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PHYTOCHEMICAL, SPECTRAL AND MOLECULAR DOCKING STUDIES ON N, N-[3-HYDROXY-5-METHYL PHENYL]-OXAMIDE FROM *PEGANUM HARMALA* SEEDS

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ABSTRACT

A new, N, N'-[3-hydroxy-5-methyl phenyl]-oxamide has been isolated from a chloroform extract of peganum harmala seeds. Preliminary screening of crude extracts obtained after solvent extract and purification by chromatographic studies. The structure of the compound was identified further by melting point determination and spectral analysis of UV-VIS, FT-IR, ^{1}H NMR & ^{13}C NMR spectroscopy and mass spectrometer studies. The structure of the isolated compound was docking with HIV integrase Protein with help of schrodinger softwere.

KEYWORDS: Peganum harmala, chromatography, NMR, molecular docking, HIV integrase

1. INTRODUCTION

Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutics, plant based natural constituents derived from any part of the plant like bark, leaves, flowers, roots, fruits seeds, etc., act as the best reservoir for products that can be explored in the treatment strategies.[1-2]

Despite the rise of combutational chemistry as an integral part of lead discovery process, natural products play a major role as starting material for drug discovery. A different set of metabolites is sometimes produced in the different anatomical parts of the plant (e.g. root, leaves and flower), and botanical knowledge is crucial also for the correct identification of bioactive plant materials.[3-6] Two main approaches exist for the finding of new bioactive

chemical entities from natural sources random collection and screening of material; and exploitation of ethnopharmacological knowledge in the selection.[7-10] The former approach is based on the fact that only a small part of Earth's biodiversity has ever been tested for pharmaceutical activity, and organisms living in a species-rich environment need to evolve defensive and competitive mechanisms to survive.[11-12] A collection of plant, animal and microbial samples from rich ecosystems might give rise to novel biological activities.[13-16] Peganum harmala is a plant of the family Nitrariaceae, native from the eastern Mediterranean region east to India. The structure was established together with harmine, harmaline and harmalol. Some naturally occurring β-carbolinnes such as harmine and harmaline have been known for a considerable time as central stimulants and inhibitors. When the seeds are extracted with water, a yellow fluorescent dye is obtained. If they are extracted with alcohol, a red dye is obtained. The stems, roots and seeds can be used to make inks, stains and tattoos.[17-18] The human immune deficiency virus (HIV) is a lent virus (a subgroup retrovirus) that cause the acquired in humans in which progressive failure of the immune system allow life threatening opportunistic infections and cancer to thrive, without treatment average survival time after infection with HIV is estimated to be 9 to 11 years on HIV subtype infection with HIV is estimated to the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk, within these bodily fluids. HIV is present as both free virus particles and the human immune system such as helper T cells or primary receptor, called "CD4". On the surface membrane of all living cells are complex protein structures called "receptors". A receptor is often compared to a lock into which a specific key or "ligand" will fit. Finally, isolated compound and associated proteins are packaged and released from the cell surface, taking with them a swatch of membrane containing viral surface proteins. These proteins will then bind to the receptors on other immune cells facilitating inhibited to infection. The structure of the compound was identified by melting point determination and spectral analysis of UV-VIS, FT-IR, ^{1}H NMR & ^{13}C NMR spectroscopy and mass spectrometer studies. The structure of the isolated compound was docking with HIV integrase Protein with help of schrodinger softwere.

2. MATERIALS AND METHODS

2.1 Plant material

The authenticated plant materials of seeds of *Peganum harmala* were obtained from VHCA Herbals, Haryana India. Seeds were air dried in the shade for several days at room temperature. Dried material were powdered and stored in air-tight containers till the use.

