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New Strategy to Find Out Microwave –Assisted Synthesis of B-Phenyl Isoserine Dipeptides as a Potential Inhibitor Candidate for HIV1 Protease and Amino Pepetides

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ABSTRACT *: This object described the synthesis of β-phenylisoserine based dipeptides (α-hydroxy β-amino cantaining dipeptides) as potential HIV-1 protease and amino peptidase inhibitors. These core structure have been synthesizes from cinnamoyl amides (derived from amino acid) using cobalt catalyzedprotocol.thepolyanilinensupported cobalt complex (PASCOS) has been prepared by reacting cobalt (n) salen with polyanline in acetic acid .thecinnamoyl amide has been converted to the corresponding epoxide on treatment with 2-methylpropanal in the presence of oxygen and catalytic amount of polyaniline supported cobalt complex.(PASCOS) This epoxide of N-cinnamoyl amide have been converted to β-phenylisoserine derivatives though microwave –assisted ring opening with various aromatic and aliphatic amine using alumina as a solid support. Application demonstrated by synthesizing a library of dipeptide derived from βphenyl isoserine derivatives and various a-amino acids. The opening of epoxides with aniline derivatives provides structure having hydrophobic enviormental around the N-terminal of the dipeptides. The synthesis of dipeptides derivatives is very useful as it may leads to the β-phenylisoserine, which may be useful as HIV protease Novalisosterea-hydroxyβamino ethylene group have been incorporated with binding elements present away from the scissile peptide bond site, which could lead to better binding interaction with the active site of aspartyl protease like hivprotease. Also these small molecule peptides are structure analogues of the potent β-phenyl isoserine based aminopeptidase inhibitors,*

KEY WORDS*: protease inhibitor, HIV-1, PASCOS, Microwave –assisted, Novel isoester,*

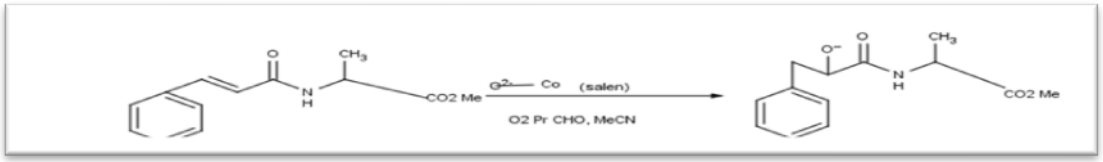


Fig: 1

Fig: 2

I. INTRODUCTION

1.1.Human immunodeficiency virus

Human immunodeficiency virus (HIV) is a lentivirus (slowly replicating retrovirus) that causes acquired immunodeficiency syndrome (AIDS),[1][2] a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Infection with HIV occurs by 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 virus within infected immune cells.HIV infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells.[3] HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms including: apoptosis of uninfected bystander cells,[4] direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocytes that recognize infected cells.[5]

1.2.HIV-1 Protease

HIV-1 protease (PR) has been extensively studieddue to its importance as a therapeutic target in AIDStherapy. It plays a key role in the maturation andinfectivity of new viral particles. In its active siteform, HIV-1 PR is a 22 kDahomodimericasparticprotease consisting of two identical 99-residue polypeptide chains that self-assemble to form anapproximately 452325 A˚dimer.The active site is formed along the interface between two subunits containing the two catalyticallyimportant Asp residues. In the virus, a single copy ofthe HIV-1 protease is synthesized as a part of thepolyprotein Gag-Pol, flanked by the highly variablep6pol sequence at its N-terminus and by reversetranscriptase (RT) at its C-terminus. The initial critical step in the maturation of the Gag-Pol precursor isthe folding and dimerization of the protease domain,which results in cleavage of peptide bonds at specificand crucial sites to release mature proteins. Thus, HIV-1 PR is responsible for processing of the Gagand Gag-Pol polyproteins to release proteins requiredfor the assembly and maturation and for subsequent replication of HIV-1 virus [9].

