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Kalia AN: A Text Book of Industrial Pharmacognosy. CBS Publishers & Distributors, First Edition 2005.

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Invited Article

Comprehensive +Ve approach three prong attack therapy for conquering Ebola virus infection.

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Ebola virus., a member of *Filoviridae*, is a human pathogen causing widespread disease for which there is no vaccine or chemotherapy. Ebola virus disease (EVD) or Ebola hemorrhagic fever (EHF) is the human disease caused by the Ebola virus. Symptoms typically start two days to three weeks after contracting the virus, with a fever, sore throat, muscle pains, and headaches. Typically nausea, vomiting, and diarrhea follow, along with decreased functioning of the liver and kidneys. At this point, some people begin to have bleeding problems. (EVD Factsheet WHO) There is an urgent need to develop inhibitors of Ebola virus. The anti Ebola drugs are selectively, picked up from Internet search with metamorphosed antiviral action and elegantly presented in the medical literature and repurposed so that they are an immediately actionable/ utilizable class of approved drugs for treatment of Filovirus infection.

First Prong of attack:

The sheet anchor among the anti Ebola virus treatment drug is Chloroquine [CQ] which I had been nurturing on from 1978 since self medication trial for Viral hepatitis infection, I myself suffered and proved as antiviral against hepatitis C virus by Huh 5-2 Cellline in collaboration with Prof. E. De Clercq of Katholike University, Belgium [2006 and J. Pharm Sci.][1] and reconfirmed by 3-D Crystallography docking study with Prof. Sureshkumar of Pondicherry University. India[2009] [2]. I had already suggested CQ's antiviral action for Swine Flu (A-H1N1)[3] infection in a letter dated May 11 2009 to Director General of W.H.O and for Dengue Viral Infection is as paper presented in Int.Conf. Anti Viral Research 2014[4].Prof. Savarino had published a detail write up regarding various antiviral action of Chloroquine[5]

Now Peter B. Madrid et al [2013][6] had indentified and published a detailed Scientific report on CQ; having Anti Ebola virus action by disturbing entry, after binding to cell receptors and replication by blocking trafficking of virus particles into cell endosome, in vitro and in vivo studies. Additional valuable

information is pharmaco kinetic studies of CQ in mice with 90mg/kg single dose and twice daily for 8 days in animal model of efficacy for Ebola Virus [EBOV] infection.

Second set of drugs identified are selective estrogen receptor modulators [SERMs] Clomiphene and Toremifen which are acting as potent inhibitors of EBOV infection by blocking virus entry after GP1,2 mediated binding the host cell surface is internalized into endosomes through micropinocytosis, both in vitro and in vivo studies. Dose of the drugs used is 60mg/kg [Lisa M. Johansen et al Sci. Trans. Med 2013][7]. All the above drugs are FDA approved and repurposed as counter measures for mass use and can be straight away utilized in treating Ebola virus infected patients.

Second Prong of attack: Of late it was observed that Nitazoxanide [NTZ] a thiazolides acting against bacteria and protozoa and found to act against a range of viruses in cell culture models E.B. keefee and J.F. Rossignol[8] had graphically described the details of antiviral mechanism of action of Nitazoxanide is that the protein kinase activated by double-stranded RNA (PKR), an interferon induced mediator of the cellular antiviral response.

The activation of PKR results in phosphorylation of its substrate, eukaryotic initiation factor 2 alpha (eIF2α). Nitazoxanide, thus, represents a new class of small molecules that modulate host antiviral pathways as interferon immune enhances. By targeting a host function, the barrier to development of antiviral resistance is significantly higher than for drugs directly targeting a viral function.

This new antiviral mechanism of action is enhancing the innate immunity of the host is increased and development of antiviral resistance is very minimal. NTZ is a known drug and is on use as anti-bacterial and anti protozoal agent; had now shown to have metamorphosed as antiviral agent and that too an innovative innate immunity booster a new angle of action contributing additive antiviral action against any invading virus infections and it will hold good for EBOV also; because of common path way of

action for any invading virus infection. The usual dose is 500mg twice a day orally and is automatically repurposed, so can be utilized to treat Ebola infection straight away.

Third Prong of attack:

The regular pathophysiological changes of any infection [Bacterial or Viral] is proinflamator agents cytokines Interleukin 1B [IL1B], Interleukin 6 [IL6] and Tumor Necrosis Factor -α [TNF-α] [9] at around physiological levels are formed and govern the inflammatory changes in the tissue and further progression is restricted or arrested by the body immunity and / or specific treatment. If the infection or inflammation is virulent or massive the cytokine formation is enhanced much more the need and reach a very high escalated level called Cytokine Storm which causes damages in the micro circulatory system of all the organ precipitating multi organ failure syndrome and death. One other mode of presentation of cytokine excess or storm is Hemophagocytic lymphohistiocytosis and multi organ failure. These are the propensity of un controlled overwhelming inflammatory changes of any cause. To put a brake at the initiation of cytokine storm we must start on the day one diagnosis of infection; the antiviral agents and anti-cytokines, so that the infecting viral load will be controlled resulting in restricted formation of cytokines commerturate with lower inflammatory changes.

