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DESIGN, SYNTHESIS AND ANTIVIRAL ACTIVITY OF 2-{[(2*E*)-3-(3, 4, 5-MONO/DI/TRI HYDROXYPHENYL) PROP-2-ENOYL] SULFANYL/AMINO} PROPANOIC ACIDS IN HUMAN PLASMA BY ROTOR GENE-Q (5 PLEX) REAL TIME PCR METHOD

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Keywords:

HIV, Integrase, Retrovirus, Chicoric Acid, Halfmer, Cinnamic Acid, Viral Load, Aza series, Mercapto Series, Real Time PCR

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ABSTRACT: Halfmer of chicoric acid derivatives in two series were planned to synthesized and evaluated for their capacity to reduce the viral load in the human plasma by ROTOR GENE-Q (5 Plex) Real Time PCR method to check their biological activity. Different substituted hydroxy cinnamic acids has been conjugated with different amino acid in N-series and in K-Series same substituted hydroxy cinnamic acids has been conjugated with thio derivative of same amino acids In Anti-HIV evaluation K series (Containing Sulfur as a linker) was found to be have highest activity in terms of in-vitro viral load. Compound K-11 (2-{[(2E)-3-(4-hydroxyphenyl) prop-2-enoyl]-sulfanyl}propanoic acid) was found to be most active with viral load 72 copies/ml and 91.69% decrease in viral load. Among two series K (Mercapto series) is more active and among all compounds K-11 is most active compounds against HIV-1 virus in terms of viral load. So, the activities of compounds constitute a strong rationale for further investigation.

INTRODUCTION: In the lifecycle of *HIV-1* virus, integration of viral genome into host cell chromosom is catalyzed by the HIV-1 Integrase. The integration of HIV-1 DNA into the host chromosome is achieved by the integrase protein performing a series of DNA cutting and joining reactions.[1-2] Literature review of chicoric acid reveals potential property of chicoric acid as HIV-IN inhibitors.

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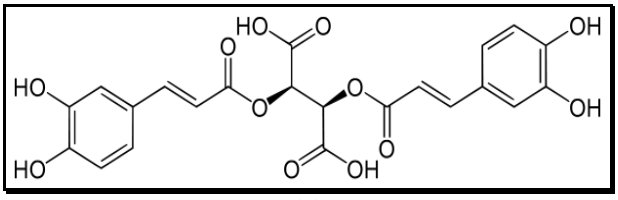
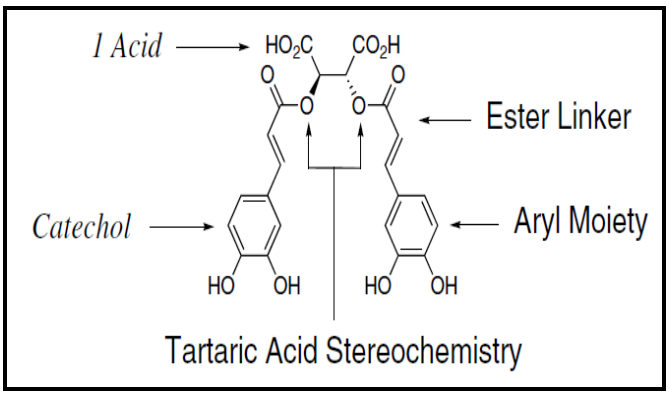
Different scientist have been found that changing the linker between the aryl moiety of chicoric acid[3-5], changing the catechol with different subsituents[4], Different substituted L-chicoric acid **(1)** with changing one substitution in place of aryl moiety with keeping catechol as one aryl moiety[6] and also simplify the structure of L-chicoric acid by developing its halfmer which have potential HIV IN inhibitory activity[7].



(1)

So after reviewing the articles of chicoric acid analogues with different substitution, we have

focused on those compounds in terms of their activity, synthesis steps. We designed the compound with different geometry and different substitution keeping the chicoric acid SAR (**Figure-1)** in mind as a core of the project for the discovery. For this, structural activity relationship of reviewed compounds was carried out by deep examination of their structures, geometry and activity.[5, 6]



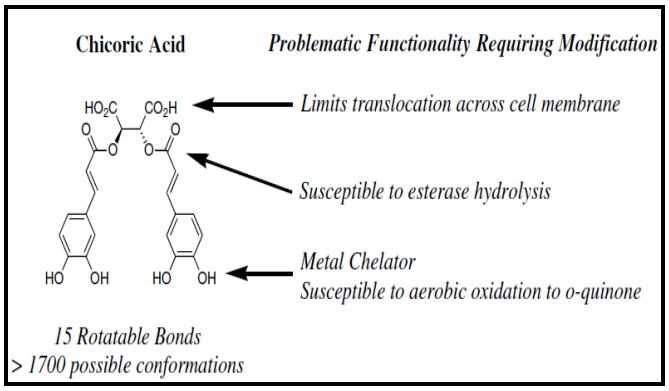


FIGURE-1: SAR OF *L*-CHICORIC ACID AND UNDESIRABLE STRUCTURAL CHARACTERISTIC OF CHICORIC ACID

MATERIALS AND METHODS:

Experiment and parameters to be evaluated:

In this section synthetic part, procedure of synthesis in detail, reaction synthesis steps structure of the final compounds. IUPAC name of final compounds and different evaluation parameters are discussed.
Evaluation will be done for various parameters those are described below:

Solubility, Melting point, %yield, Thin layer chromatography, UV/Visible spectrophotometry, Infra-red spectroscopy, Mass spectroscopy, Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy.
Reaction steps, procedure and evaluation of all final compounds are described individually for each two series. Series name are given according to linker used.

Chemicals

All chemicals for synthesis work were procured from below listed companies:
Sigma Aldrich, U.S.A., Loba Chemie, Mumbai, S.D. Fine chem., Mumbai, Finar chem. Ltd., Ahmedabad, National Chemicals, Vadodara.