II. MATERIALS AND METHOD

2.1.Experimental section

Materials and Method: Acetonitrile, ethyl acetate, hexane, dichloromethane, tetrahydrofuran and all other solvents were purified by slandered procedure. $CoCl_2$ was purchased from LOBA Indian Limited and dried at 140^0 C for 4 h before use. All the amino acids were brought from Spectrochem India Limited and use as such. Cinnamic acid, cinnamic acid derivative, primary aromaticamines, triethylamine, methylchloroformate, 2-methylpropanal (isobutyraldehyde) were all procured commertcially and were purified before use. The aldehydes were distilled before use. Amines were re-crystallized before use. Polyaniline supported Co (II) salen was prepaired according to procedure develop in our lab. Column chromatography was performed on ACME silica-gel eluent. TLC was performed on ACME silica gel-G coated glass plates and was visyalized in iodine chamber. TLC was also performed on MERCK, silica gel, TLC grade 60 $GF_{254\ with}$ gypsum binder and fluorescent. Coated glass plates and was irradiated using UV lamp. ^1H NMR spectra was recorded using Jeol PMX-60 system, Bruker WP- 80 Joel LA 400 FT NMR machines in CCL_4 / $CDCL_3$. Chemical shift are given relative to TMS in ppm $_{(\delta)}$. Multiplicity is indicated using the following abbreviations: s (singlet), bs (broad singlet), d (double), dd (doublet of a doublet), dt (doublet of a triplet), td (triplet of a doublet), q (quartet), and m (multiplet). The mass spectrum was recorded on Hawlett Packard GS-MS model no. 5989 A mass spectrometer with ionization electron beam energy of 70 eV. Optical rotations were measured in Autopol II / Autopol III polarimeters. All the known compounds were charactereized by comparing with the literature data. Ir spectra were recorded on Perkin – Elmer 1600 FT-IR spectrometer, using either a neat sample or a solution in CCl_4 /Ch_2Cl_2 and solids were examined as KBr pellets and the values are reported in V_{max} (cm^{-1}). HPLC analysis were done with water 745 integrator, water 510 pump and detected with Shimadzu Spd-10 AVP, UV- vis detector. The microwave oven used inn experiments was MLS 1200 mega high performance microwave digestion unit.

2.2.Preparation of polyaniline

Freshly distilled aniline 10mL(109.5 mol) was dissolved in 125mL of 1.5 M HCL, and solution of ammoniumpersulfied (54.8mmol) in 1.5MHCL (125Ml) was added to it at 0°C. Since aniline polymerization is strongly exothermic, the oxidant must be aided slowly over a period of 1h. After the addition of the oxidant, the reaction was stirred further for 4h. Thepolyaniline hydrochloride peptide was separated by filtration and washed consecutivrly with water (3x15 ml) ,methanol (2x 25 ml), and diethyl ether (2x 15 ml) to remove the oligomer and any of the reaction side products. The polymer wasthen vacuum dried until constant mass .deprotonationofpolyaniline hydrochloride was achieved with aqueous ammonia (3wt %) deprotonated polymer was again washed with water ,methanol, and diethyl ether dried until constant mass (~3gm).polyaniline is quite stable to air and can be stored indefinitely in closed glass vials

2.3.Preparation of Polyaniline Supported Cobalt (II) salen (PASCOS)

Cobaltous salen (200mg) and polyaniline (200mg) were added to a solution of acetic acid (25ml) in acetonitrile (25ml) and stirred at ambient temperature for 36 h. The resultant catalyst was filtered off and washed first with acetic acid (3 x 10ml) and then thoroughly with acetonitrile until the filtrate was colorless. The resulting residue was dried in an air oven at 100^0C for 2 h to afford the black (or blackish brown) coloured catalyst. Polyaniline supported cobalt (II) salen is stable to atmosphere and can be stored indefinitely in closed vials.General procedure for the synthesis of Methyl-N-cinnamoyl-amino-ester 2

III. METHOD (A)

To a stirring, ice-cold solution of cinnsasmic acid (1 equivalent) and triethylamine (1 equivalents) in THF (1.5 ml/ mmol) was added methylchloroformate (1 equivalent) and the mixture was stirred vigourously for 2 minutes . After which, a solution of the amino ester hydrochloride(1.1 equivalents) in DMSO (0.5ml/ mmol) was added followed by triethylamine(2.2 equivalent)dissolved in THF(1 ml/mmol) . The reactin vessel allowed to warm to room temp. andvigourously stirred for further 3-4 hr . triethylamine hydrochloride was filterd off on a sintered funnel under suction and washed with THF . Removal of solvent from the filterate under vacuo yielding a residue , which was dissolved i9n EtoaC (~2 ML/MMOL) and washed with saturated aqueous solution of Nhco3 , water and brine . A Drying (Na2so4) and evaporation o0f solvent under vacuo yielding the crude product, which was further purified usually as good solids in good yields .on prolonging beyond this time , usually methyl cinnamate is formed in healthy quantities as a side product.

