently followed by the anisotropic refinement. The anisotropic refinement for a few cycles of full matrix least-squares refinement was continued and the R-factor further reduced down to 0.06, 0.09 and 0.11 for the compounds I, II and III respectively. After a few cycles of anisotropic refinement, the positions of all the hydrogen atoms were fixed geometrically and allowed to ride on the corresponding non-hydrogen atoms for all the compounds, with aromatic C-H distances of 0.93Å and methyl C-H distances of 0.96Å with Uiso (H)=1.5 Ueq (C) for methyl H and 1.2 Ueq (N,C) for other H atoms. For compound III, the benzene ring (C10 - C15) and methyl acrylate (C16/C17/O3/O4) group of the phenylacrylate unit are disordered over two orientations with site-occupancy ratio of 0.705 (5):0.295 (5) and 0.683 (3):0.317 (3) representing major and minor components respectively. The command EADP was used in SHELXL-97 (Sheldrick,
2008) to constrain the $U_{eq}$ disordered atoms. The refinement converged to a final R-factor of 0.041 for compound I, 0.044 for compound II and 0.052 for compound III respectively.

2.3 Results and Discussion

The crystal data, intensity data collections and refinement details of compounds I, II and III are given in Table 2.1. The atomic coordinates of the non-hydrogen atoms with their equivalent temperature factors are presented in Tables 2.2a, 2.2b and 2.2c for compound I, II and III, respectively. The anisotropic displacement parameters are listed in Table 2.3a for compound I, Table 2.3b for compound II and 2.3c for compound III. The bond lengths of compounds I, II and III for the non-hydrogen atoms are presented in Table 2.4a, 2.4b and 2.4c. The bond angles of compounds I, II and III for the non-hydrogen atoms are presented in Table 2.5a, 2.5b and 2.5c. The atomic coordinates and their isotropic displacement parameters involving hydrogen atoms are given in Tables 2.6a, 2.6b and 2.6c for compounds I, II and III, respectively.

The torsion angles involving non-hydrogen atoms are listed in Table 2.7a, 2.7b and 2.7c for compounds I, II and III, respectively. The least-squares planes calculated using the program PARST (Nardelli., 1995) for various groups of atoms in the molecules of compounds I, II and III are presented in Tables 2.8a, 2.8b and 2.8c. The hydrogen bonding and non-bonded interactions for compounds I, II and III are presented in Table 2.9.
Fig 2.2a shows the ORTEP plot of the molecule drawn at 30% probability displacement ellipsoids level with the atom numbering scheme. The structure contains a methyl-phenylacrylate moiety and bromo-formyl phenoxy group. The methyl-phenylacrylate and the bromo-formyl phenoxy group are linked through CH$_2$ group.

The molecule adopts an E configuration along C7=C8 double bond of 1.338(4) Å, which is comparable with the reported value (Wang et al., 2011). The bond distance 1.890 (2) Å of Csp$^2$ – Br agree with the literature value (Allen, 1987).

The torsion angles of $[C13-C12-O3-C11] = 11.5(3)$ ° and $[C17-C12-O3-C11] = -168.7(2)$ ° show that O3 is slightly twisted away from the best plane of the bromo-formyl phenyl ring.

The methyl acrylate group takes up an extended conformation which is evident from the torsion angle values $[C8-C9-O2-C10] = 179.9^\circ$ (1); $[O1-C9-O2-C10] = -0.2^\circ$ (5). The significant difference in the length of the C9 – O1 and C10 – O2, bonds is attributed to a partial contribution from O$^-$ - C = O$^+$ - C resonance structure of the O1 = C9 – O2 – C10 group (Merino; 1971). This feature, commonly observed in the carboxylic ester groups of substituents in various compounds, gives average values of 1.340(1) Å and 1.447(1) Å, respectively, for these bonds (Varghese et al., 1986). The bond angles involving the carbonyl O atom are invariably expanded (Dunitz and
The O2 – C9 – C8 and O1 – C9 – C8 exocyclic bond angles are comparable with corresponding values for the structure of ethyl 2-acetyl 3-(5-(p-tolyl)-2-furyl)acrylate (110.2(1) and 125.6(1) (Lokaj et al., 1990).

The formyl group (C18/H18/O4) makes a torsion angle [C16-C17-C18-O4] = -2.5(4)° with the attached phenyl ring. The dihedral angle between the phenyl ring and the acrylate of the methyl-phenylacrylate unit is 16.1(1)°, which shows that the phenyl ring is slightly twisted in its position.