Instruments:

All the melting points were determined in open capillaries on chemiline CL-725 melting point apparatus and are uncorrected. Thin layer chromatography was performed on microscopic plates (2×7.5cm) coated with silica-gel-60 F_{254} prepared by Merck KGaA, Germany and spots were visualized under UV light and by exposure to iodine vapour. UV spectra were recorded in UV-1800 Shimadzu spectrophotometer.

IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer using KBr. Mass spectra was obtained using 2010EVMS Shimadzu instrument. The ^{1}H-NMRwas recorded on Bruker Advance - IINMR400MHzinstruments using DMSO-d_6 as solvent and Tetramethylsilane (TMS) as internal standard, chemical shifts were expressed as δ values (parts per million). Splitting patterns are as follows: s (singlet), d (doublet), m (multiplet), b (broad), dd (doublet in doublet).

Synthesis of Substituted Cinnamic Acids from Substituted Benzaldehydes: [8]

0.12 mol of malonic acid was dissolved in 25 ml of pyridine, 1 ml of piperidine was added with 0.055 mole of substituted benzaldehyde. Heated on the water bath at temp. 95-100°C for 2-6 hours. Reaction was monitored by TLC at every 1 hour. After completion of reaction which was confirmed by TLC with single spot, the reaction mixture was diluted with water and conc. HCl was added to neutralize pyridine & got the precipitates of product. Confirm with melting point and TLC.

Synthesis of Thio Acids from Different Amino Acids: [9]

0.05 Mole of amino acid was dissolved in required quantity of 5 N HCl, Cooled to 0°C in ice bath. Precooled solution of 0.08 mole of $NaNO_2$was added drop wise at about 2 ml/min, temperature

cooled below 5°C. Reaction is allowed to stand overnight at room temperature after addition completed. The flask was stirred for 3 hours to remove NO, color changed from yellowish brown to pale yellow. 5 gm of Na_2CO_3 was added carefully to prevent excessive foaming.

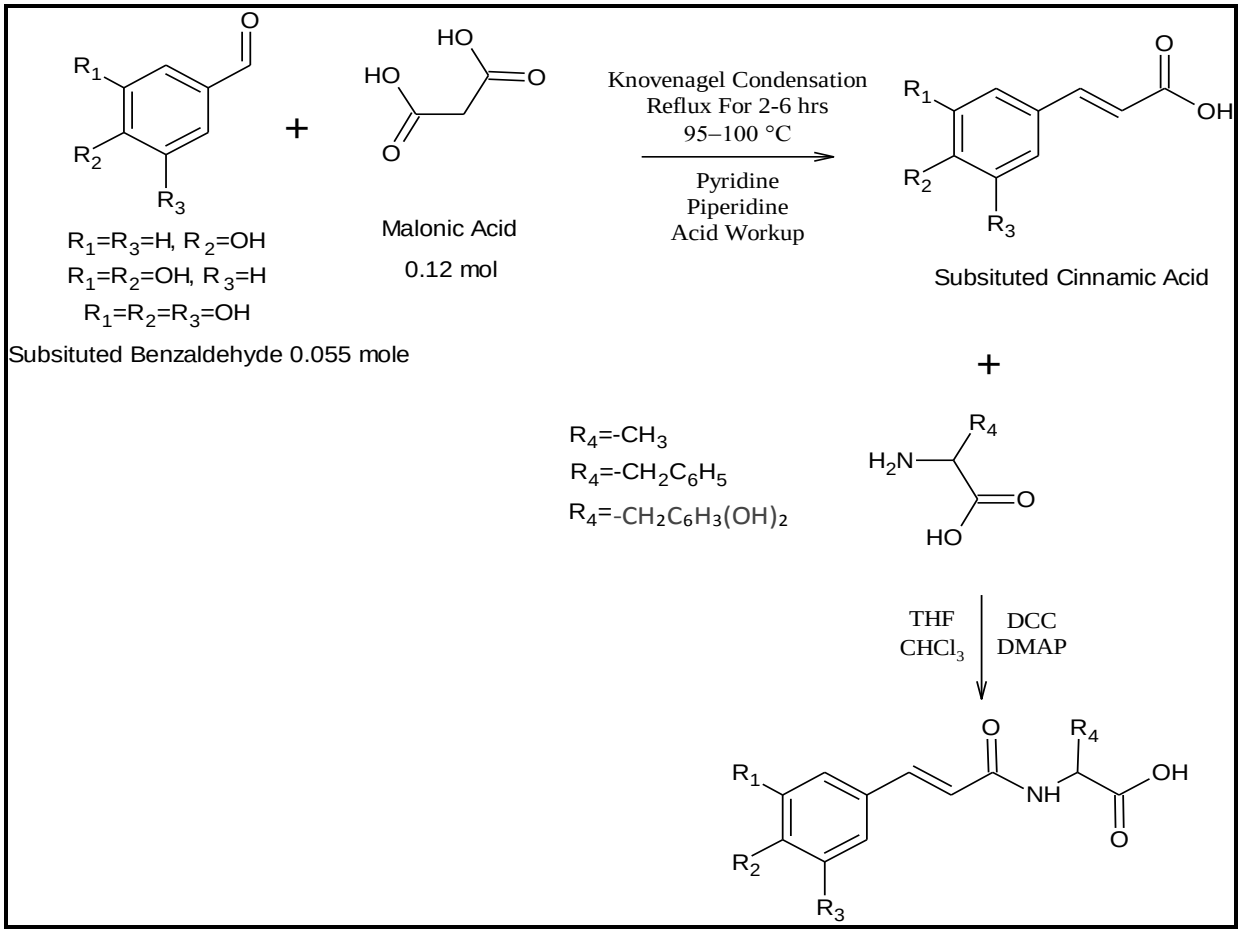
Solution was treated with 4 portions of 20 ml diethyl ether, the combine ether layer were concentrated. Solution washed with 3 ml of Brine solution, which re-extracted with 3 portions of 5 ml ether. The ether is distilled to get oily residue, transferred and the again redistilled. H_2S gas was bubbled through the solution of chloro derivative, got the liquid product which was extracted with hexane, hexane was distilled to get the final thio substituted acids.