3.1.Method (B)

To a stirring , ice cold solution of cinnamoyl chloride (1 equivalent) in dichloromethane (1 ml/ mmol) was added the amino ester hydrochloride (1.1 equivalents) followed by a solution of triethylamine (2.2 equivalent) in dichloromethane (1ml/mmol) drop wise through a dropping funnel. After complete addition of triethylamine , the reactin mixtures was vigourously stirred for a further 5-6 and then diluted with dichloromethane (1 ml/ mmol) . workup as describe in method A , With SaturadAquous Solution of $NaHCO_3$, Water and brine and purification by column chromatiography yielding the N- cinnamooyl amino easter in good yields. In generals it was observed that yields. Ingeneral, it was observed that yields of methyl –N-cinnamoyl-amino esters were better by method A , Than by method B.

3.2.Synthesis of 4a

To a solution of methyl –N (3 phenyl glycidyl) –valinate (200mg, 0.722mmol) and anisidine (355mg,2088mmol) in dichloromethane (5ml)neutral alumina (Al2O3) was added .this slurry was tranfered to a Teflon veaael. The vessel was kept under microwave oven for 18 minutes at 50 watt and 50˚C after the alumina was filtered off and the solvent was removed in vacuo. This residue was taken in CCl4 solution .to this CCl4 solution hexane was added to precipitate the product aqnd leaving the amine and the epoxide in the mother liquor. This brown powder was then subjected to column chromatography (silica gel ;ethyl acetate;hexane-1:3)

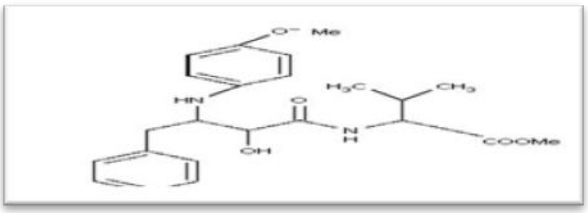


Fig: 3(4a)

3.3.Diastereomerof low R_f

1H NMR (400 MHz, CDCl3):δ7.34-7.32 (m, 2H), 7.24-7.18(m, 2H), 6.90(db., 1H), 6.64 (s,4H),6.69(bs,1H),4.59(d,j=4.4Hz,1H),4.29(DD,J=4.88,4.64Hz,1H),3.64(s,6H),0.80(m,1H),0.59(D,J=6.84 Hz,3H),0.53 (d,j=6.84Hz,3H).
MS(m/z,%:212(M+,100).
IR(neat):Vmax 3342(s), 2924,1744,1669,1510,1033,816,702cm-1

3.4.Diastereomer of high Rf

mp 145˚C

1H NMR(400 MHz, CDCl3):δ7.31-7.15(m,5H),6.93-9.91(bd,IH),6.81-6.75(db,1H),6.62(D,J=8.8 Hz,2H),6.52(d,j=8.8Hz,2H),4.65(dd,J=4.8, 4.64Hz 1H) ,4.43(D,J=4.64Hz,iH),4.31(dd,j=5.4,5.34 Hz,1H), 3.61(s,3H),3.54(s,3H),1.99-1.94(m,1H),0.77 (D,J=6.84 Hz, 3H), 0.74 (D, J=6.8 Hz,3H).
MS (m/z,%):212(M+,100).
IR(KBr):Vmax 3383(s), 2964(s) ,1750(s),1650(s),1548,1513,1020,807,702cm

3.5.Synthesis of 4b

To a solution of N(3phenyl glycidyl) valinate (125mg,0.45mmol) and bromoaniline (310mg,1.80mmol) in dichoromethane (5ml), neural alumina (Al2O3) was added .this slurry was transferred to a Teflon vessel. The vessel was kept under microwave oven for 20 min at 50watt and 50˚C after the completion of the reaction ethyl acetate was aided and stirred for 30 min. the alumina was filtered off and the solvent was removed in

vacuo.t5his residue was taken in CCl4 solution . To this CCl4 solution hexane was aided to precipitate the product and leaving the amine and the epoxide in the mother liquor. This brown powder was then purify by column chromatography(silica gel; ethyl acetate: hexane-1:3) (TLC:Rf=0.40,0.37; ethyl acetate; hexane -1:4) to yield two diastereomeric product as good.solid

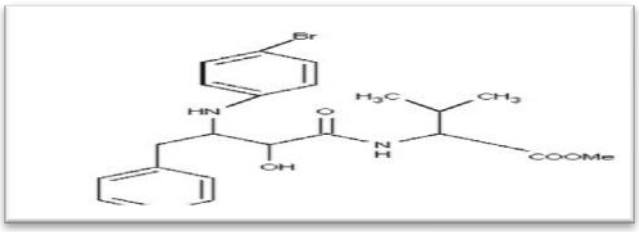


Fig: 4(4b)