The best plane through the central unit (C8/C11/O3) makes a dihedral angle of 10.3(1)° with bromo-formyl phenyl ring and 86.1(2)° with the methyl-
phenylacrylate unit. The dihedral angle between the best planes through the bromo-formyl phenoxy group (C11/O3/C12—C18/Br1) and methyl-phenylacrylate group (C1-C6/C7/C8/C9/O1/O2/C10) is 85.3(2)°. The formyl group (C18/H18/04) is axial to the plane of the benzene ring to which it is attached as evidenced by the torsion angle C12-C17-C18-O4 of 174.9 (3)°.

The phenyl ring of the methyl-phenylacrylate unit and bromo-formyl phenyl unit are in planar conformation, the maximum deviations of their C atoms form the best fit planes describing them are -0.021Å and -0.006Å respectively

**Compound II**

Fig 2.2b shows the ORTEP plot of the molecule drawn at 30% probability displacement ellipsoids level with the atom numbering scheme. The structure contains a methyl-phenylacrylate moiety and methoxy-formyl phenoxy group. The methyl-phenylacrylate and the methoxy-formyl phenoxy group are linked through CH₂ group.

The asymmetric unit of the compound II contains the two independent molecules, A and B (Fig. 2.2b). The compound II adopts an E configuration along C7=C8 double bond of 1.335(2) Å and 1.333(2) Å for the molecule A and molecule B respectively, which are comparable with the reported values (Wang et al., 2011).
The torsion angles of [C11-O3-C12-C13] = -106.9(2) ° and 119.2(1) ° for molecule A and B, [C11-O3-C12-C17] = 75.5(1) ° and -66.3(2) ° for molecule A and B show that O3 is slightly twisted away from the best plane of the methoxy-formyl phenyl ring.

The methyl acrylate group takes up an extended conformation which is evident from the torsion angle values [C10-O2-C9-C8] = 177.3(1) ° and -178.9(2) °; [C10-O2-C9-O1] = -2.1(2)° and 0.7(1) ° for molecule A and B respectively. The significant difference in the length of the C9 – O1 and C10 – O2, bonds is attributed to a partial contribution from O\(^-\) - C = O\(^+\) - C resonance structure of the O1 = C9 – O2 – C10 group (Merino; 1971). This feature, commonly observed in the carboxylic ester groups of substituents in various compounds, give average values of 1.336(1) Å and 1.440(1) Å for

**Figure. 2.2b.** ORTEP plot of compound II showing the atom numbering scheme and thermal ellipsoids are drawn at 30% probability level.
molecule A, 1.327(1) Å and 1.450(1) Å for molecule B, respectively, for these bonds (Varghese et al., 1986). The bond angles involving the carbonyl O atom are invariably expanded (Dunitz and Schweizer, 1982). The O2 – C9 – C8 and O1 – C9 – C8 exocyclic bond angles are comparable with corresponding values for the structure of ethyl -2- acetyl -3-(5-(p-tolyl)-2-furyl)acrylate (110.2(1) and 125.6(1); Lokaj et al., 1990).

The acrylate group in molecule A is +syn periplanar with respect to central unit (O1/C9/C8/C11 = 2.8 (1)°) whereas in molecule B, the acrylate group is –antiperiplanar with respect to the central unit (O1/C9/C8/C11 = -176.7 (2)°) as evidenced by torsion angles.

The formyl group (C18/H18/O4) makes a torsion angle [C14-C13-C18-O4] = 10.3(3)° and -2.7(3)° with the attached phenyl ring for the molecule A and B respectively. The methoxy group (C19/O5) makes torsion angle of [C19-O5-C17-C12] = -170.7(2)° and -179.1(2)° for the molecule A and B respectively.

The central unit of the molecule A and B adopts a different conformation with the methoxy-formyl phenyl ring which are shown by the torsion angles [C11-O3-C12-C13] = -106.9(2)° and 119.2(2)° and [C11-O3-C12-C17] = 75.5(2)° and -66.3(2)° for molecule A and B respectively.