2.2 Experimental Methods

The collected seed materials were washed with distilled water and shade dried at room temperature. The seeds were grinded to fine powder with the help of mixer grinder and stored in an airtight container. The powdered (250 g) plant seed materials were extracted successively using Soxhlet apparatus (500 ml of 24-neck round bottom flask) with 250 ml of chloroform. The extraction was carried out for 24 hours at room temperature. The extracts were filtered and concentrated. The extract solution was applied to the column chromatographic technique. The fractions were evaporated to dryness and the residues were collected**.** The oxamide deposit (light brown color compound) was predicted at the composition of petroleum ether 90% and ethyl acetate 10%. The collected material was used for further techniques. Each fraction collected and checked for thin layer chromatography techniques.

2.3 Characterization Techniques

Melting points were recorded with melting point apparatus Macro Scientific Works (Delhi). Thin Layer Chromatography (TLC) was performed on glass plates coated with silica gel 60 (E.Merck, India Ltd.). UV-VISIBLE spectrum of the compound was recorded employing systronics double beam spectrophotometer: 2202 FT IR spectrum was recorded employing Perkins Elmer FT IR spectrometer using the KBr pellet technique. The ^{1}H NMR and ^{13}C NMR spectra of the compound were recorded using the AMX 300 spectrometer with CDCl3 as the internal standard reference. The structure of the isolated compound was docking with HIV integrase Protein with help of schdinger softwere.

3. RESULTS AND DISCUSSIONS

The isolated compound identified with help of chromatography techniques**.** This thin layer chromatography technique used to find out the single spot in the solvent mixture of 10 ml of petroleum ether (non-polar: 60-80^{o}C) few drops of ethyl acetate (polar). The selected spot analyzed and concluded is an oxamide. The melting point of the compound was found to be 224-225 º C.

3.1 UV-Vis absorption spectra of the compound

UV visible spectrum was used to determine the transmission range as well as to find the accuracy of oxamide (Fig.1). The expected compound was recorded and reproduced in UV-Visible absorption spectrum. Figure shows the UV-visible spectrum of oxamide from seeds of Peganum harmala. The compound revealed in absorption band in the entire visible region

from 200-800nm. The spectrum exhibited strong absorption bands due to π-π* and n-π* transitions in the near UV-region of the spectrum 240nm and 300.50nm respectively; although it include the visible region is 324-663nm. Here, enough double bond identified in conjugation, and also compound color was confirmed into visible region range (λ_{max}=663nm).

