



## Synthesis, Characterization and Molecular docking of Bi and Tridentate Chiral molecules for HIV-Native protease

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### ABSTRACT

Six potential Bi and Tridentate chiral molecules were synthesized and characterized, in that the amino ester and 2-amino-3,5-dibromobenzaldehyde were linked by C=N bond which can be specifically reduced using Tetrabutylammonium borohydride and ester further convert into Alcohol using lithium borohydride. The synthesized compounds are evaluated for molecular docking to check potency of the synthesized compounds for HIV-protease.

**Keywords:** Bi- and Tridentate chiral molecules, molecular docking, HIV-protease.

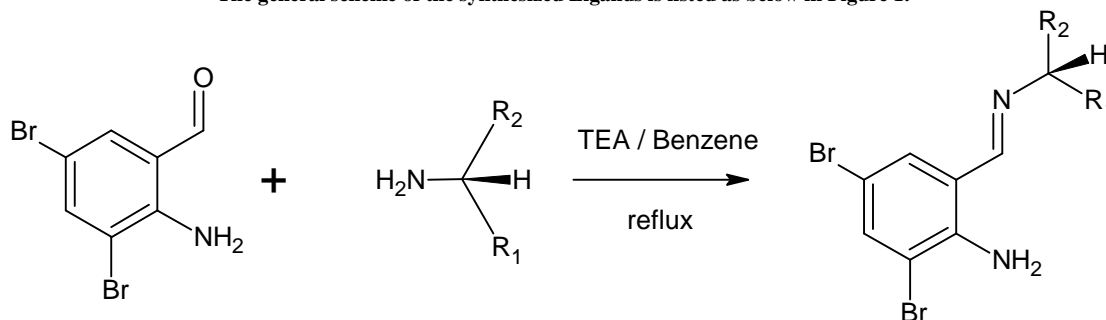
### INTRODUCTION

Organic chemists working over the past 10 years to the Design, synthesis, characterization and application of diverse chiral Schiff bases reagents are becoming increasingly important in the pharmaceutical, plastic and dye industries. In 1992 the Food and Drug Administration issued a policy which stressed the importance of enantiomerically pure drugs, and in 2003 six of the top ten best selling pharmaceutical drugs were marketed as single enantiomer[1]. Additionally, chiral alcohols are important intermediates in the synthesis of the NK1 receptor antagonist Aprepitant, which is marketed as Emend used to treat vomiting in chemotherapy patient[2]. Most promisingly the chiral molecules have been explored for their interesting physicochemical and biological properties, the compounds like Imidazole have two nitrogen atoms serves as coordination part of the molecule and is frequently found as part of a large number of biologically and medicinally significant substances[3,4]. The amine functionality is present in many natural products and due to its interesting physiological activity it is an extremely important pharmacophore in many biologically active compounds[5]. Same time HIV is an inevitable one of the most studied and unsolved human diseases. So far 5 million HIV positive people are on treatment, a majority of them in countries like India, Brazil, Thailand and South Africa. Thus Patents on lifesaving drugs can end access for them and so activists across the world are fighting against multinationals from stopping the production of generics recently most of the scientist and doctors are continuously working to resolve this diseases by new effective drugs for this Anti HIV treatment. At present study we synthesized the chiral ligands, generally the chiral compounds has two enantiomers which elicit different properties and responses. Most of the compounds reveal that some Pharmacophores are indeed essential to impact desired therapeutic effect in the molecules. the significant Pharmacophores like halogen, Phenolic -NH<sub>2</sub>,-CH<sub>2</sub>OH,-CH=N-, -C<sub>6</sub>H<sub>5</sub> and chiral centre in the molecules could exhibit broad spectrum activities. Some of the FDA approved Anti-HIV active compounds are lopinavir, Ampenavir and Indinavir [7] etc.