The dihedral angle between the phenyl ring and the acrylate of the methyl-phenylacrylate unit is 41.7(1)° for molecule A and 49.1(1)° for molecule B. The dihedral angle between the C1 – C6 and C12 – C17 benzene
rings is 41.7 (1)° in molecule A and it is 35.6 (1)° in molecule B. The methoxy and formyl group at the meta positions of the benzene group are close to being coplanar with the ring (C12-C17) (5.7 (1)° and 4.2 (1)° in molecule A and 1.5 (1)° and 2.3 (1)° in molecule B). The central unit (C8/C11/O3) is equatorial with respect to the methyl phenylacrylate and methoxy-formylphenyl (C1- C6/C7/C8/C9/O1/O2/C10) = 76.9 (1)° and C8/C11/O3/C12 – C17 =63.1 (1)° in molecule A and 76.6 (1)° and 54.4 (2)° in molecule B).

The phenyl ring of the methyl-phenylacrylate unit and methoxy-formyl phenyl unit are in planar conformation, the maximum deviations of their C atoms form the best fit planes describing them are -0.006Å and 0.008Å for molecule A and 0.006 Å and 0.015 Å for molecule B respectively.

**Compound III**

Fig 2.2c shows the ORTEP plot of the molecule drawn at 30% probability displacement ellipsoids level with the atom numbering scheme. The structure contains a methyl-phenylacrylate moiety and nitro-phenoxy group. The methyl-phenylacrylate and the nitro-formyl phenoxy group are linked through CH₂ group.

The molecule adopts an E configuration along C7=C8 double bond of 1.325(3) Å, which is comparable with the reported value (Wang et al., 2011). The benzene ring (C1–C6) and methyl acrylate (C9/O1/O2/C10) group of the
phenylacrylate unit are disordered over two orientations with site-occupancy ratios of 0.705 (5):0.295 (5) and 0.683 (3):0.317 (3) representing major and minor components, respectively.

Figure. 2.2c. ORTEP plot of the compound III, showing the atom - numbering scheme with 30% probability displacement ellipsoids. H atoms are shown as spheres of arbitrary radius. The minor fractions of the disordered benzene ring and methylacrylate have been represented by broken bonds.
The mean plane through the benzene ring of the phenyl acrylate makes dihedral angles of 88.4 (8)° (major component) and 86.7 (8)° (minor component) with the nitrophenoxy (C12–C17/N1/O4/O5) ring; the dihedral angle between the two components is 3.6 (6)°.

The major and minor components of the methylacrylate (C8/C9/O1/O2/C10) are essentially planar with maximum deviations for atoms O2 and O2' being -0.015 (1) and 0.015 (1) Å, respectively.

The central unit (C8/C11/O3) is almost equatorial to the major component of methylphenylacrylate group (C1–C6/C7/C8/C9//O1/O2/C10) whereas axial to the nitrobenzene (C12–C17/N1), making dihedral angles of 88.4 (1) and 8.1 (1)°, respectively.

The methyl acrylate group takes up an extended conformation which is evident from the torsion angle values [C10-O2-C9-C8] = 179.8(3)° and [C10-O2-C9-O1] = -3.8(6)°. The significant difference in the length of the C9 – O1 and C10 – O1, bonds is attributed to a partial contribution from O' - C = O° - C resonance structure of the O1 = C9 – O2 – C10 group (Merino; 1971). This feature, commonly observed in the carboxylic ester groups of substituents in various compounds, gives average values of 1.353(1) Å and 1.471(1) Å, respectively, for these bonds (Varghese et al., 1986). The bond angles involving the carbonyl O atom are invariably expanded (Dunitz and Schweizer, 1982).
The O2 – C9 – C8 and O1 – C9 – C8 exocyclic bond angles are comparable with corresponding values for the structure of ethyl-2-acetyl-3-(5-(p-tolyl)-2-furyl)acrylate (110.2(1) and 125.6(1); Lokaj et al., 1990).

The phenyl ring of the major and minor component of methyl-phenylacrylate unit and nitro-phenyl unit are in planar conformation, the maximum deviations of their C atoms form the best fit planes describing them are -0.007 Å, 0.001Å and -0.011Å

2.4. Hydrogen Bonding and Crystal Packing

Compound I

For the compound I the pattern of hydrogen bonding and the packing of the molecules in the crystal are shown in the Fig 2.3a1, Fig 2.3a2 and Fig 2.3a3 respectively. Hydrogen bonding details are listed in Table 2.9a.