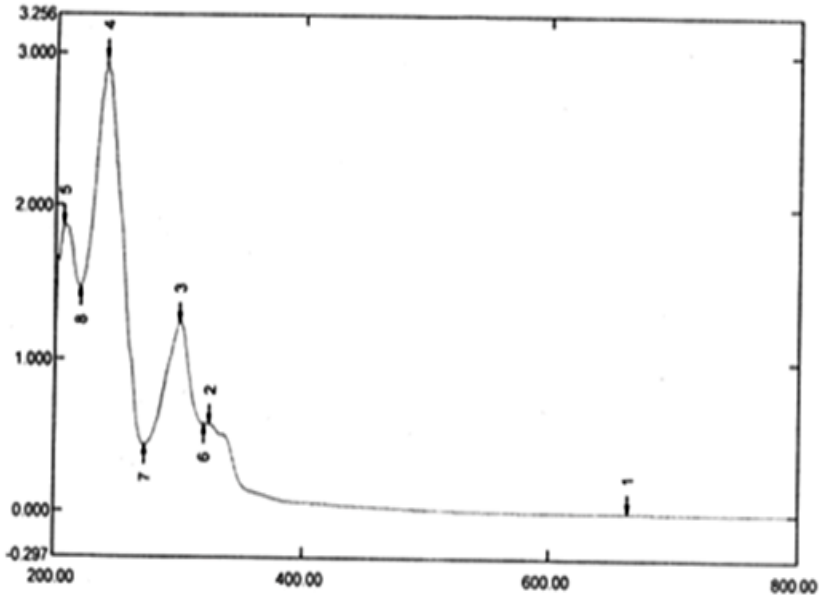


Fig 1. UV spectrum of the compound

3.2 IR Spectrum

IR spectrum was recorded employing Perkins Elmer IR spectrometer using the KBr pellet technique. The vital application of infrared spectroscopy to oxamide compound is to identify the presence of various functional groups which in turn supports the determination of the molecular structure (Fig.2). The band appearing at 3425.34 cm^{-1} and appearing at 3363.62 cm^{-1} were ascribed to the asymmetric stretching vibration of the N–H group. The aromatic C–H symmetric stretching vibration is observed at 2923.88 cm^{-1} and the corresponding symmetric stretching vibration is observed at 2854.45 cm^{-1}. The strong and sharp band appeared at 1627.81 cm^{-1} is assigned to the carbonyl (>C=O) stretching vibration. The spectral data are given in the Table 1.

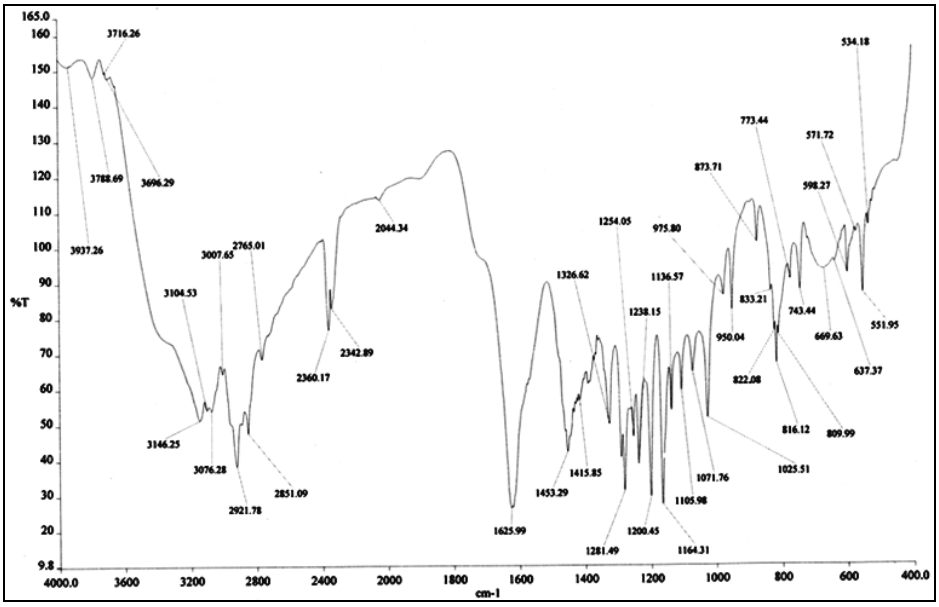


Fig 2. IR spectrum of the compound of N, N′-[(3-hydroxy-5-methyl) phenyl]-oxamide

Table:1 - IR spectral data of N, N′-[(3-hydroxy-5-methyl) phenyl]-oxamide

Bond stretching characteristics	Wave number (cm^{-1}
O-H asymmetric stretching vibration	3425.34
N-H asymmetric stretching vibration	3363.62
Aromatic C-H asymmetric stretching vibration	2923.88
Stretching vibration =C=O Kenotic group	1627.81

3.3 ^{1}H NMR

A seed extract was analyzed by ^{1}H-NMR (Fig.3) and ^{13}C-NMR (Fig.4) in order to identify the major components.