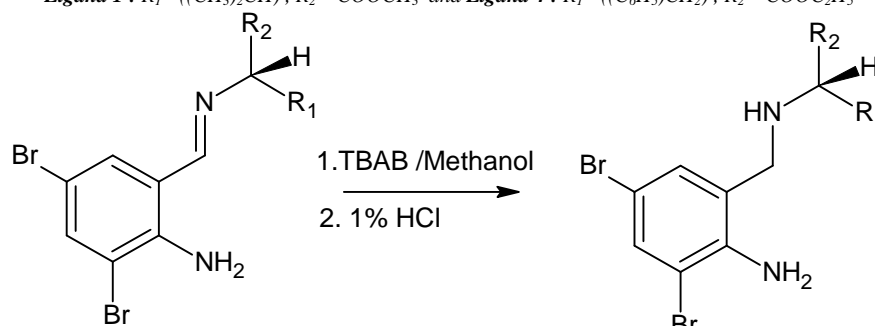
Lopinavir is a compound used as an Anti-HIV molecule in the commercial market, it has bicyclic acidic amide bond with hydroxyl group and it possesses chiral centre for both the compounds are biologically important [6-7]. In view of the above important factors, the present work is undertaken to synthesize Schiff bases by incorporating 2-Amino-3,5-dibromobenzaldehyde moiety with Amino acid derivatives containing the above mentioned significant pharmacophores. This could result in possessing more potential Anti-HIV activity. [8- 11]

In the latest investigation into the structure and reactivity of tridentate Schiff base ligands and complexes derived from L-tert-Leucine, were recently reported derived from ligand, which contained an unprecedented anti-skew carboxylate bridging group, previously some of the bi and tridentate amino alcohol ligands as Schiff bases were reported[12-15]. in the present study bromo substituted amino chiral ligand were synthesized by treating valine methyl ester hydrochloride or phenyl alanine ethyl ester hydrochloride along with 2-amino-3,5-dibromo benzaldehyde to form schiffs bases and it has reduced C=N bond specifically using Tetrabutyl ammonium borohydride ,followed by ester has reduced to its alcohol by using lithium aluminium hydride. The schematic diagram of the ligands mentioned in (Figure 1).

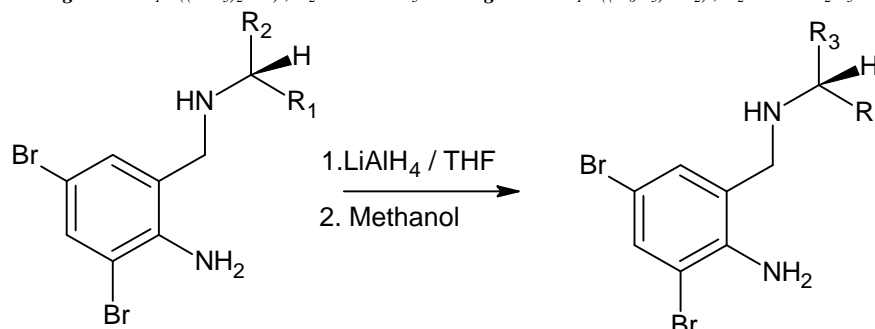
The general scheme of the synthesized Ligands is listed as below in Figure 1.



*Ligand 1* : R<sub>1</sub> - ((CH<sub>3</sub>)<sub>2</sub>CH), R<sub>2</sub> - COOCH<sub>3</sub> and *Ligand 4* : R<sub>1</sub> - ((C<sub>6</sub>H<sub>5</sub>)CH<sub>2</sub>), R<sub>2</sub> - COOC<sub>2</sub>H<sub>5</sub>



*Ligand 2* : R<sub>1</sub> - ((CH<sub>3</sub>)<sub>2</sub>CH), R<sub>2</sub> - COOCH<sub>3</sub> and *Ligand 5* : R<sub>1</sub> - ((C<sub>6</sub>H<sub>5</sub>)CH<sub>2</sub>), R<sub>2</sub> - COOC<sub>2</sub>H<sub>5</sub>