The molecular structure and crystal packing are stabilized by C-H…O interactions. In this structure four intramolecular interactions C5-H5…O3 is present. There are three intermolecular interactions C4-H4…O1, C13-H13…O1 and C14-H14…O4. The oxygen atoms of acrylate group and formyl group are involved in these interactions by acting as the acceptor atoms.

The C4-H4…O1 intermolecular hydrogen bond links the molecule along c-axis and the C14-H14-O4 form a chain running along [100] direction.
shown in the Fig 2.3a1. The C13-H13…O1 and C11-H11…O1 hydrogen bonds along symmetry operation ½+x, 3/2-y,-1/2+z forms graph-set chain shown in the Fig 2.3a2.

**Compound II**

For the compound II the pattern of hydrogen bonding and the packing of the molecules in the crystal as viewed along the b-axis are shown in Fig 2.3b1, Fig 2.3b2, Fig 2.3b3, Fig 2.3b4 and Fig 2.3b5 respectively. Hydrogen bonding parameters are listed in Table 2.9b.


The hydrogen atom (H18A) of carbon(C18A) forms bifurcated intramolecular hydrogen bond with two oxygen atoms (O1A and O3A) resulting in formation of $R_{2}^{2}(6)$ rings(Bernstein et al., 1995) and the hydrogen bond C18A-H18A…O1A forms a chain running along [100] direction shown in the Fig 2.3b3 and Fig 2.3b4 respectively. Packing of the molecules viewed down the c-axis is shown in the Fig 2.3b5.

In intermolecular interactions Oxygen atom O4 of molecule A and O3 of molecule B act as an acceptor atoms for the hydrogen bonds C10B-
H10D…O4A and C11A-H11A…O3B along the symmetry operations 1-x, 1-y, 2-z and 1-x, 2-y, 2-z respectively. (Fig 2.3b1 and Fig 2.3b2).

**Compound III**

For compound III the geometric details of the hydrogen bonds are listed in the Table 2.9c. The pattern of hydrogen bonding and the packing of the molecules in the crystal are shown in Fig 2.3c1, Fig2.3c2 and Fig 2.3c3.

The crystal structure is consolidated by C-H…O hydrogen bond interactions of which C5-H5…O3, C7-H7…O1 are intramolecular interaction and C14-H14…O5, C15-H15…O1 are intermolecular interaction with [x, 1+y, z], [1/2+x, 1/2+y, 1/2+z] symmetry operations respectively.

The oxygen atoms O3, O1, O5 act as acceptors of the corresponding hydrogen bond interactions. The crystal structure is stabilized by intermolecular bifurcated C—H···O hydrogen bonds involving two hydrogen atoms (H15/H16) of the benzene ring (C12—C17) and O1 of the acrylate resulting in an \( R_{2}^{2}(5) \) ring motif (Bernstein et al., 1995) shown in the Fig 2.3c1 and C14—H14···O5 interactions resulting in a chain of molecules running along the b-axis (Fig. 2.3c2) making symmetry operation x, 1+y, z and also forms \( R_{2}^{2}(25) \) ring motif (Fig. 2.3c3)

**2.5. Comparison**

In all the three compounds, the methyl phenyl acrylate moiety and phenoxy group are the common constituents figure below. In the compound I
and II, formyl group is attached to the meta position of the phenyl ring and in compound III nitro group is attached.

For all the three compounds the crystal packing is stabilized by C-H…O inter and intra molecular hydrogen bond interactions.

\[
\text{\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{compound_structure.png}
\caption{Structure of compound.
}\end{figure}}
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<th>Compound III</th>
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<th>Mrcos-Sermek, et al., 2006</th>
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Figure 2.2a. ORTEP plot of compound I showing the atom numbering scheme and thermal ellipsoids are drawn at 30% probability level.
Figure 2.3a1 Part of the crystal structure of the compound I showing C-H…O hydrogen bonds

Figure 2.3a2. Part of the crystal structure of the compound I showing the $R_2^2(8)$ graph-set linked by C-H…O hydrogen bonds
Figure 2.3b1. Part of the crystal structure showing intermolecular C10B-H10D…O4A hydrogen bond interactions.

Figure 2.3b2. Part of the crystal structure showing intermolecular C11A-H11A…O3B hydrogen bond interactions.
Figure 2.3b3. Part of the crystal structure showing intramolecular bifurcated hydrogen bond interactions along bc plane.