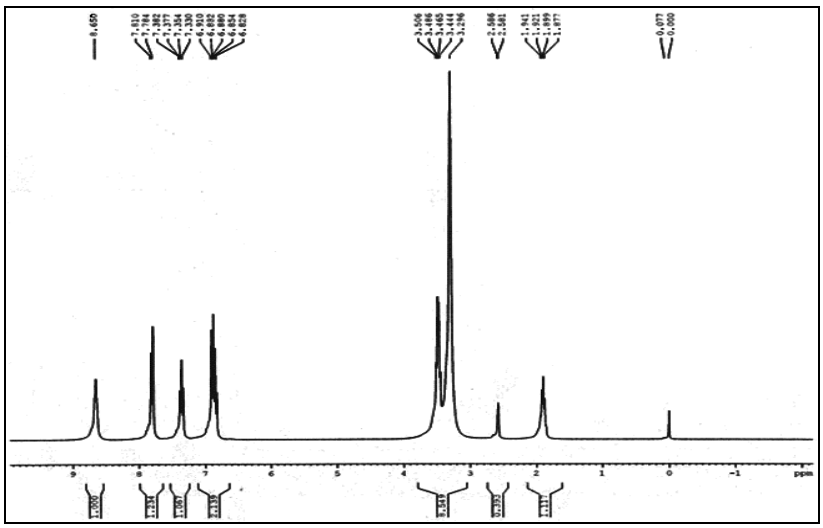


Fig.3: - ^{1}H NMR spectrum of the compound of N, N′-[(3-hydroxy-5-methyl) phenyl]-oxamide.

The ^{1}H NMR spectrum of the synthesized compound show well-resolved signals appeared. The compound showed the resonance with the integrated intensities (Fig.3). The chemical shift of the compound appeared at δ with the value of 8.65 for OH peak. Then aromatic signals were observed up to 7.810 1H and 6.828 2H. The 2.58 s1H was NH peak and 1.90 t, 3H methyl group was observed.

^{13}C NMR

^{13}C NMR spectrum revealed the presence of 8 signals 165.43 (>C=O) was observed and then 147.48 (Ar-C-OH), 141.88 (Ar-C-NH), (127.21, 116.74, 114.22) aromatic carbons and 24.83 for methyl carbon was observed(fig.4).

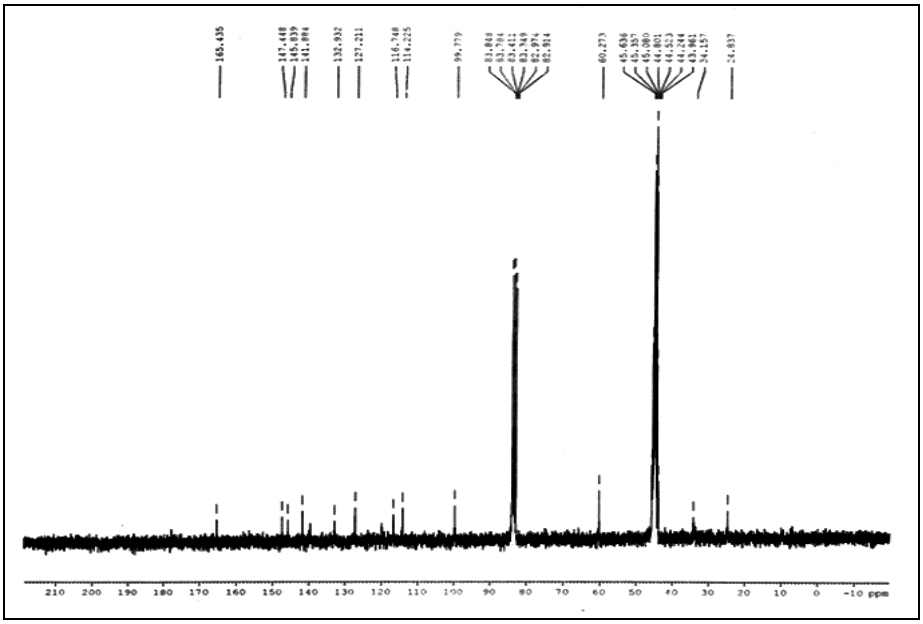


Fig.4: - ^{13}C NMR spectrum of the oxamide compound.