3.6.Diastereomer of low R_f: (mp216˚C)

^{1}H NMR (400MHz, CDCl3):δ7.38-7.34 (m,2H),7.29-7.21(m,3H),7.15(d,J=8.52Hz, 2H),6.89 (bd,1H),,4.54(d,j =5.12 Hz,1H) ,4.45(D, J= 5.88 Hz, 1H),4.34(dd, J=8.8 NHz, 4.88 Hz ,1H), 3.71(s,3H), 0.88-0.82 (M, 1H), 0.63 (d, J=6.84 Hz, 3H), 0.58 (d, j=6.84 Hz,3 H) MS (m/z,%):260 (M+, 100), 262 (M+,100).
IR (KBr):Vmax 3389,2964,2888,1750,1653,1022,801,703 cm-1.

3.7.Diastereomer of high R_f: (mp182˚C)

^{1}H NMR(400MHz,CDCl3):δ7.34-7.30(m,2H).7.27-721(m,3H), 7.13(d, j= 8.8 Hz,2H), 7.02(bd,1H),6.46(d, j= 8.8 Hz, 2H) , 4.93 (D, J = 5.4 Hz, 1H),4.76 (d, j=4.16 Hz,), 4.49 (d, j = 4.4 Hz, 1H), 4.36 (dd, j =8.92 Hz, 5.5 Hz, 1H),3.59 (s, 3H), 2.17 (s,1H), 2.09-2.02 (m,1H), 0.87 (d,j= 6.84 Hz, 3H), 0.84 (d,j=6.84 Hz, 3H).MS(m/z %):260(M+99), 262 (M+, 100).
IR (KBr) :Vmax 3380, 2970, 1737,1644,1548,802,700cm-^{1}.

3.8.Synthesis of 4c

To the solution of methyl –N-(3 phenyl glycidyl –leucinate (100mg, 0.344 mmol)and bromoline (236mg, 1.376 mmol) in dichloromethane (5 ml), neutral alumina (Al2O3) was aided .this slurry was transferred to a Teflon vessel . The vessel was kept under microwave oven for 18 min at 50watt and 50℃ after the completion of the reaction ethyl acetate was aided and stirred for 30 min the alumina was filtered off and the solvent was removed in vacuo .thisresiduer was taken in CCl4 solution .to this CCl4 solution hexane was added to precipitate the product and leaving the amine and the epoxide in the motherloquor. This brown powder was then purify by column chromatography ?(silica gel:ethyl acetate: hexane -1:4) (TLC: Rf= 0.60, 0.55; ethyl acetate:hexane -2:3) to yield two diastereomeric products as good solid .

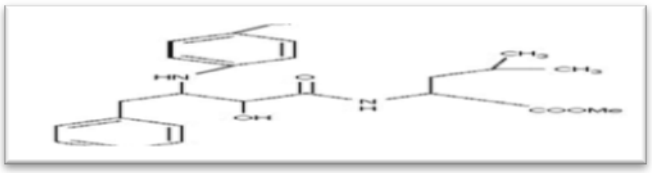


Fig: 5 (4c)

3.9.Diastereomer of low R_f: (152˚C)

^{1}H NMR (400 MHz, CDCl3)δ7.32-7.31(m,2H), 7.24-7.18 (m,3H), 6.64 (d, j= 9.04 Hz, 2H), 6.57 (d, j = 4.16 Hz, 2H), 4.72 (d, j= 4.16 Hz , 1H), 4.49 (D, J = 4.16 Hz 1H), 4.42- 4.37 (m, 1H), 3.63 (s, 3H), 1.38-1.31 (m, 2H) , 1.18 (m 1h), 0.69 (D, J = M2.96 Hz 3H _, 0.67 (d, j= 2.92 Hz, 3H).MS (m/z,%): 260 (M+,100), 262 (M+99).IR (neat): Vmax 3387,2927,2853,1742,1653,1539,1512 cm-^{1}.

3.10.Diastereomer of high R_f: (mp 120˚C)

^{1}H NMR (400MHz,CDCl4): δ7.36-7.20(m,5H)7.17(dj=8.8Hz,2H),6.46(d,j=8.8 Hz, 2H), 4.72 (d,j=5.12 Hz, 1H),4.56-4.51 (m,1H) 4.42 (bs, 1H),3.66 (s,3H), 1.46-141 (m, 2H), 1.25 (m,1H) , 0.91- 0.88 (bd, 6H). MS(m/z,%) : 260(M+,100), 262 (M+,100).