Synthesis of Chicoric Acid Halfmer by Coupling: [10]

To the 4.8 mmol solution of cinnamic acid in 12 ml THF, cooled to 0°C were added successively 4.8 mmol α-amino or α-thio acids & solutions of 4.8 mmol Triethylamine in 5 ml $CHCl_3$ & 5.3 mmol dicyclohexylcabodimide in 10 ml $CHCl_3$. After stirring for 1 hr at 0°C, the mixture was warmed to R.T. and stirred overnight. After filtration of insoluble material (dicyclohexylurea) from the mixture, the filtrate was evaporated and dissolved in ethyl acetate. The ethyl acetate solution was washed with water, 10% citric acid aqueous solution, 10% $NaHCO_3$ aqueous solution, and finally, with brine. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by recrystallization.

Scheme for Synthesis of Aza Series (Scheme-1) and Scheme for Synthesis of Mercapto series (Scheme-2) and the physical properties of all synthesized compound had been mentioned in **Table-1** for Aza series and in **Table-2** for mercapto series.

Synthesis Scheme for Aza series (N-11-N-13, N-21-N-23, N-31-N-33):



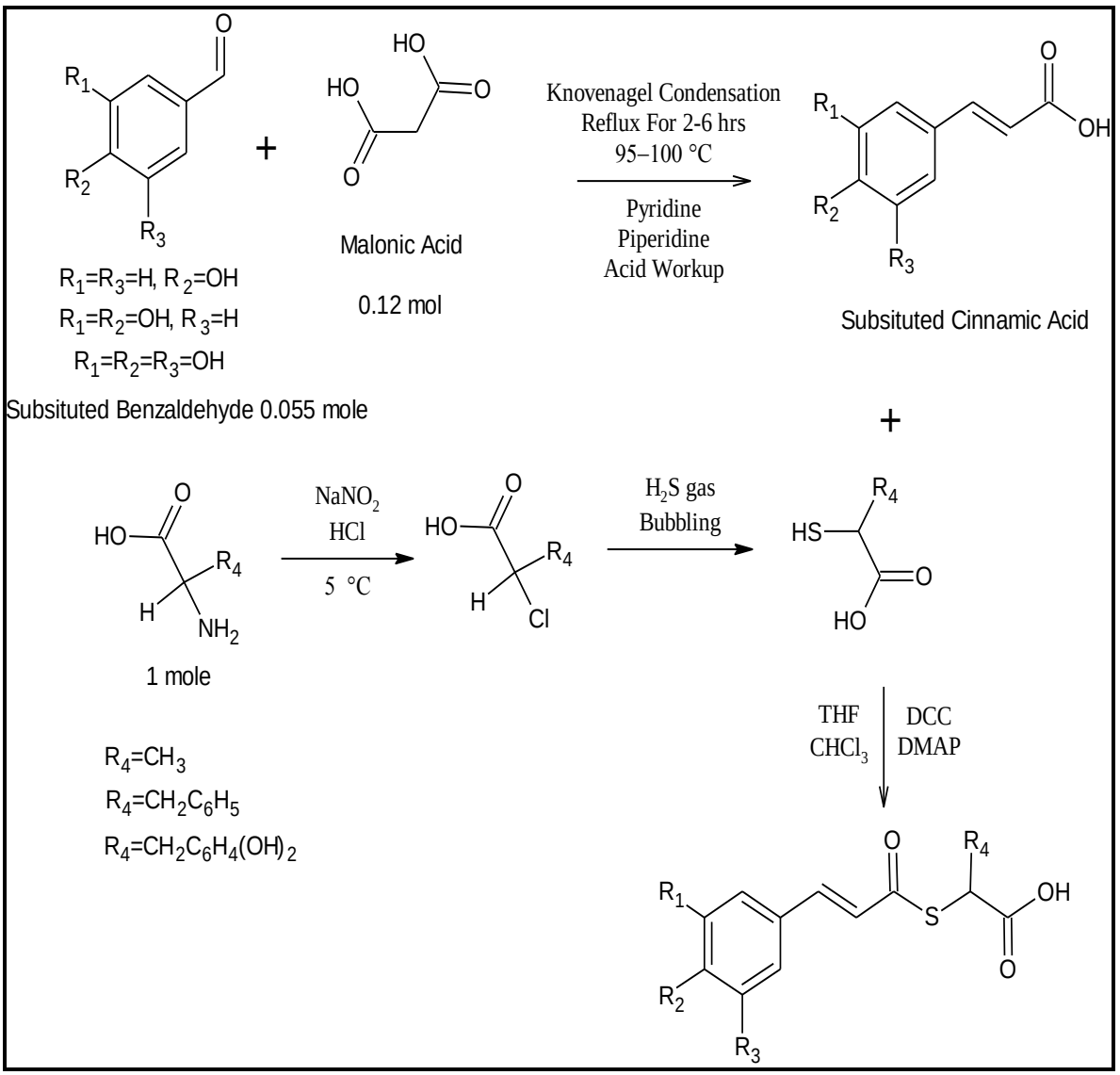
DCC = N, N'-Dicyclohexylcarbodiimide, DMAP = 4-Dimethylaminopyridine, THF = Tetrahydrofuran

SCHEME-1

TABLE 1: PHYSICAL CHARACTERIZATION DATA OF AZA SERIES

Compound	R_1	R_2	R_3	R_4	Molecular Formula	Molecular Mass (g/mol)	Melting Point (°C)	%Yield
N-11	-H	-OH	-H	$-CH_3$	$C_{12}H_{13}NO_4$	235.23	216-218	55
N-12	-H	-OH	-H	$-CH_2C_6H_5$	$C_{18}H_{17}NO_4$,	311.13	180-182	52
N-13	-H	-OH	-H	$-CH_2C_6H_3(OH)_2$	$C_{18}H_{17}NO_6$	343.33	196-198	67
N-21	-OH	-OH	-H	$-CH_3$	$C_{12}H_{13}NO_5$	251.23	160-162	52
N-22	-OH	-OH	-H	$-CH_2C_6H_5$	$C_{18}H_{17}NO_5$	327.33	146-148	42
N-23	-OH	-OH	-H	$-CH_2C_6H_3(OH)_2$	$C_{18}H_{17}NO_7$	359.33	186-188	85
N-31	-OH	-OH	-OH	$-CH_3$	$C_{12}H_{13}NO_6$	267.23	94-96	58
N-32	-OH	-OH	-OH	$-CH_2C_6H_5$	$C_{18}H_{17}NO_6$	343.33	138-140	60
N-33	-OH	-OH	-OH	$-CH_2C_6H_3(OH)_2$	$C_{18}H_{17}NO_8$	375.32	172-174	65