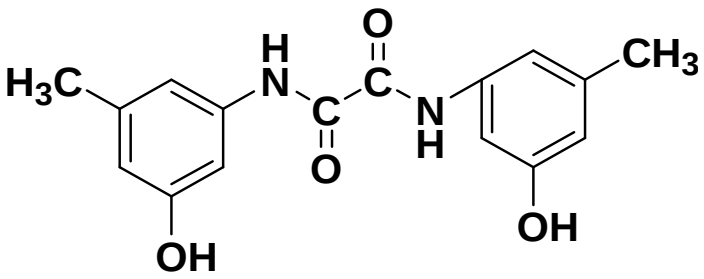


Fig:5 – Chemical structure of the compound of N, N′-[(3-hydroxy-5-methyl) phenyl]-oxamide.

4. Molecular Docking studies

4.1 *In silico* analysis

The amino sequence of target protein HIV Protease.

>1HXW:A|PDBID|CHAIN|SEQUENCE
PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMNLPGRWKPKMIGGIGGFIKVR
QYDQILIEICGHKAIGTVLVGPT
PVNIIGRNLLTQIGCTLNF
>1HXW:B|PDBID|CHAIN|SEQUENCE
PQITLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMNLPGRWKPKMIGGIGGFIKVR
QYDQILIEICGHKAIGTVLVGPT
PVNIIGRNLLTQIGCTLNF

The primary structure of target protein HIV Protease.

PQITLWQRPL VTIKIGGQLK EALLDTGADD TVLEEMNLPG RWKPKMIGGI GGFIKVRQYD

70 80 90 100 110 120
QILIEICGHK AIGTVLVGPT PVNIIGRNLL TQIGCTLNFP QITLWQRPLV TIKIGGQLKE

130 140 150 160 170 180
ALLDTGADDT VLEEMNLPGR WKPKMIGGIG GFIKVRQYDQ ILIEICGHKA IGTVLVGPTP

190
VNIIGRNLLT QIGCTLNF

- Number of amino acids: 198
- Molecular weight: 21621.6
- Theoretical pI: 8.98
- Total number of negatively charged residues (Asp + Glu): 16
- Total number of positively charged residues (Arg + Lys): 20.

4.1 Atomic composition

Carbon	C	980
Hydrogen	H	1608
Nitrogen	N	262
Oxygen	O	269
Sulfur	S	8

- Formula: $C_{980}H_{1608}N_{262}O_{269}S_8$
- Total number of atoms: 3127
- Extinction coefficients**:**
- Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.
- Ext. coefficient 25230
- Abs 0.1% (=1 g/l) 1.167, assuming all pairs of Cys residues form cystines
- Ext. coefficient 24980
- Abs 0.1% (=1 g/l) 1.155, assuming all Cys residues are reduced.

4.2 Estimated half-life

The N-terminal of the sequence considered is P (Pro).

The estimated half-life is: >20 hours (mammalian reticulocytes, in vitro).

>20 hours (yeast, in vivo).

? (Escherichia coli, in vivo).

4.3 Instability index

- The instability index (II) is computed to be 44.11
- This classifies the protein as unstable.
- Aliphatic index**:** 119.09
- Grand average of hydropathicity (GRAVY): 0.182

Visualization: All the visualization of the structure files was done using XP visualize.