R<sub>2</sub> - COOCH<sub>3</sub>, - COOC<sub>2</sub>H<sub>5</sub>  
*Ligand 3* : R<sub>1</sub> - ((CH<sub>3</sub>)<sub>2</sub>CH), R<sub>3</sub> - CH<sub>2</sub>OH and *Ligand 6* : R<sub>1</sub> - ((C<sub>6</sub>H<sub>5</sub>)CH<sub>2</sub>), R<sub>3</sub> - CH<sub>2</sub>OH

## EXPERIMENTAL SECTION

Melting points were determined in open capillary tubes on melting point apparatus (Veego, Shankar scientific) and are uncorrected. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker NMR 400 MHz using CDCl<sub>3</sub> as solvent. Mass spectra were recorded on JEOL GC mate mass spectrometer. The IR Spectra of the synthesized compounds were recorded on FT-IR Spectrophotometer. TLC checked the purity of the compounds on pre-coated silica Gel Plates by using methanol: Ethyl acetate (1:9) as a mobile phase and visualized in iodine vapour. Optical rotations of the ligands were recorded using Redolph Polarimeter.

Molecular docking was performed using the structure based drug designing tools available in Accelrys discovery studio 2.1V software. The Chemsketch software used to draw molecular structures for converting in to small molecular input line system (SMILES).

**RESULTS AND DISCUSSION**

Synthesis of methyl 2-[(2-amino-3,5-dibromophenyl)methylidene]amino-3-methylbutanoate (**ligand 1**):

L-Valine methyl ester hydrochloride (5.0g, 0.03mol) taken with 100ml of benzene and 2-amino-3,5-dibromobenzaldehyde (8.30g, 0.03mol) was added, to this triethylamine (1.0ml) was added drop wise at 30°C, slowly raise the temperature to reflux and started collection of water using Dean stark apparatus, maintained the reflux for 4hrs and cool to room temperature then formed triethylamine hydrochloride was removed by passing the reaction mass to silica flush column, the organic layer removed under vacuum, which afford methyl 2-[(2-amino-3,5-dibromophenyl)methylidene]amino-3-methylbutanoate (10.01g, 0.025mol) as yellow gel liquid, Yield : 85.0%,

Synthesis of methyl 2-[(2-amino-3,5-dibromobenzyl)amino]-3-methyl butanoate (**ligand 2**):

methyl 2-[(2-amino-3,5-dibromophenyl)methylidene]amino-3-methylbutanoate (5.0g, 0.0127mol) dissolved in 30ml of methanol and cool to 0-5°C and Tetra butyl ammonium borohydride (1.2g, 0.005mol) added in a lot with same temperature, the completion of the reaction was monitor by TLC (ethylacetate:Hexane) 5:5, after completion the reaction quenched with 10ml of 1% dil.HCl solution and degassed the methanol completely then extracted with 50ml of ethyl acetate and washed with 50ml of water and removed the organic layer under vacuum afford the methyl 2-[(2-amino-3,5-dibromobenzyl)amino]-3-methyl butanoate as crude material which is further purified by CC(ethyl acetate and Hexane as eluent) and isolated (3.02g, 0.007mol) as pale yellow gel liquid. Yield : 60.0%,

Synthesis of 2-[(2-amino-3,5-dibromobenzyl)amino]-3-methylbutanol (**ligand 3**):

methyl 2-[(2-amino-3,5-dibromobenzyl)amino]-3-methyl butanoate (2.0g, 0.005mol) is dissolved in 20ml of THF and cool the reaction to 0-5°C and lithium aluminium hydride (0.095g, 0.0025mol) was added lot wise over 30 min and stir at same temperature for 2hr and slowly raise the temperature to 40°C and it maintained for 1hr till completion of the reaction. after completion confirmed by TLC the reaction cool to 10°C and quenched with methanol and removed the solvent under vacuum and the residue purified by CC (ethylacetate and methanol as eluent) and isolated the pure 2-[(2-amino-3,5-dibromobenzyl)amino]-3-methylbutanol (1.2g, 0.003mol). Yield : 65.0%, white crystalline solid,