We are very happy to state that our sheet anchor newly metamorphosed antiviral Chloroquine had shown another functional metamorphosis as an effective anti cytokine acting against the IL1B, IL6 and TNF-α with additional advantage; it can be given parenterally. Dose oral 5 to 15mg/kg as single dose; intramuscular injection at 2mg/kg/day. same anti-cytokine actions; of CQ is reported for the popular antibiotic Doxycycline[11] and will be having an additive action dose 100mg b.d orally. As an add on drug, Montelukast anti allergy drug blocking cysteniyl receptor; dose 10mg once a day oral. Zileuton anti asthmatic agent blocks synthesis of cystenieyl leukotriene and leukotriene B4; dose 600mg b.d. with additional anti-cytokine action.

We are in a desperate situation and my +ve approach mode of treatment for Ebola virus [EBOV] infection will be a very valuable approach of three prong attack which comprises multi level and multi modal attack to give us positive results. All the drugs are already in use and repurposed for the new metamorphosed action and can be directly utilized to treat EBOV infection. In this situation poly pharmacy is mandatory and is in a way implementing old saying of BENCH TO BEDSIDE namely the useful scientific work of laboratory bench is made available for the benefit of suffering patients in the hospital bed. The new antiviral agents and the immunity booster shall be utilized as prophylaxis for all the susceptible persons in the premises of the sufferers and Medical and Paramedical People treating the sufferers with full benefit of prevention of the dreaded infection.

We appeal that, we will resolve to utilize all the drugs delineated in the three prong attack be started on "DAY ONE OF THE DIAGNOSIS OF EBOLA VIRUS INFECTION" and expect laudable result since it is a comprehensive multi drug regimen different level of actions which are additive and supplementary to each other.Treating and curing disease of endemic and epidemic in nature is the best way of arresting the spread and stamp out endemic or epidemic manifestation of the disease.

So we will all join together and coordinate for the successful victory against the dreaded EBOLA virus infection and discard the very idea of cordon sanitaria [New york times news service – Donald G Meneil J – well shall fulfill our social obligation to the suffering society members and keep ourselves "Out of bound" from criminal negligence. Expecting best attention and taking up the task of treating EBOV infection in "WAR FOOTING" and Stamp Out the epidemic.

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Design, Synthesis and Assay of Novel Mercaptobenzimidazole Derivatives Against the West Nile Protease Target

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ABSTRACT

Background: The mosquito-borne viral pathogens of global significance include the members of flavivirus genus of Flaviviridae family. Two important human pathogens are dengue and West Nile viruses which cause considerable morbidity and mortality throughout tropical and subtropical regions of the world. No vaccines or antiviral therapeutics are available for these two pathogens. The overall goal of our study is to develop potent inhibitors of West Nile virus serine protease, which is an excellent viral target as it is required for viral replication. In this study, we examined whether derivatives of Mercaptobenzimidazole could be versatile lead compounds for structure-activity relationship study.

Methods: Novel Mercaptobenzimidazole derivatives synthesized and screened for their inhibitory activities of WNV protease in vitro. Molecular modeling was performed by computational methods to understand mode of action of the compounds.

Results: The N-Sulphanoamidomethyl-Mercaptobenzimidazole (MBZ-SN) exhibited significant inhibitory activities against the WNV protease (IC_{50} values of 2.5 µM). Modeling suggests that MBZ-SN could bind at the active site of WNV protease although co-crystallization of the viral protease with the active compound is required to confirm the modeling data. Free $–SO_2NH_2$ group is essential for activity and any substitution decreases the inhibitory activity (for example, MBZ-SDM and MBZ-SAC).

Conclusions: To our knowledge, this is the first report regarding the inhibitory activities of Mercaptobenzimidazole derivatives against the WNV serine protease. Further work on SAR study for lead optimization is in progress.

Keywords: Mercabtobenzimidazole, Sulphanilamide, West Nile virus NS2B-NS3 protease

INTRODUCTION

West Nile virus (WNV), a mosquito-borne member of *Flaviviridae*, is a human pathogen causing widespread disease for which there is no vaccine or chemotherapy. The two-component viral serine protease consists of a heterodimeric complex between the hydrophilic domain of the cofactor, NS2B (NS2BH) and the protease domain (NS3-pro). The protease is essential for polyprotein processing followed by assembly of viral replicase and genome replication. Therefore, the protease is an excellent target for development of antiviral therapeutics. There is an urgent need to develop inhibitors of mosquito-borne flaviviruses, including dengue hemorrhagic fever type viruses, yellow fever, Japanese encephalitis and West Nile (WNV) viruses. (Beasley 2005; Hayes CG :2001; Van der Meulen *et al.,* 2005) and Mukhopadhyay S et al 2005)