Synthesis Scheme for Mercapto series (K-11-K-13, K-21-K-23, K-31-K-33):



SCHEME-2

TABLE 2: PHYSICAL CHARACTERIZATION DATA OF MERCAPTO SERIES

Compound	R_1	R_2	R_3	R_4	Molecular Formula	Molecular Mass (g/mol)	Melting Point (°C)	%Yield
K-11	-H	-OH	-H	$-CH_3$	$C_{12}H_{12}O_4S$	252.28	158-160	30
K-12	-H	-OH	-H	$-CH_2C_6H_5$	$C_{18}H_{16}O_4S$	328.38	124-126	30
K-13	-H	-OH	-H	$-CH_2C_6H_3(OH)_2$	$C_{18}H_{16}O_6S$	360.38	200-202	67
K-21	-OH	-OH	-H	$-CH_3$	$C_{12}H_{12}O_5S$	268.28	141-143	42
K-22	-OH	-OH	-H	$-CH_2C_6H_5$	$C_{18}H_{16}O_5S$	344.38	134-136	74
K-23	-OH	-OH	-H	$-CH_2C_6H_3(OH)_2$	$C_{18}H_{16}O_7S$	376.38	160-162	62
K-31	-OH	-OH	-OH	$-CH_3$	$C_{12}H_{12}O_6S$	284.28	122-124	65
K-32	-OH	-OH	-OH	$-CH_2C_6H_5$	$C_{18}H_{16}O_6S$	360.38	196-198	65
K-33	-OH	-OH	-OH	$-CH_2C_6H_3(OH)_2$	$C_{18}H_{16}O_8S$	392.37	156-158	70

Spectral Data for Aza Series are given below:

N-11:

2-{[(2*E*) -3 - (4-hydroxyphenyl) prop – 2 - enoyl] amino} propanoic acid (N-11): Mol. Wt: 235.23, Yield: 55%, M.P.: 216-218ºC, IR (KBr) (cm^{-1}): 3325.05-3029.96 (Phenolic –OH stretching), 1731.96 (Carboxylic -C=O bending), 1645.17(Amide –C=O bending) and 1568.02 (-N-H stretching). MASS: m/z 236.4 (M+1).

N-12:

2-{[(2*E*) – 3 - (4-hydroxyphenyl) prop - 2 - enoyl] amino}-3-phenylpropanoic acid (N-12): Mol. Wt: 311.13, Yield: 52%, M.P.: 180-182ºC, IR (KBr) (cm^{-1}): 3305.76-2808.16 (Phenolic –OH stretching), 1716.53 (Carboxylic -C=O bending) and1701.10 (Amide –C=O bending), 1623.95 (-N-H stretching). Mass: m/z 310.1 (M-1). ^{1}H NMR (DMSO): δ 11.589-11.421 (s, 1H, COO**H**), 7.926-7.184 (S, 1H, CON**H**), 7.724-7.085 (d, 9H, Ar-**H**), 6.565-6.325 (d, 2H, **H**C=C**H**), 5.691-5.521 (m, 1H, Ph-**H**), 1.671-1.603 (m, 3H, Aliphatic).

N-13:

3-(3,4-dihydroxyphenyl) -2 - {[(2*E*) - 3- (4-hydroxyphenyl) prop-2-enoyl] amino} propanoic acid (N-13): Mol. Wt: 343.33, Yield: 67%, M.P.: 196-198ºC, IR (KBr) (cm^{-1}): 3323.12-3029.96 (Phenolic –OH stretching), 1714.60 (Carboxylic -C=O bending), 1681.81 (Amide –C=O bending) and 1556.45 (-N-H stretching). MASS: m/z 342.3 (M-1).

N-21:

2-{[(2*E*)-3-(3, 4-dihydroxyphenyl) prop -2-enoyl] amino} propanoic acid (N-21): Mol. Wt: 251.23, Yield: 52%, M.P.: 160-162ºC, IR (KBr) (cm^{-1}): 3255.62-3060.82 (Phenolic –OH stretching), 1693.38 (Carboxylic -C=O bending), 1645.17 (Amide –C=O bending) and 1581.52 (-N-H stretching). MASS: m/z 251.1(M), 251.9(M+1).

N-22:

2-{[(2*E*) -3- (3, 4 - dihydroxyphenyl) prop-2-enoyl]amino}-3-phenyl propanoic acid (N-22): Mol. Wt: 327.33, Yield: 42%, M.P.: 146-148ºC, IR (KBr) (cm^{-1}): 3255.62-3002.96 (Phenolic –OH stretching), 1693.38 (Carboxylic -C=O bending), 1645.17 (Amide –C=O bending), 1581.52 (-N-H stretching). MASS: m/z 328.3 (M+1).

N-23:

3-(3,4-dihydroxyphenyl)-2-{[(2*E*) -3 - (3, 4-dihydroxyphenyl) prop - 2-enoyl] amino} propanoic acid (N-23): Mol. Wt: 359.33, Yield: 85%, M.P.: 186-188ºC, IR (KBr) (cm^{-1}): 3259.47-2991.39 (Phenolic –OH stretching), 1691.48 (Carboxylic -C=O bending), 1631.67 (Amide –C=O bending) and 1598.88 (-N-H stretching). MASS: m/z 360.0 (M+1)**.** ^{1}H NMR (DMSO): δ 11.482-11.452 (s, 1H, COO**H**), 8.330-8.252 (S, 1H, CON**H**), 7.468-7.414 (d, 6H, Ar-**H**), 6.797-6.597 (d, 2H, **H**C=C**H**), 5.611-5.592 (m, 4H, Ph-**H**), 1.770-1.555 (m, 3H, Aliphatic).