Synthesis of ethyl 2-((2-amino-3,5-dibromophenyl)methylidene)amino-3-phenyl propanoate. (**ligand 4**):

L-Phenyl alanine ethyl ester hydrochloride (7.0g, 0.030mol) taken with 100ml of benzene and 2-amino-3,5-dibromobenzaldehyde (8.30g, 0.03mol) was added, to this triethylamine (1.0ml) was added drop wise at 30°C, slowly raise the temperature to reflux and started collection of water using Dean stark apparatus. It maintained the reflux for 4hrs and cool to room temperature then formed triethylamine hydrochloride was removed by passing the reaction mass to silica flush column, the organic layer removed under vacuum, which afford ethyl 2-((2-amino-3,5-dibromophenyl)methylidene)amino-3-phenyl propanoate (10.01g, 0.022mol) as yellow gel liquid, Yield : 73.2%.

Synthesis of ethyl 2-((2-amino-3,5-dibromobenzyl)amino)-3-phenylpropanoate. (**ligand 5**)

ethyl 2-((2-amino-3,5-dibromophenyl)methylidene)amino-3-phenyl propanoate (6.0g, 0.0132mol) dissolved in 80ml of methanol and cool to 0-5°C and Tetrabutyl ammonium borohydride (4.2g, 0.016mol) added in a lot with same temperature, the completion of the reaction was monitor by TLC (ethylacetate:Hexane) 5:5, after completion the reaction quenched with 10ml of 1% dil.HCl solution and degassed the methanol completely and extract with 100ml of ethyl acetate and washed with 100ml of water and removed the organic layer under vacuum afford the ethyl 2-((2-amino-3,5-dibromobenzyl)amino)-3-phenylpropanoate as crude material which is further purified by CC(ethyl acetate and methanol as eluent) and isolated (5.03g, 0.011mol) as pale yellow gel liquid. Yield: 83.0%.

Synthesis of 2-((2-amino-3,5-dibromobenzyl)amino)-3-phenylpropanol (**ligand 6**):

ethyl 2-((2-amino-3,5-dibromobenzyl)amino)-3-phenylpropanoate (3.0g, 0.006mol) is dissolved in 30ml of THF and cool the reaction to 0-5°C and lithium aluminium hydride (0.24g, 0.0063mol) was added lot wise over 20 min and stir at same temperature for 2hr and slowly raise the temperature to 40°C and it maintained for 1hr till completion of the reaction. after completion confirmed by TLC the reaction cool to 10°C and quenched with methanol and removed the solvent under vacuum and the residue purified by CC (Ethylacetate and methanol as eluent) and isolated the pure 2-((2-amino-3,5-dibromobenzyl)amino)-3-phenylpropanol (1.9g, 0.004mol). Yield : 70.0% as white crystalline solid,

**Characterization of Synthesized compounds**

The structures of the synthesized compounds were confirmed by IR, <sup>1</sup>HNMR, <sup>13</sup>CNMR and Optical rotation analysis are presented in (Table 1)