Figure 2.3b4 Crystal packing of the molecules viewed along a-
Figure 2.3b4. Part of the crystal structure showing intermolecular C18A-H18A…O1A hydrogen bond interactions forms chain running along [100] direction.
Figure. 2.3c1. Part of the crystal structure showing intermolecular bifurcated hydrogen bond interactions with $R^{2}_{2}(5)$ ring motif.

Figure. 2.3c2 C14—H14···O5 interactions resulting in a chain of molecules running along the $b$-axis
**Figure 2.3c3.** C14—H14⋯O5 interactions resulting in $R_2^2(25)$ ring motif.

**Figure 2.3b5.** Crystal packing of the molecules viewed along c-axis.
Figure 2.3a3. Crystal packing of the molecules viewed along b-axis.
2.6. Molecular Docking Studies of Phenylacrylate – evidencing its antimicrobial activity by Inhibition of mushroom tyrosinase

Introduction

Microbes are the main pathogens of foodborne illnesses. Microbial contamination of food is a major concern for the food industry regulatory agencies and consumers. In most foods, including fruits, vegetables and some seafoods (crustaceae), the discoloration and browning process has two components: enzymatic and non enzymatic oxidation.

The enzymatic oxidation is considered to be deleterious to the color quality of foods, especially to bruised or cut fruits and vegetables. This unfavorable darkening from enzymatic oxidation has been of great concern (Friedman, M., 1996). The enzymatic oxidation is mainly caused by tyrosinase (EC 1.14.18.1) and is usually initiated by the enzymatic oxidation of monophenols into o-diphenols and o-diphenols into quinones, which undergo further nonenzymatic polymerization leading to the formation of pigments. Tyrosinase inhibitors could be useful as antibrowning agents. Therefore, it is of great importance to search for compounds which possess antimicrobial activity.

A lot of tyrosinase inhibitors have been used commercially (Kubo et al., 1998), but the safety regulation limits their applications. Methyl trans-cinnamate or E-Methyl phenylacrylate (Chemical Abstracts Service Registry Number, 103-26-4; European Inventory of Existing commercial Chemical Substances Number, 203-093-8) is a fragrance ingredient used in many fragrance compounds and
decorative cosmetics. Different kinds of experiments have been done to confirm its safety, such as acute toxicity, skin irritation, mucus membrane irritation, skin sensitization. Its use worldwide is in the region of 10-100 metric tonnes per annum (Bhatia et al., 2007) for food preservation.

Methyl trans-cinnamate has antimicrobial activity against *E. coli*, *B. subtilis*, *St. aureus* and *C. albicans*. It was found that methyl trans-cinnamate could inhibit the proliferation of these four different kinds of microbes to different extents. The result shows that the antimicrobial activity of methyl trans-cinnamate is broad-spectrum. It can inhibit G+ bacteria, G- bacteria and fungi (Qian-sheng huang et al., 2009).

In view of this medicinal importance, the three derivatives of phenylacrylate namely (E)-Methyl 2-[(4-bromo-2-formylphenoxy) methyl]-3-phenylacrylate (compound I), (E)-methyl-2-[(2-formyl-6-methoxyphenoxy)methyl]-3-phenylacrylate(compound II) and Methyl (E) -2- [ 2-(2-nitrophenoxy)methyl]-3-phenylacrylate(compound III) are subjected for *In silico* antimicrobial activity analysis. Pymol representations of the compounds are shown in Fig 2.6a-c.

**Material and methods**

Molecular modeling studies have been carried out using GLIDE (*Grid-based Ligand Docking with Energetics*) software v5.5 developed by Schrödinger (Glide v5.5, 2009) running on Red Hat Enterprise Linux 5 (RHEL5) workstation.
Maestro v9.0 Graphical User Interface (GUI) workspace was used for all the steps involved in ligand preparation, protein preparation and Induced Fit Docking.

**Ligand Preparation**

The ligands used in this study were prepared using LigPrep module of v2.3 of Schrödinger Suite 2009. LigPrep follows OPLS-AA (Optimized Potential Liquid Simulations for All Atoms) force fields for energy minimization. In this study we used the crystal coordinates of the ligand Tropolone (Fig. 2.6d) retrieved from their corresponding PDB complex structures and the crystal coordinates of phenyl acrylate derivatives obtained from the crystal data. The crystal structure retrieved from PDB lack hydrogen atoms and hence hydrogen atoms were added to Tropolone structures and energy minimized.