3.11.Synthesis of 5a

To a solution of methyl-n-(3-phenylglycidyl)-leucinate (150 mg, 0.515 mmol)and allylamine (235 mg, 4.12 mmol) in dichloromethane (5 ml) neutral alumina (Al_2o_3) was added (which acts as a solid support). This slurry was transferred to a Teflon vessel . The vessel was kept under microwave oven for 20 min at 100 watt and 80 c . After the completion of the reaction ethyl acetate was added and swtirred for 30 min. the aqlumina

was filterd off and the solvent was removed in vacuo . This residue was taken in CCl4 solution . To this CCl_4solution .to this CCL_4 solution hexane was added to ppt the product and leaving the amine and the epoxide in the mother liquor . this brown powder was then chromatographed (silica gel ; ethyl acetate :hexane -1:3) (TLC:R_f=0.25,0.20;ethyl acetate :hexane-3:2) to get the bestatin analog (2- diastereomers).

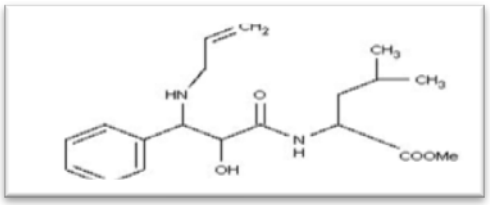


Fig: 6 (5a)

3.12.Diastereomer of low R_f:

1H NMR (400 MHZ, CDCL3):δ 7.41-7.39 (M, 2H) , 7.39 (m, 2H) , 7.29-7.27(m, 3H), 5.93-5.87 (M , 1H) ,5.26(d, j=10.28Hz ,1H), 5.17 (d, j=16.84 Hz , 1 H), 4.84 (d, J= 4.16 Hz 1H) , 3.18(dd,j=13.68,7.56Hz,1H),1.31-1.10 (m, 2H) , 0.92-MS (m/z,%):349(M++1,100),146(M+,57).

3.13.Diastereomers of high R_f

1H NMR (400MHZ, CDCL3):δ 7.61 (bd ,1H) , 7.31-7.26 (m, 5H),5.90-5.81 (m, 1H) , 5.18 (d, J=16.08 HZ,1H),5.13(d,J=10Hz,1H),4.15 (m,1 H) 4.29 (d,J=6.39,1H), 3.98 (d,J=6.39, 1H) , 3.61 (s,3 H), 3.23 (m, 2H),1.61 (bs,2H),1.25 (bs, 1H),0.92(d,j+4.88HZ,3H),0.92(d,j+5.36HZ,3H)

3.14.Synthesis of 5b

To a solution of methyl-N-(3-phenylglycidyl)-phenylalaninate (150 mg, 0.46mmol) and benzylamine (98 mg, 0.923 mmol) in dichloromethane (5ml) neutral alumina(AL2o3)was added . This slurry was transferred to a Teflon vessel . The vessel vessel was kept under microwave oven for 20 min at 100 watt and 80 c .After the completion of the reaction ethyl acetate was added and swtirred for 30 min. The aqlumina was filterd off and the solvent was removed in vacuo . This residue was taken in ccl4 solution .to this CCL_4solution . To this CCL4 solution hexane was added to ppt the product and leaving the amine and the epoxide in the mother liquor . This brown powder was then chromatographed (silica gel ; ethyl acetate :hexane -1:3) (TLC:R_f=0.40,0.35;ethyl acetate :hexane-3:2) to get the bestatin analogs (2- diastereomers).

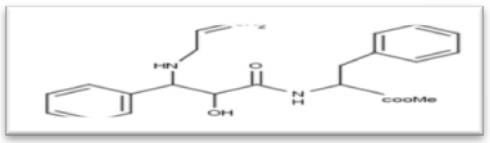


Fig: 7(5b)

Diastereomers of low R_f: mp 134c

1H NMR (400MHZ, CDCL3):δ 7.43 -6.97 (m,1 5H),6.64 (s, 1H) , 6.63(s,1H),4.67(bs,1H),4.30 (d, J=5.6 HZ,1H),4.23 (d,J=6.36 Hz,1H),) ,3.66(d,J=11.56HZ,2H), 3.54 (s,3 H), 2.77 (dd, j =13.8,5.6HZ ,2H).
MS (m/z ,%): Vmax 3404, 3323,3323 ,3030 ,2956,2928,2850,1737,1648,1531,1027,746,698cm^{-1}.