**Table 1: Spectral data of the synthesized compounds**

| Compounds | IR( $\nu$ , $\text{cm}^{-1}$ )    | $^1\text{H}$ NMR ( $\text{CDCl}_3$ )   | $^{13}\text{C}$ NMR(ppm)( $\text{CDCl}_3$ )  | Optical rotation @25°C                            |
|-----------|-----------------------------------|--|--|---|
| Ligand 1  | 3456, 3240, 1733, 1526, 1450, 681 | 0.9(m, 6H, -CH <sub>3</sub> ), 2.3(m, 1H, -CH), 3.6(d, 1H, -CH), 3.7(s, 3H, -OCH <sub>3</sub> ), 7.2(b, 2H, -NH <sub>2</sub> ), 7.3-8.2(2H, ArH), 9.8(s, 1H, N=CH)   | $\delta$ 18.34, 19.43, 31.95, 52.02, 79.55, 105.97, 110.19, 118.80, 135.15, 136.09, 145.04, 163.70, 172.10.                                  | $\alpha_D = -87.60$<br>(C=1 in $\text{CHCl}_3$ )  |
| Ligand 2  | 3449, 3315, 3252, 1728, 678       | 0.9(m, 6H, -CH <sub>3</sub> ), 1.82(s, 1H, -NH), 1.9-2.06(m, 1H, -CH), 3.0(d, 1H, -CH), 3.74(dd, 2H, -CH <sub>2</sub> ), 3.78(s, 3H, OCH <sub>3</sub> ), 5.3(s, 2H, -NH <sub>2</sub> ), 7.3-7.6(s, 2H, ArH)                  | $\delta$ 18.40, 19.50, 31.90, 52.02, 65.96, 79.5, 110.1, 110.30, 125.75, 131.80, 133.74, 143.80, 163.65, 175.02.                             | $\alpha_D = -101.0$<br>(C=1 in $\text{CHCl}_3$ )  |
| Ligand 3  | 3429, 3308, 2950, 683             | $\delta$ 0.9(m, 6H, -CH <sub>3</sub> ), 1.7(b, 1H, OH), 2.0(m, 1H, CH), 2.4(m, 1H, CH), 3.5(m, 1H, NH), 3.7-3.9(dd, 4H, -2CH <sub>2</sub> ), 5.2(b, 2H, NH <sub>2</sub> ), 7.1(s, 1H, ArH), 7.5(s, 1H, ArH)                  | $\delta$ 18.70, 19.20, 28.80, 50.9, 61.5, 64.0, 108.49, 110.45, 126.80, 131.50, 133.37, 143.50.  | $\alpha_D = -65.00$<br>(C=5 in $\text{CHCl}_3$ )  |
| Ligand 4  | 3520, 3315, 1750, 1423, 690       | 1.2(t, 3H, -CH <sub>3</sub> ), 1.8(s, 2H, -NH <sub>2</sub> ), 3.13.3(m, 2H, CH <sub>2</sub> ), 4.1(t, 1H, -CH), 4.2(m, 2H, -CH <sub>2</sub> CH <sub>3</sub> ), 7.1-7.5(s, 7H, ArH), 9.4(s, 1H, -N=CH)                        | $\delta$ 14.17, 40.24, 61.27, 74.60, 105.90, 110.13, 118.70, 128.43, 128.54, 129.32, 136.12, 137.03, 137.09, 144.98, 163.94, 171.38, 191.96. | $\alpha_D = 176.83$<br>(C=5 in $\text{CHCl}_3$ )  |
| Ligand 5  | 3433, 3315, 1726, 687             | 1.3(t, 3H, -CH <sub>3</sub> ), 2.7-3.0(m, 2H, -CH <sub>2</sub> NH), 3.45(m, 1H, -CH), 3.5-3.8(dd, 2H, -CH <sub>2</sub> ), 4.2(m, 2H, -CH <sub>2</sub> CH <sub>3</sub> ), 4.84(s, 2H, -NH <sub>2</sub> ), 7.0-7.4(s, 7H, ArH) | $\delta$ 14.28, 39.61, 51.13, 61.09, 61.33, 108.03, 110.38, 125.31, 126.83, 128.54, 129.14, 131.74, 133.50, 137.42, 143.99, 174.17           | $\alpha_D = -90.01$<br>(C=5 in $\text{CHCl}_3$ )  |
| Ligand 6  | 3300, 2890, 2540, 680             | 1.29(s, 2H, -NH <sub>2</sub> ), 2.8-3.0(m, 2H, -CH <sub>2</sub> ), 3.50(m, 1H, -CH), 3.8(m, 2H, -CH <sub>2</sub> OH), 7.04(s, 1H, -OH), 4.84(s, 2H, -NH <sub>2</sub> ), 7.1-7.5(s, 7H, ArH), 8.0(s, 1H, N=CH)                | $\delta$ 32.0, 39.40, 60.10, 66.21, 74.61, 104.69, 107.59, 122.00, 126.43, 128.40, 129.48, 134.52, 135.87, 143.72, 163.20, 203.01            | $\alpha_D = -189.01$<br>(C=5 in $\text{CHCl}_3$ ) |