Molecular dynamics or simulation

QSAR

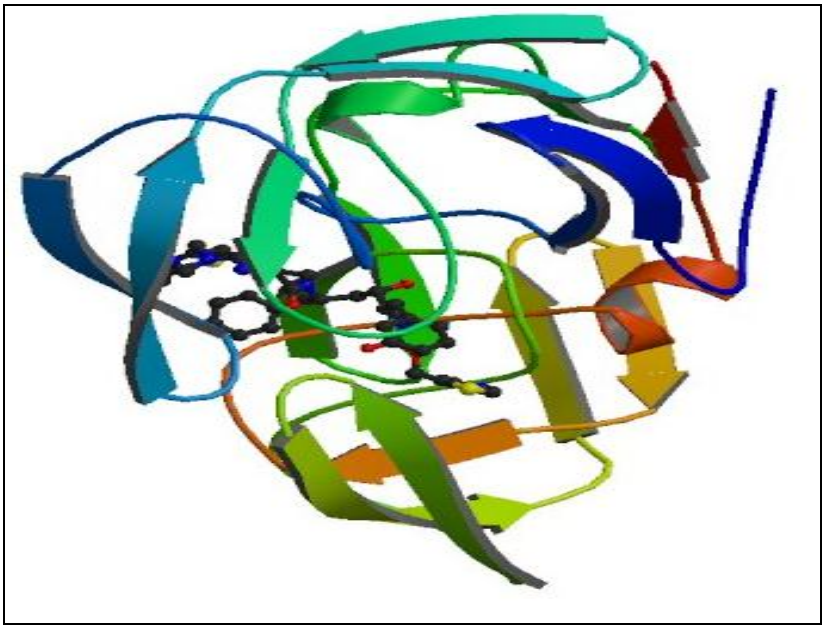
Preparation of protein

The crystal structure of HIV integrase were obtained from Protein databank (PDB ID: 1HXW) with a resolution factor Å. Before docking, the crystal structure of the protein were cleaned by removing the water molecule, added the hydrogen atom to the protein structure

for tautomeric and ionization states of amino acid residues using OPLS-2005 forcew field. Finally the protein structure energy was minimized reached untill the average root mean square deviation non bonded hydrogen atom 0.30Å. The prepared protein were input file for molecular docking.

Active site prediction Using CAST p

The HIV protease (PDB ID: 1HXW) was retrieved from protein data bank further taken to CASTp for binding site prediction which revealed different binding site. The best binding residues from the CASTp result given below. R8, 23L, 25D, 26T, 27G, 28A, 29D, 30D, V32, 47I, 48G,49G, 50I, R8, P9,L10, L23, D25, T26, G27, A28, D29, D30, P31, V32, I47, 48G, R87..



3-Dimensional Structure of Target protein HIV Protease (PDB ID: 1HXW).

Preparation of Ligand Structure

The five synthesised compounds were drawnusing chem draw version. The ligand structure were further carried out into ligprep for ligand preparation version. Ligprep convert 3D structure from 2D structure, include different tautomers and ionized form at a pH range 7.0± 2.0. the prepared ligands were input file for molecular docking studies.

4.4 Docking

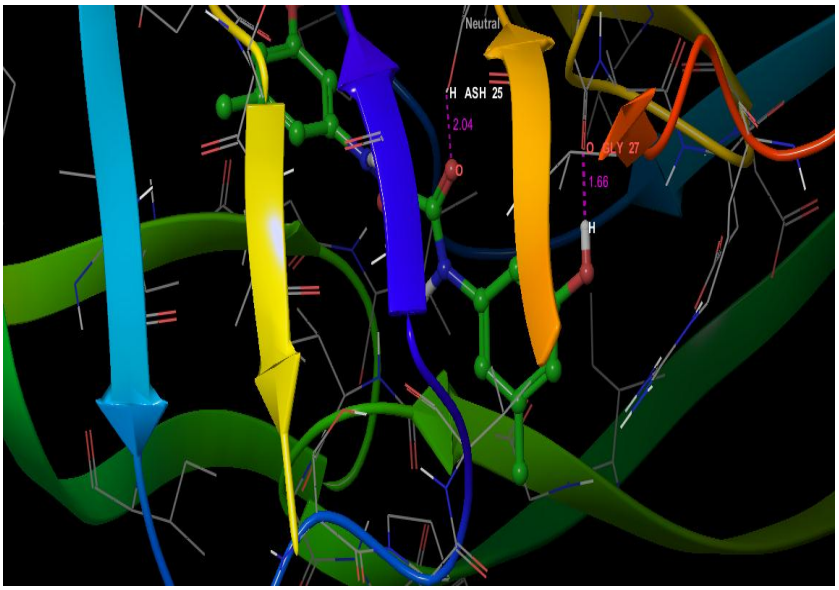
The isolated compounds were docked in to the active site of the HIV protease (PDB ID: 1HXW) using Glide from Schrodinger.[26] Grid was prepared for p HIV protease with the