N-31:

2-{[(2*E*)-3-(3, 4, 5 -trihydroxyphenyl) prop - 2-enoyl] amino} propanoic acid (N-31): Mol. Wt: 267.23, Yield: 58%, M.P.: 94-96ºC, IR (KBr) (cm^{-1}): 3257.55-3062.75 (Phenolic –OH stretching), 1716.53 (Carboxylic -C=O bending), 1683.74 (Amide –C=O bending) and 1542.95 (-N-H stretching). MASS: m/z 268.5 (M+1). ^{1}H NMR (DMSO): δ 11.345-11.231 (s, 1H, COO**H**), 8.451-8.431 (S, 1H, CON**H**), 7.629-7.085 (d, 2H, Ar-**H**), 6.901-6.632 (d, 2H, **H**C=C**H**), 5.526-5.213 (m, 3H, Ph-**H**), 1.895-1.562 (m, 4H, Aliphatic).

N-32:

3-phenyl-2-{[(2*E*)-3-(3, 4, 5 - trihydroxyphenyl) prop-2enoyl] amino} propanoic acid (N-32): Mol. Wt: 267.23, Yield: 60%, M.P.: 138-140ºC, IR (KBr) (cm^{-1}): 3321.19-3060.82 (Phenolic –OH stretching), 1731.96 (Carboxylic -C=O bending), 1693.38 (Amide –C=O bending) and 1581.52 (-N-H stretching). MASS: m/z 344.4 (M+1).

N-33:

3-(3, 4-dihydroxyphenyl) - 2- {[(2*E*)- 3- (3, 4, 5-trihydroxyphenyl) prop - 2-enoyl] amino} propanoic acid (N-33): Mol. Wt: 375.32, Yield: 65%, M.P.: 172-174ºC, IR (KBr) (cm^{-1}): 3261.40-3002.96 (Phenolic –OH stretching), 1731.96 (Carboxylic -C=O bending), 1693.98 (Amide –C=O bending) and 1620.09 (-N-H stretching). MASS: m/z 374.7(M-1). ^{1}H NMR (DMSO): δ 11.423-11.531 (s, 1H, COO**H**), 8.428-8.448 (S, 1H, CON**H**), 7.460-7.498 (d, 5H, Ar-**H**), 6.849-6.627(d, 2H, **H**C=C**H**), 5.120 (m, 5H, Ph-**H**), 1.671-1.603 (m, 3H, Aliphatic)

Spectral Data for Mercapta Series are given below:

K-11:

2- {[(2*E*) - 3- (4-hydroxyphenyl) prop – 2 - enoyl] sulfanyl} propanoic acid (K-11): Mol. Wt: 252.28, Yield: 30%, M.P.: 158-160ºC, IR (KBr) (cm^{-1}): 3255.05-3029.96 (Phenolic –OH stretching), 1731.96 (Carboxylic -C=O bending) and 710.12 (C-S stretching). MASS: m/z 253.4(M+1), 251.1(M-1). ^{1}H NMR (DMSO): δ 11.562-11.534 (s, 1H, COO**H**), 8.428-8.393, 7.449-7.084 (m, 4H, Ar-**H**), 6.898-6.626 (m, 2H, **H**C=C**H**), 5.108 (m, 1H, Ph-**H**), 1.685-1.532 (m, 4H, Aliphatic).

K-12:

2-{[(2*E*)-3-(4-hydroxyphenyl) prop – 2 - enoyl] sulfanyl} -3 - phenylpropanoic acid (K-12): Mol. Wt: 328.38, Yield: 30%, M.P.: 124-126ºC, IR (KBr) (cm^{-1}): 3305.76-3012.60 (Phenolic –OH stretching), 1716.63 (Carboxylic -C=O bending) and 750.26 (C-S stretching). MASS: m/z 328.3(M), 329.4(M+1).

K-13:

3-(3, 4- dihydroxyphenyl) – 2 - {[(2*E*) – 3 - (4-hydroxyphenyl) prop - 2-enoyl] sulfanyl} propanoic acid (K-13): Mol. Wt: 360.38, Yield: 67%, M.P.: 200-202ºC, IR (KBr) (cm^{-1}): 3290.33-3029.96 (Phenolic –OH stretching), 1622.02 (Carboxylic -C=O bending) and 640.32 (C-S stretching). MASS: m/z 361.4 (M+1).

K-21:

2-{[(2*E*)-3-(3, 4-dihydroxyphenyl) prop-2-enoyl] sulfanyl} propanoic acid (K-21): Mol. Wt: 268.28, Yield: 42%, M.P.: 141-143ºC, IR (KBr) (cm^{-1}): 3257.55-3062.75 (Phenolic –OH stretching), 1629.74 (Carboxylic -C=O bending), 756.04 (C-S stretching). MASS: m/z 267.4 (M-1). ^{1}H NMR (DMSO): δ 11.383-11.328 (s, 1H, COO**H**), 7.469-7.106 (m, 3H, Ar-**H**), 6.780-6.761 (m, 2H, **H**C=C**H**), 5.617 (s, 2H, Ph-**H**), 1.711-1.004 (m, 4H, C**H**$_3$).

K-22:

2-{[(2*E*)-3-(3, 4-dihydroxyphenyl) prop-2-enoyl] sulfanyl} -3 -phenyl propanoic acid (K-22): Mol. Wt: 344.38, Yield: 74%, M.P.: 134-136ºC, IR (KBr) (cm^{-1}): 3259.47-3056.96 (Phenolic –OH stretching), 1730.03 (Carboxylic -C=O bending) and 705.90 (C-S stretching). MASS: m/z 345.1 (M+1).