### Structure based drug designing

It is an iterative process, in which both ligands and drug target were known and further proceeded for the receptor-ligand interaction studies. In this current study crystal structure of HIV-1 protease with Pbd id -1ODW resolution of 2.10 Å were docked with the ligands 1 to 6 with standard Indinavir using ligand fit protocol available in Accelrys discovery studio 2.1 v

### Receptor-ligand interaction

The binding site search was carried out in the shape-based mode of receptor using the eraser and flood-filling algorithm among 3 active sites of increasing volume. The site 1 was chosen with binding site attributes of Volume 376.375 Å with Point Count 3011 in 3D view direction of 6.031 (X), 0.459 (Y) 12.704 (Z) and Grid Spacing 0.5 x 0.5 x 0.5 surrounded by radius 11.2 Å. The conformations of each ligands were generated using Monte Carlo simulation of about 2 500 120, 4 1200 300, 6 1500 350, 10 2000 500, 25 3000 750 trails. The docked complex was evaluated based on the dock score and internal energy. The following table 2 and the respective interactions were shown in the Figure 2 and binding of ligands to the active site is shown in the Figure 3.

**Table 2: Receptor- ligand interaction of protein and ligands**

| Ligands   | Dock score | Internal energy | Interacting amino acid        |
|-----------|------------|-----------------|-------------------------------|
| 1         | 53.447     | -4.198          | A:GLY27                       |
| 2         | 51.720     | -4.188          | B:ASN25                       |
| 3         | 47.099     | -3.155          | B:GLY48                       |
| 4         | 64.651     | -6.031          | B:ASN25                       |
| 5         | 24.67      | 2.456           | Vander Waals                  |
| 6         |            |                 | No docking                    |
| Indinavir | 85.115     | -9.024          | A:B:25,A:GLY27,A:ASP29,A:ARG8 |

Due to the structure activity belong to the above molecules the nitrogen atom in the amine and oxygen atom of the carbonyl ester group make more hydrogen bonding interaction and the chiral nature of compound may possess the character of increasing the solubility and the docking results suggest that the ligand 4 exhibit the potential to act as HIV protease inhibitor.

Figure 2: Interaction of ligands in the active site of the drug target.