**Protein Preparation**

Protein Preparation Wizard of GLIDE software was used to process and prepare the protein. This Wizard allows one to properly prepare a protein for docking studies. This also follows the Optimized Potential for Liquid Simulations-All Atoms (OPLS-AA) force fields for energy minimization.

**Preparation of Mushroom Tyrosinase**

The X-ray crystal structure of Agaricus bisporus Mushroom Tyrosinase (PDB id: 2Y9X) retrieved from PDB is a tetramer structure. The complex comprises two H subunits of ~392 residues and two L subunits of ~150 residues with four inhibitor molecule tropolone, 25 water molecules, 8 copper ions and 4
Ho ions. The protein was prepared by removing all the water molecules and Ho ions present in the structure. Chain A was retained from the complex structure. Since the raw data do not contain any hydrogen in it, the implicit hydrogen atoms were added to the atoms to satisfy their appropriate valancies. Then the structure was optimized by assigning the bond orders, bond angles and topology. The formal atomic charges were fixed for the amino acid residues. The optimized structure was then energy minimized to remove the steric clashes between the atoms. The energy minimization was done till it reached a root mean square deviation (rmsd) cutoff of 0.18 Å and the resulting structure was used for docking.

**Induced Fit Docking (IFD)**

IFD of the prepared ligands with the prepared proteins was performed using *Induced Fit Docking* protocol of *GLIDE* v5.5 (Schrödinger Suite 2009). It is based on *GLIDE* and *Prime* Refinement module.

The prepared structure of 2Y9X was used for induced fit docking simulations. Initially a receptor grid, where the ligand has to be docked with the receptor was set by picking the centroid of the co-crystallized inhibitor (Troplone in 2Y9X) present at the active site. It creates a grid box and the size of the grid box was limited to 20 Å. The generation of different conformations of the docked complexes (poses) was set to a maximum of 20. Then compound I, II and III were docked at the active site of 2Y9X individually. The poses generated were ranked based on G-score. The poses that made the maximum hydrogen bond (H-bond) interactions from compound I-2Y9X, compound II-2Y9X and compound III –
2Y9X docked complexes were considered for further analysis and the results are compared.

Results and Discussion

Substrate-Binding Site of Tyrosinase

The binuclear copper-binding site is located at the bottom of a spacious cavity in the surface of the H subunit. The active site is lined with His244, part of the carboxylate of Glu256, Asn 260, the main chain atoms of residues 279-282, and Ala 286. The ligands of the first copper ion, Cu-A, are the N2 atoms of His 61 (end of helix α3), His85 (in the loop connecting α3 and α4), and His94 (beginning of α4). The second copper ion, Cu-B, has as ligands the N2 atoms of His259, His263 (α10), and His 296 (α11). The covalent thioether bond with Cys 83 fixes the orientation of the His 85 side chain. The space of the active site cavity is large enough to even accommodate phenolic steroids as substrates. (Wangsa et al., 2011).

IFD of phenylacrylate compounds with 2Y9X

Induced fit docking studies show that the three phenylacrylate compounds binds nearer to the binuclear Cu- binding site located at the bottom of a spacious cavity in the surface of H-subunit. The site is located at the heart of two pairs of antiparallel R-helices (R3/R4 and R10/R11, respectively), which make an angle of nearly 90° with each other (Fig 2.6a-c1).
During IFD of the three phenylacrylate compounds the geometry of binuclear Cu-binding site is changed along with their active site ligands. In native state CuA is bonded to two ligands His61 and His85 whereas in IFD with phenylacrylate compounds, CuA shows with different ligand sets say Asn81, Cys83 and Thr84. (Fig 2.6a-c2) The O1 atom of the acrylate group in all the compounds makes H-bond interaction with the active site residues say O atom of Asn260 for compound I (Fig2.6a2) N atom of Val283 for compound III (Fig2.6c2) at a distance 2.81 Å and 3.24 Å respectively.