K-23:

3-(3,4-dihydroxyphenyl) - 2 - {[(2*E*) - 3- (3, 4-dihydroxyphenyl) prop – 2 - enoyl] sulfanyl} propanoic acid (K-23): Mol. Wt: 376.38, Yield: 62%, M.P.: 160-162ºC, IR (KBr) (cm^{-1}): 3257.55-3060.82 (Phenolic –OH stretching), 1629.74 (Carboxylic -C=O bending) and 624.89 (C-S stretching). MASS: m/z 375.2 (M-1).

K-31:

2-{[(2*E*)-3-(3, 4, 5 - trihydroxyphenyl) prop-2-enoyl] sulfanyl} propanoic acid (K-31): Mol. Wt: 284.28, Yield: 65%, M.P.: 122-124ºC, IR (KBr) (cm^{-1}): 3325.05-3004.89 (Phenolic –OH stretching), 1693.38 (Carboxylic -C=O bending) and 624.89 (C-S stretching). MASS: m/z 285.1(M+1). ^{1}H NMR (DMSO): δ 12.391-12.377 (s, 1H, COO**H**), 7.551-6.531 (d, 2H, Ar-**H**), 7.114-6.531 (d, 2H, **H**C=C**H**), 5.312-5.124 (m, 3H, Ph-**H**), 1.571-1.532 (m, 4H, Aliphatic).

K-32:

3-phenyl-2-{[(2*E*)-3-(3, 4, 5-trihydroxyphenyl) prop-2-enoyl] sulfanyl} propanoic acid (K-32): Mol. Wt: 360.38, Yield: 65%, M.P.: 196-198ºC, IR

(KBr) (cm^{-1}): 3325.05-3004.89 (Phenolic –OH stretching), 1716.53 (Carboxylic -C=O bending) and 692.7 (C-S stretching). MASS: m/z 361.1 (M+1)

K-33:

3-(3,4-dihydroxyphenyl) -2 - {[(2*E*) - 3 - (3, 4, 5 - trihydroxyphenyl) prop – 2 - enoyl] sulfanyl} propanoic acid (K-33): Mol. Wt: 392.37, Yield: 70%, M.P.: 156-158ºC, IR (KBr) (cm^{-1}): 3257.55-3060.82 (Phenolic –OH stretching), 1629.74 (Carboxylic -C=O bending) and 742.54 (C-S stretching) MASS: m/z 393.4 (M+1)

RESULTS AND DISCUSSION:

Method: ROTORGENE-Q (5Plex) Real Time PCR

Procedure for Anti-HIV activity by checking Viral Load:

The viral load of reference sample was obtained. 1mgof each of the test compounds was dissolved in 1mlof 100% DMSO to prepare test compound suspensions. Each compound suspension was added in reference plasma and incubated overnight.

Results of Anti-HIV activity:

Mentioned results **(Table-3)** include Anti-HIV activity against the HIV virus in terms of viral load in blood plasma in human HIV-infected blood.

TABLE 3: RESULT OF ANTI-HIV ACTIVITY

Sr. No.	Compound Code	Viral Load (IU/mL)	% Decrease in Viral Load	Sr. No.	Compound Code	Viral Load (IU/mL)	% Decrease in Viral Load
1	Reference	867	0	11	K-11	72	91.69
2	N-11	721	16.83	12	K-12	343	60.43
3	N-12	632	17.10	13	K-13	568	34.48
4	N-13	561	35.29	14	K-21	517	40.36
5	N-21	823	5.07	15	K-22	453	47.75
6	N-22	758	12.57	16	K-23	314	63.78
7	N-23	692	20.18	17	K-31	865	0.23
8	N-31	843	2.76	18	K-32	654	23.56
9	N-32	811	6.45	19	K-33	532	38.63
10	N-33	795	8.30				

RESULT INTERPRETATION:

HIV RNA >72 IU/ML:

The result is within the determined test range. The detection probability of HIV RNA is >95%.The result is statistically ensured.

HIV RNA <72 IU/ML:

The result is outside the determined test range. The reproducibility of the positive. Result is not assured.

HIV RNA NEGATIVE:

No HIV RNA was detected.

Artus (QIAGEN) HIV-1 RG RT-PCR, Analytical Sensitivity:

95% Probability that 66.9IU/ml will be detected.

Analytical Specificity:

No Cross reactivities appeared with mixed infections.

Robustness: > 99% for the artus HI Virus-1 RG RT–PCR.

Linear Range: 1 × 108 IU/ml Precision: 1.66%.

Diagnostic Sensitivity: 98.1 % Diagnostic Specificity: 84.3%.