Synthesis of 5c

To asolution of metyl-N-(3-phenylglycidyl)n –phenylalaninate (100mg,0.307mmol) and allylamine (140mg,2.46mmol) in di chloromethane (5ml) neutral alumin(Al_2O_3)was added .this slurry was transferred to a Teflon vessel the vessel was kept under microwave oven for 18 minu at 100watt and 80˚C.after the completion of the reaction ethyl acetate was added and stirred for 30 min the alumina was filtered off and the solvent was removed in vacuo. This residue was taken in CCl4 solution to this CCl4 solution hexane was added to precipitate the product and leaving the amine and the epoxide in the mother liquor. This brown powder was then chromatographed 9silica gel;ethylacetate;hexane -1:4) (TLC:Rf=0.30,0.25,ethyl acetate ;hexane -3:2) to get the beatatin analogs (two diastereomers).

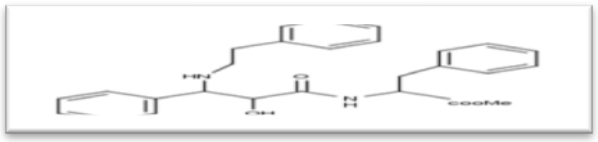


Fig: 8 (5c)

Diasrereomer of low R_f

^{1}H NMR (400MHz,CDCl3):δ 7.36-7.03(m,10H) ,6.64-6.61(m,2H),5.80-5.73 (m,1H),5.15-5.05 (m,2H) 4.59(d,j=5.12Hz,1H),4.24 (d,j= 4.88Hz,1H) ,3.55 (s,3H),3.331(m,2H),2.75(m,2H).
MS (m/z,%:383 (M+1,100),146(M+,51),100(M+,33),90(M+,58).
IR (KBr):Vmax 3395,3063,2926,2853,1746,1667,1530,746,700-^{1},

Diasrereomer of high R_f: (mp 128˚C)

^{1}H NMR (400 MHz,CDCl3):δ 7.29-7.00(m,10H),5.73-5.67 (m,1H),5.04(m,2H) 4.63 (dd,j=14.02, 6.36Hz1H), 4.29 (d,J= 6.39Hz, 1H),4.29(d ,j=6.39 Hz,1H),3.52 (s,3H),3.14 (d, j=13.97 5.88 HZ,1H),3.04 (D,J=1392,612 HziH), 2.96-2.91 (m,2H),2.78 (BS,1H).
MS (m/z%): 383 (m+1,100),245 (m+,43),146 (m+,33),90(M+,58).
IR (KBr):Vmax 3347,2925,2853,17421661,1517,747,700cm-^{1}.

IV. RESULTS AND DISCUSSION

As discussion in the previous section , hiv protease (PR) is one of the three crucial viral enzyme ,viz, reversetrascriptase (RT).PRandintegrase is essential for replication of HIV .it has been shown that the inhibition of HIV PR result in the production of viral particles that are immature and non-infectious,this is particularly important for the occurrence of the mutation in HIVprotease and subsequent inhibitor resistance.as a part of our ongoing study to the synthesis of small molecules library of HIV protease inhibitor , we thought of making some structural divers small libraries which will have the potency of protease inhibition.

TABLE 1: synthesis of N-cinnomoyl dipeptides 2

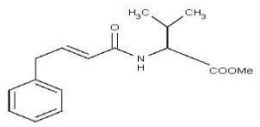
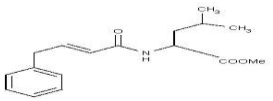
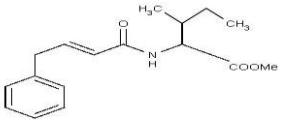
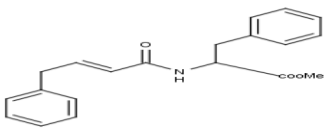
R	STRUCTURE	REACTION TIME (A)THF:(B)DCM	YIELD (%)A:B
(CH3)CH	 2a	5:3	78:82
(CH3)CHCH2	 2b	5.5:3.5	80:85
(CH3)CH2CH(CH3)	 2c	5:3	85:88
C6H5CH2	 2d	6:4	68:78