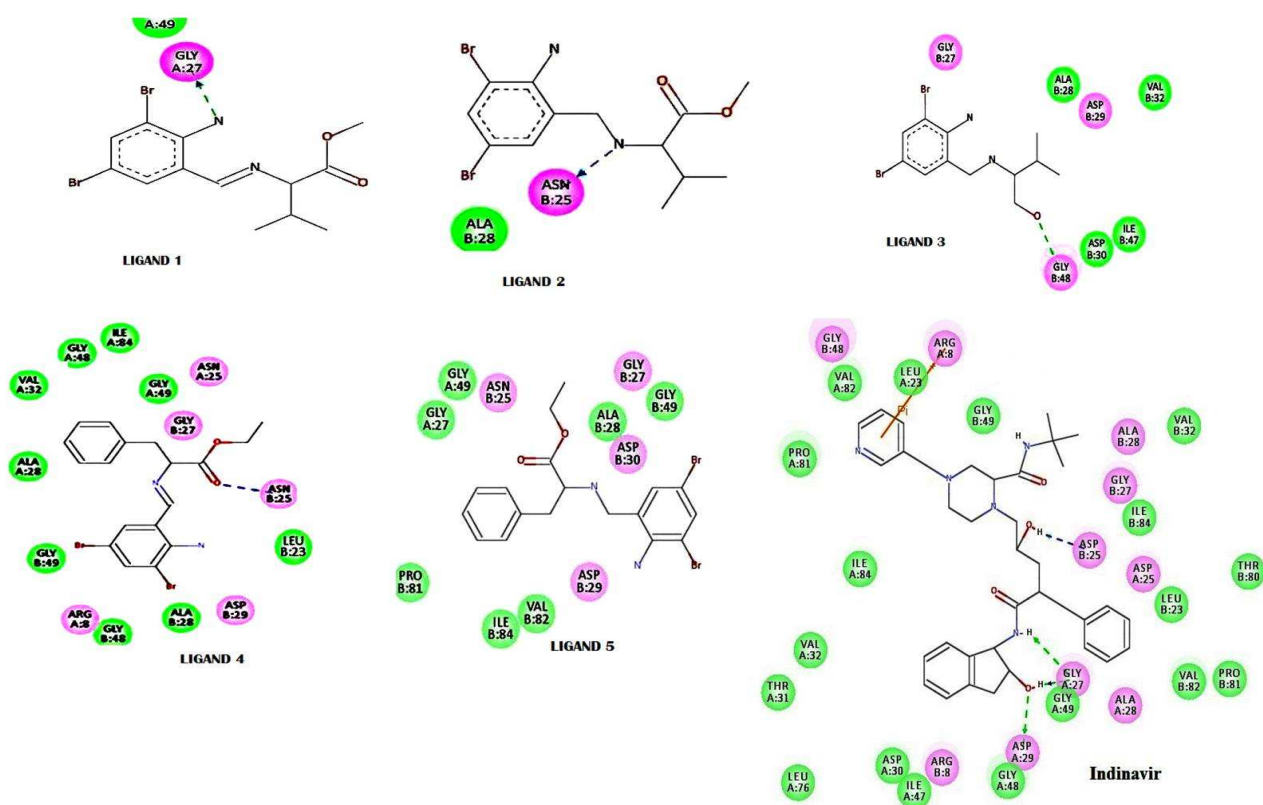
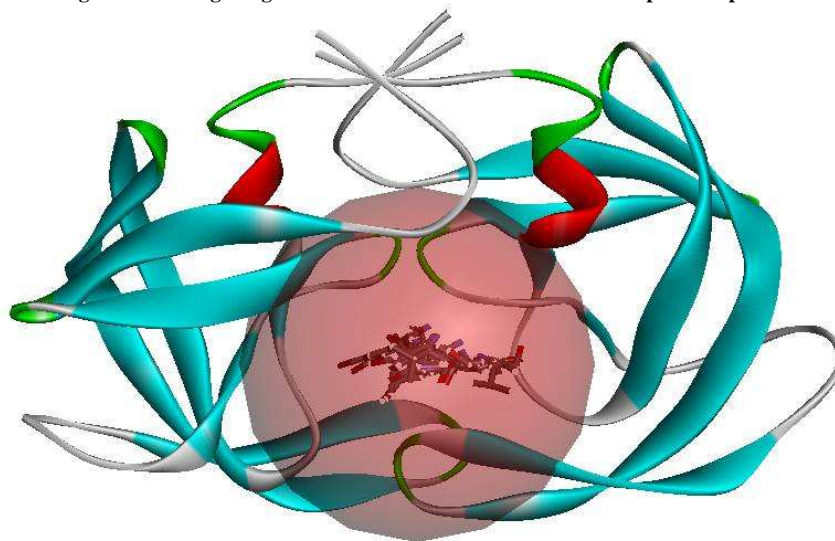


Figure 3: Binding of ligands to the active site of the HIV native protease protein



## CONCLUSION

In the present work we have synthesised six chiral molecules and characterized by different analytical techniques, molecular docking is a key tool in structural molecular biology and computer assisted drug design. The chiral ligand suits the structure complementarily with the active site of the protein and shows specific binding with ASP25and it is crucial amino acid for the inhibition of HIV-protease .The nitrogen and oxygen present in various functional groups are the important pharmacophore feature of the chiral compounds to this drug target protein .It can be concluded that this class of compounds certainly holds great promise towards the pursuit to discover novel classes of Anti HIV drug candidates in future.

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