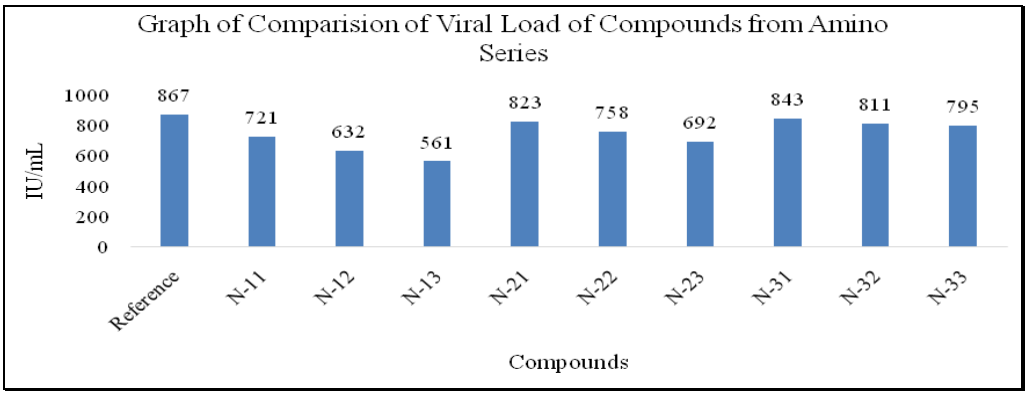


FIGURE 2: GRAPH OF COMPARISON OF VIRAL LOAD OF COMPOUNDS FROM AZA SERIES

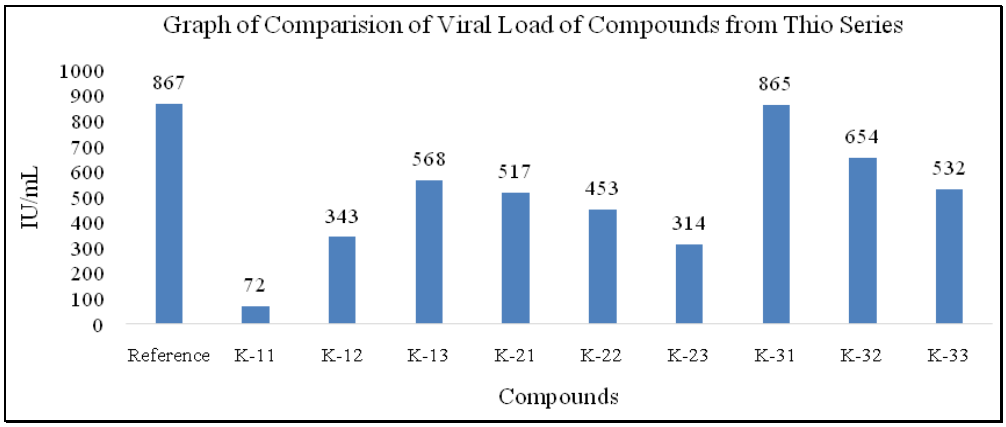


FIGURE 3: GRAPH OF COMPARISON OF VIRAL LOAD OF COMPOUNDS FROM MERCAPTO SERIES

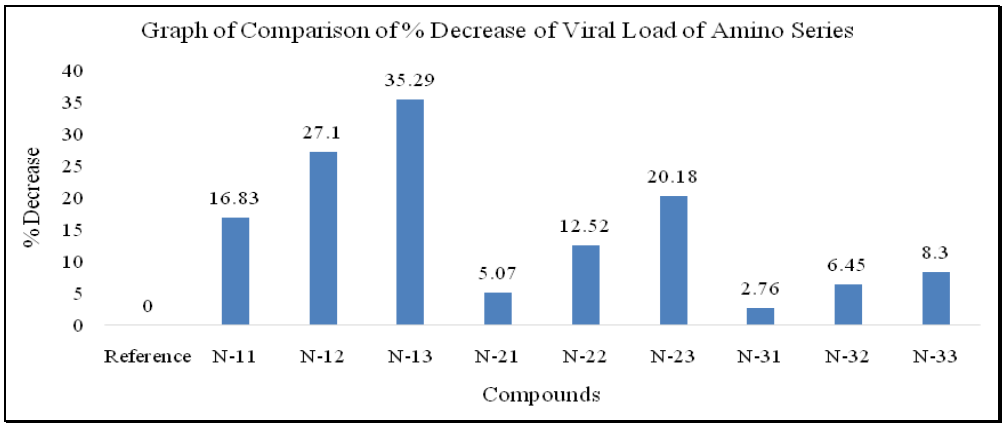


FIGURE 4: GRAPH OF COMPARISON OF % DECREASE OF VIRAL LOAD OF AZA SERIES

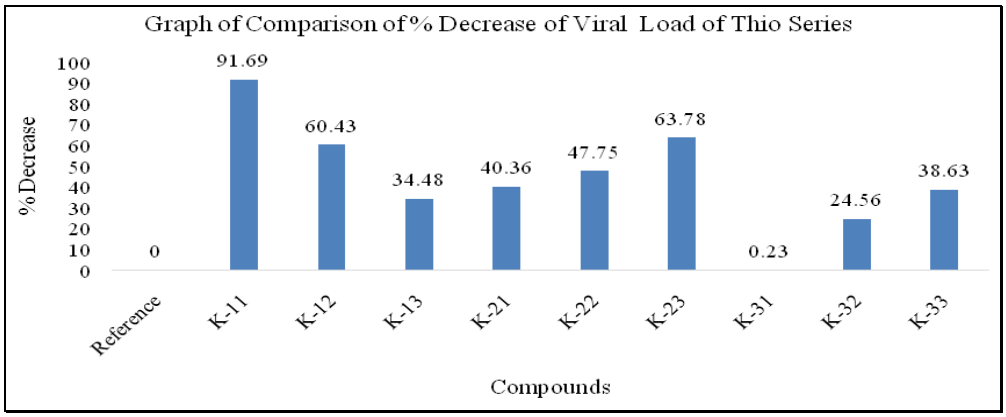


FIGURE-5: GRAPH OF COMPARISON OF % DECREASE OF VIRAL LOAD OF MERCAPTO SERIES

Discussion of Anti-HIV activity:

Synthesis of novel chicoric acid halfmer derivatives was carried out in two different series. All were evaluated for anti-HIV activity against the HIV virus in plasma by *in-vitro* testing method named ROTOR GENE-Q (5 Plex) Real Time PCR assay.

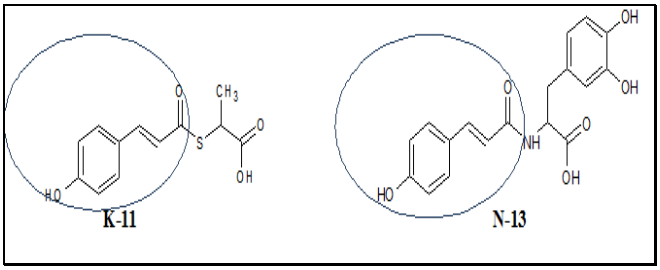
Viral load of all compounds were determined by the procedure described in previous chapter. From that viral load in the HIV infected patient's blood plasma was calculated and their results tabulated in **Table-3**.

Intra-Series Comparison

Series 1 (Aza series): The % decrease of viral load of this series of compounds had been described in **Table-3**. Comparison of viral load described in **Figure-2** and % decrease described in **Figure-4**. Among all compounds of this series N-13 shows the highest % decrease in viral load which is

35.29% so it possesses the highest Anti-HIV activity among the series. So the N-13 was found to best in this series in terms of viral load which was tested in human plasma.