exact same center and the size of the bounding box set on 20 Å. The Glide algorithm is based on a systematic search of positions, orientations, and conformations of the ligand in the receptor binding site using funnel type approach. The search begins with a rough positioning and scoring phase that significantly limits the search space and reduces the number of poses to be selected for minimization on the pre-computed OPLS-2005 van der Waals and electrostatic grids for the protein. The glide score and glide energy was analyzed using XP visualize.

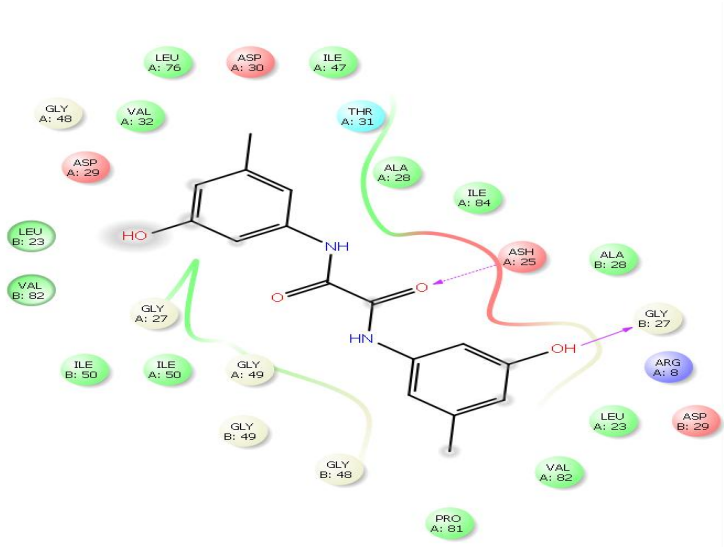
4.5 Binding Mode Analysis

Docking Simulation of oxamide compound into HIV Protease

Docking simulation of isolated compound into HIV Protease resulted in the formation of only two hydrogen bond interactions with bond distance of (2.04Å, 1.66Å) and it was observed that side chain hydrogen atom of ASH 25 were interacted with oxygen atom of the isolated compound, the hydrogen atom of the GLY 27 were strongly interacted with oxygen atom of the compound 2 Glide Score and Glide Energy value for compound 2 were observed -5.745Kcal/mol and -55.404Kcal/mol. Furthermore LEU 23, VAL 82, ILE 50, ALA 28 a number of hydrophobic interaction were bound between compound 2 into HIV Protease.



This figure shows docked structure of target protein HIV Protease with isolated compound.



This figure shows docked structure of target protein HIV Protease with isolated compound.

CONCLUSION

In conclusion, this study is a complementary survey to phytochemical and spectral studies carried out upon N, N′-[(3-hydroxy-5-methyl) phenyl]-oxamide. This project work involves extraction of the peganum harmala seeds with petroleum ether from which a pure component has been identified by crystallization with composition of petroleum ether 90% and ethyl acetate 10%. TLC report also gives the indication of a pure component. Melting point determination, UV, IR, ^{1}H-NMR and ^{13}C-NMR spectral techniques identify the purity of the compound and also the spectral data's indicate that the compound is N, N′-[(3-hydroxy-5-methyl) phenyl]-oxamide. So that identified compound may be correctly predicted as N, N′-[(3-hydroxy-5-methyl) phenyl]-oxamide. More recently has been to have an antimicrobial studies have been carried out. Docking simulation of isolated compound into HIV Protease resulted in the formation of only two hydrogen bond interactions target protein HIV Protease.

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