Table 1 Explains : synthesis of N-cinnamoyl dipeptides2 via isolated yield after column chromatography the first part of the synthesis involves preparation of N-cinnamoyl dipepetide2.in atypical reaction ,cinnamoyl chloride was reacted with methyl ester hydrochloride of amino acid 1 in the presence of triethyl amine as the base in dichloromethane at 0 ℃ .the reaction was monitored by TLC. after 5-6 hours the amide derivatives 2of the valine 2a,leucine2b, isoleucine 2c and phenyl alanine 2d were obtained.When THF was used as solvent the reaction proceeded faster and was complete in 3-4 hours. The structure of each of the N-cinnamoyl dipeptide 2 was confirmed its spectral analysis. Among the various N-cinnamoyl dipeptide, the product abtained in the case of isoleucine 2c was highest ,approximately 88%. After getting the N-cinnamoyl 1 dipeptide 2, the next step was to carry out the epoxidation of these amide .an easier and higher yielding method of epoxidation has been developed in our laboratory using polyaniline supported co(2)salen was prepared by stirring an equal mixture (w/w)of polyaniline co(2) salen in acetonitrile at ambient condition for36 hours. The filtration and washing with acetonitrile and acetic acid followed by drying at 110-120 ˚C afforded black powdery mass. The metal

incorporation into polymer was studied though UV-visible spectrum. It has been reported from our group that cobalt complex efficiency catalyse the epoxidation of electron deficient α,β unsaturated system
In atypical reaction condition the different amides 2 were taken along with catalytic amount of catalyst (nearly 5mg) in the presence of isobutyraldehyde in dry acetonitrile .the reaction mixture was stirred at room temorature for 25-30 hours under the atmospheric of O2 .after the completion of the reaction the epoxy derivatives 3 of the corresponding amides 2 were obtained .although the reaction time was alittle longer , but the yield of the products were high and in case of 3a the yield was98% the structure of each epoxy derivatives was confirmed by its spectral analysis and the spectra compound have been given in the experimental section for conformity.

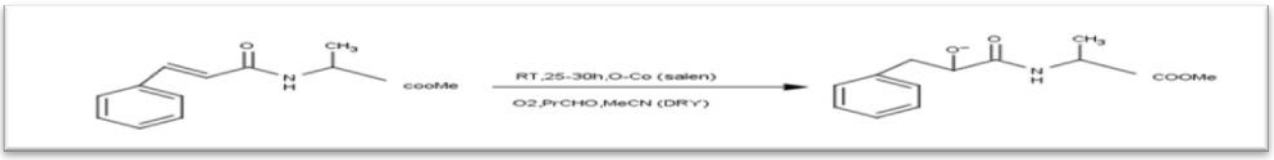


Fig : 5

TABLE: 2: Epoxides 3 of corresponding N-cinnamoyldipepdide 2.

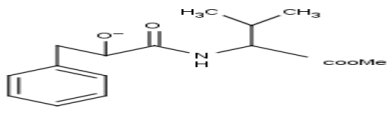
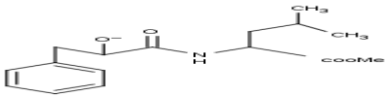
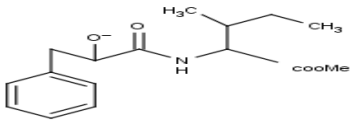
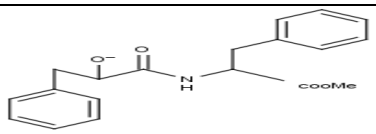
R	STRUCTURE	REACTION TIME (h)	YIELD (%)
(CH3)2CH	 3a	28	98
(CH3)2CH2CH2	 3b	30	95
(CH3)CH2CH(CH3)	 3c	26	97
C6H5CH2	 3d	25	96

Table 2: Explainsalthough the stereochemistry of the epoxidation is not confirmed ,but it was expected to be trans as the stereochemistry of the olefin is retained in the reaction .various other modification of the reaction procedure were unsuccessful .the results are compiled in table 2.the epoxide ring opening by use of cocl2 has been developed in our laboratory ,typically to the epoxide 3 (1mmol)on 10mL CH3CN was aided AMINE (1.1mmol)followed by 10%of dry anhydrous COCl2 .the progress of the reaction was monitoring through TLC. Typically the conversion to the 1,2 amino alcohol took 15-20hrs .the dipeptides were obtained as a mixture of diastereomers in which the anti-isomers was found to be predominant .due to the longer reaction time ,in the reaction ,microwave assisted reaction was planned microwave assisted organic transformation are well known to speed up the.reaction.

Fig: 6

Where
R1=9CH3)CH,(CH3)2CHCH2,(CH3)CH2CH(CH3),C6H5CH2.
R2=Br,OMe

Table 3: Microwave mediated opening of epoxides3 with aromatic amines.