Series 2 (Mercapto series): The % decrease of viral load of this series of compounds had been described in **Table-3**. Comparison of viral load described in **Figure-3** and % decrease described in **Figure-5**. Among all compounds of this series K-11 shows the highest % decrease in viral load which is 91.69% so it possesses the highest Anti-HIV activity among the series. So the K-11 was found to best in this series in terms of viral load which was tested in human plasma. So Compounds which are most potent in their series have some similarity which can be explained by common structure



Compounds with 4-Hydroxy cinnamoyl group possess higher anti-HIV activity compared to 3, 4-dihydroxy cinnamoyl and 3, 4, 5-trihydroxy cinnmoyl group.

Inter-Series Comparison

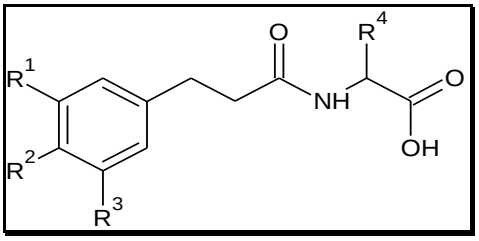
Inter-series comparison (**Table-4)** of the activity of the compounds provides the idea for the best active series among both series.

TABLE 4: INTER-SERIES COMPARISON OF SYNTHESIZED COMPOUNDS

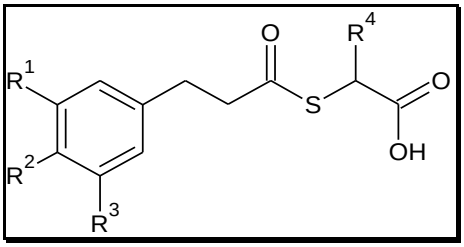
Aza series Compound	% Decrease of viral load	Mercapto series Compound	% Decrease of viral load
N-11	16.83	K-11	91.69
N-12	17.10	K-12	60.43
N-13	35.29	K-13	34.48
N-21	5.07	K-21	40.36
N-22	12.57	K-22	47.75
N-23	20.18	K-23	63.78
N-31	2.76	K-31	0.23
N-32	6.45	K-32	23.56
N-33	8.30	K-33	38.63

As per result and comparison graph of viral load and % decrease of viral load compared to reference plasma of all synthesized compounds. Compounds of mercapto series found to be best among both series in terms of viral load so series of thio linkage was found active. Inter-series comparison of % decrease of viral load depicated in the **Table 4**.

From above inter-Series comparison, Mercapto series was found more active towards HIV virus, Aza series was found to be second most active series. The Results can be explained by comparing structure of compounds. It is due to the difference of linker which is used between the substituted hydroxyl cinnamic acid and *L*-amino acids. In aza series amide link was formed while in mercapto series thio link was formed.



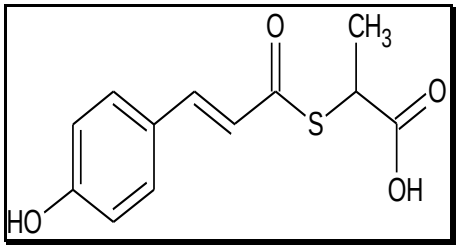
AZA SERIES



MERCAPTO SERIES

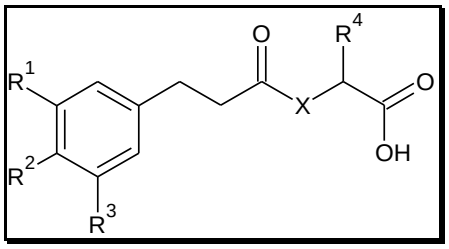
Compounds of mercapto series containing Sulfur as a linker compared to Nitrogen as a linker for aza series which make the compound more non-polar as Sulfur is more lipophilic than Nitrogen in terms of logP. So it can easily cross the biological membrane and it is also more robust to cleavage than amide linkage so it has the higher potency than aza series as compared in **Table 3** and **Table 4**.

From the above two intra-series and inter series comparison of all synthesized compounds, compound K-11 was found most active anti-HIV agent against the HIV-1 virus because low viral load in its in-vitro study that is 72 and higher % decrease of viral load that is 91.69%. So, overall compound K-11 was found best in terms of activity.



K-11

Structure activity relationship of compound can be carried out as below after examination of results:



Putting S in place of N as a linker atom (X) between the moiety lead to better anti-HIV activity as mercapto series is more active and more robust linkage. Substitution at R_1 with –OH group and keeping R^2 and R^3 as H will lead to increase in activity rather than keeping 2 or 3 –OH group on aryl moiety. Keeping one carboxylic acid compared to diacid in parent structure of *L*-Chicoric Acid will leads to better cell permeation. Substitution of R^4 with $–CH_3$ will lead to most potent compound but changing the substitution at R^4 as aryl ring or 3, 4-dihydroxy aryl side chain will lead to increase in potency.

CONCLUSION: After performing all the relevant experiments we have synthesized and tested the inhibitory activity of new type of HIV IN inhibitors, which has different substituted hydroxyl acid group instead of catechol and also the single acid and different linker than the structure of *L*-Chicoric acid. It has been concluded that synthesized compounds of both series have anti-HIV activity. Among them mercapto series was found more active in terms of % decrease of viral load in human plasma as Sulfur have 2 lone pair of electron compared to Nitrogen which having only 1 lone pair of electron. Compound K-11 [2-{[(2*E*)-3-(4-hydroxyphenyl) - prop - 2- enoyl] - sulfanyl} propanoic acid] was found most active which constitute a strong rationale for further investigation. Electro negativity of sulfur ($_{16}S^{32}$: $1s^2 2s^2 2p^6 3s^2 3p^4$) is 2.58 having two lone pair of electrons whereas electro negativity of nitrogen ($_7N^{14}$: $1s^2 2s^2 2p^3$) is 3.04 having one lone pair of electrons, so the electron density at sulfur is higher than nitrogen and due to lower electro negativity mercapto group it becomes more concentrated for 3´-Processing unit of RNA and inhibit the replication

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