Compound I makes H-bonds with Ne2 atom of His85 and His61 of CuA ligands at a distance of 2.75 Å and 3.01 Å respectively. The CuB exhibits H-bond at a distance of 2.2 Å with O2 atom of compound II (Fig2.6b2). Compound III shares an H-bond with Asn260 at a distance of 2.8 Å. (Fig2.6c2). In all three IFD of phenylacrylate compounds the binding region is lined with the residues His61, Cys83, Thr84, His85, Phe90, His94, His244, Glu256, His259, Asn260, His263, Phe264, His279, Met280, Gly281, Ser282, and Val283 formed an array of hydrophobic interactions shown in the (Fig2.6a-b3). The Glide scores of compound I, II and III are -6.36, -6.24 and -4.78 respectively and Glide energy is -42.69 Kcal/mol, -38.63 Kcal/mol and -29.68 Kcal/mol respectively. (Table 2.10)

**IFD of native ligand tropolone with 2Y9X**

Induced Fit Docking (IFD) of tropolone into the active site of 2Y9X has not influenced any significant structural changes in the overall conformation of the protein backbone (Fig2.6d1). The docked orientation of tropolone was similar
to the orientation found in the PDB complex. The binuclear copper site remains the same with their Ligands and hydrophobic active site residues but the H-bond interactions are disrupted (Fig 2.6d3). The binding mode of tropolone in the PDB represents a pre-Michaelis complex which showed no H-bonds. In IFD of tropolone exhibited two H-bonds with the active site residues. The O atom of the tropolone formed H-bonds with the N atom of His259 at a distance of 3.22 Å and O atom of Asn260 residue at a distance of 2.91Å, respectively (Fig 2.6d2). The geometry of the active site as surface representation with troplone in ball and stick is shown in (Fig2.6a-c4). The G-score of is -4.653 and Glide energy is -31.533 Kcal/mol.

**CONCLUSION**

Molecular docking studies reveal that Methyl E-phenylacrylate derivatives could inhibit both ortho-hydroxylation of monophenolase activity and subsequent oxidation of the diphenolase activity of mushroom tyrosinase by disrupting catalytic center formed by binuclear copper. IFD with 2Y9X confirms that phenyl acrylate can be co-crystallized with the 2Y9X and the *in vitro* binding mode and energy with the protein could be revealed. The exploration and characterization of new inhibitors are not only useful for the medicinal purposes. But their potential applications in improving food quality and nutritional value, controlling insect pests, etc., are also important. So it is very important to discover novel and potent inhibitors of the enzyme tyrosinase. Thus, we believe that methyl *trans*-cinnamate could be a potential compound used in antibrowning food additive.
Figure 2.6d1 Cartoon representation of complex tropolone with AbTyr (PDB ID:2Y9X.)

Figure 2.6d2 Pymol representation of H-bond exhibited by tropolone with active site residues of AbTyr(PDB ID: 2Y9X)
Figure 2.6d3 Pymol representation of interactions exhibited by tropolone with active site residues of AbTyr (PDB ID: 2Y9X)

Figure 2.6d4 Surface diagram showing the binding of tropolone with hydrophobic pocket of AbTyr (PDB ID: 2Y9X)
Figure 2.6a1 Cartoon representation of complex compound I with AbTyr (PDB ID: 2Y9X.)

Figure 2.6a2 Pymol representation of H-bond exhibited by compound I with active site residues of AbTyr (PDB ID: 2Y9X)
Figure 2.6a3 Pymol representation of interaction exhibited compound I with the active site residues of AbTyr (PDB ID: 2Y9X)

Figure 2.6a4 Surface diagram showing the binding of compound I with the hydrophobic pocket of AbTyr (PDB ID: 2Y9X)
Figure 2.6b1 Cartoon representation of complex compound II with AbTyr (PDB ID: 2Y9X.)

Figure 2.6b2 Pymol representation of H-bond exhibited by compound II with active site residues of AbTyr (PDB ID: 2Y9X)
Figure 2.6b3 Pymol representation of interaction exhibited compound II with the active site residues of AbTyr (PDB ID: 2Y9X)

Figure 2.6b4 Surface diagram showing the binding of compound II with the hydrophobic pocket of AbTyr (PDB ID: 2Y9X)
Figure 2.6c1 Cartoon representation of complex compound III with AbTyr (PDB ID: 2Y9X.)

Figure 2.6c2 Pymol representation of H-bond exhibited by compound III with active site residues of AbTyr (PDB ID: 2Y9X)
**Figure 2.6c3** Pymol representation of interaction exhibited compound III with the active site residues of AbTyr (PDB ID: 2Y9X)

**Figure 2.6c4** Surface diagram showing the binding of compound III with active site residues of AbTyr (PDB ID: 2Y9X)