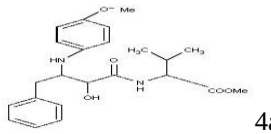
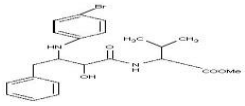
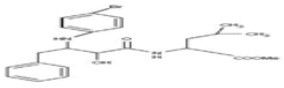
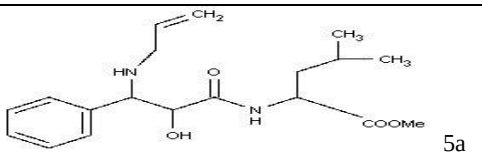
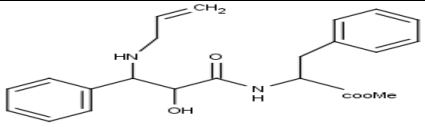
ENTRY	PRODUCT	YIELD (%) [a]	[α]D25(conc)[b]
1.	 4a	60	-20(0.001) -6(0.001)
2.	 4b	62	-1.5(0.001) -20(0.006)
3.	 4c	68	+20(0.002) -11(0.001)

Table: 3 Explains αisolated combine yield of diastereomers confirmed by HPCL and columnchromatograpy isolation .yield on the basis of epoxide. b Optical rotation of major and minor diastereomers in CH2Cl2.
It was planned to carry out the opening of epoxides in the solid phase using neutral alumina or clay without the use of COCl2 under microwave irradiation .in atypical reaction condition neutral alumina was added to a solution of methyl-N-(3-phenylglycidyl)-valinate(100mg,0.3mmL)and p-anisidine (170mg,1.3mmol)in dichoromethane .the slurry was transferredto a Teflon vessel.thevesselwas kept under microwave oven for 0.5hour at 50watt and 50 ℃.after that completion of the reaction ,ethyl acetate was added and stirred for 30 minutes. The alumina was filtered out and the solvent was removed from the filtrate in vacuo. This residue was taken in CCl4 and hexane solution to precipitate the product leaving the amine and the epoxide in the mother liquor .the product was further purified via column chromatography to give two products4a, which were characterized as two diastereomers of the α-hydroxy –βamino analogs of bestatin the [α]D 25 for the major product was found to be -20 ˚ (c=0.001,CH2Cl2)and for minor one was found to be 6 ˚(c=0.006,CH2Cl2)subsequently it was found that the reaction completed within 20 min thus microwave irradiation has drastically reduced the time required for the reaction .similarly when p-bromoaniline was used two diastereomers 4b were again formed in 62% combined yield .the optical rotation [α]D25 -1.5 ˚(c=0.001,CH2Cl2)for major isomer -20 ˚(c=0.006,CH2Cl2) for minor isomer .the epoxy derivatives of leucine 3b,isoleucine 3c and phenylalanine 3d were similar treated with p -anisidine and p bromoaniline .at 50 ℃at the power of 50 watt for 10-20 minutes under similar condition asdescribed above . in each case two diastereomers were formed in good yield .the result are compiled in table 3.

Table .4: Microwave mediated conversion of epoxide 3 to β phenylisoserine derivatives 5.

ENTRY	R1	R3	STRUCTURE	YIELD (%) [a]
1.	(CH3)2CHCH2	CH2CHCH2	 5a	32:28
2.	C6H5CH2	CH2CHCH2	 5b	37:31
3.	C6H5CH2	C6H5CH2	5c 	35:30

[a]isolated yield of diastereomers .

Table 4 : Explains the epoxy derivatives of N-cinnamoyl phenylalanine 3d was similary treated with allylamine and benzyl amine at 80 ˚C at the power of 100watt for 15-20 min . in each case two diastereomers5b and 5c were formed in good yield . similarly epoxide of N-cinnomoylvaline 3a and isoleucine 3c when treated with aliphatic amines resulted in two diastereomers under condition as described above (table 4 entry 1-3). Both products were characterised by spectrum analysis .in case of valine and isoleucine both the epoxy open proton were observed more down field i.e near δ 5ppm. The data is given in experimental section along with details experimental procedure .the results are compiled in table 4. Under the aforesaid condition the opening of tripeptide epoxide could not be achived .reason there of opening of tripeptide epoxide could not be synthesized on the lines of dipeptide synthesis

V. CONCLUSION

In this new strategy research manuscript. the microwave mediated epoxide ring opening reaction by various amines to corresponding β-phenylisoserine derivatives provides a general and efficient protocol to potentially useful inhibitors of HIV protease and amino peptide.

VI. ACKNOWLEDGMENT

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