Flaviviruses such as West Nile virus have a single-strand, positive-polarity, RNA genome. The 11-kb RNA genome encodes a single polyprotein precursor. The precursor comprises three structural proteins (capsid [C], membrane [M], and envelope [E]) and seven nonstructural (NS) proteins arranged in the order C-prM-E-NS1-NS2ANS2B-NS3-NS4A-NS4B-NS5 (Mukhopadhyay *et al* 2005).Post-translational proteolytic processing of the polyprotein precursor is required to produce the functional viral proteins that assemble a new viral progeny. The flaviviral NS2-NS3 proteinase cleaves the polyprotein precursor at multiple sites, including the NS2A/NS2B, NS2B/NS3, NS3/NS4A and NS4B/NS5 junctions. (Chappell *et al.,* 2005 & 2006; Lin *et al.,* 2007; Shiryaev et al., 2006). The N-terminal and the C-terminal portions of NS3 represent protease (NS3pro) and RNA helicase domains, respectively. The upstream NS2B protein functions as a cofactor of NS3pro (Bera *et al.,* 2007; Chambers *et al.,* 2005; Nall *et al.,* 2004; Niyomrattanakit *et al.,* 2004). The NS2B sequence is essential for the activation of NS3pro (Aleshin *et al.,* 2007 ; Erbel *et al.,*2007).without the NS2B cofactor, NS3pro is inert. Inactivation of NS2B-NS3pro by mutations blocks the viral infection (Yusof *et al.,* 2000). These parameters suggest

that NS3pro is a promising drug target for flaviviral therapy (Ganesh *et al.,* 2005; Knox *et al.,* 2006; Lohr et al.,2007; Yin *et al.,* 2006).

Mercaptobenzimidazole, a versatile lead molecule for potential bioactive agents and its derivatives were reported to possess wide spectrum of activity. Mercaptobenzimidazole derivatives were reported to possess inhibitory activity against a variety of pathogenic viruses. In earlier studies, some novel benzimidazole derivatives were synthesized and evaluated for antiviral and cytotoxicity (Periyasamy selvam *et al.,* 2010). In this study we describe the West Nile Virus NS3 protease inhibitory activity of some novel of Mercaptobenzimidazole Derivatives (Scheme 1).

Experimental

Melting points were determined using Thomas melting point apparatus and are uncorrected. The purity was checked by TLC using silica gel G as stationary phase. The structure of the synthesized compounds was elucidated using a Perkin Elmer FT-IR in KBr disc and PMR was taken on a Bruker AMX-(400 MHz) FT-NMR. Mass spectra were obtained on a Varian Atlas CH-7 Mass spectrometer at 70 eV.

Synthesis of N-Sulphanoamidomethyl-Mercaptobenzimidazole

Equimolar quantities (0.01 mole) of mercaptobenzimidazole, formaldehyde (37%) and sulphonamide (sulphanilamide, sulphacetamide and sulphadimidine) were dissolved in warm ethanol. The reaction mixture was stirred in magnetic stir at room temperature for 3 hours and kept in refrigerator overnight. The resultant solid was washed with dilute ethanol and recrystallized from ethanol-chloroform mixture. The structure of synthesized compounds were elucidated by spectral analysis

4-[(1H-Benzoimidazol-2-ylsulfanylmethyl)-amino]-benzenesulfonamide(MBZ-SN): yield: 62 %, mp: 276^0, IR (KBr)$\square_{max}$ in Cm^{-1} 3350 (NH), 1620 (C=C), 1580 (C=N), 1160 (SO_2); ^{1}H NMR (DMSO-d_6) in $\square$ ppm 2.5 (s, 1H, NH-Benzimidazole) 4.6 (s,2H,-NCH_2 N-) 6.7-7.8 (m, 9H, Ar-H) 10 (b, 1H, -SO_2 NH-) EIMS (M/z) 334.

N-Acetyl-4-[(1H-benzoimidazol-2-ylsulfanylmethyl)-amino]-benzenesulfonamide(MBZ-SAC): yield: 68 %, mp: 210^0, IR (KBr) cm^{-1}: 3320 (NH), 1720 (C=0), 1695 (C=N), 1540 (C=C), PMR (DMSO-d_6) □ ppm: 2.02 (s, 3H, -CH_3), 2.5 (s, 1H, NH-Benzimidazole) 4.6 (s,2H,-NCH_2 N-) 6.7-7.9 (m, 9H, Ar-H) 10 (b, 1H, - SO_2 NH-); EI-MS (m/e):376

4-[(1H-Benzoimidazol-2-ylsulfanylmethyl)-amino]-N-(4,6-dimethyl-pyrimidin-2-yl)benzene sulfonamide (MBZ-SDM): yield: 60 %, mp: 242^0, IR (KBr) cm^{-1}: 3320 (NH), 1690 (C=N), 1587 (C=C), PMR (DMSO-d_6) □ ppm: 2.04 (s, 6H, -2XCH_3), 2.5 (s, 1H, NH-Benzimidazole) 4.6 (s,2H,-NCH_2 N-) 6.7-7.9 (m, 9H, Ar-H), 8.2 (d, 1H, pyrimidinyl-H) 10 (b, 1H, - SO_2 NH-); EI-MS (m/e): 440

WNV NS2B-NS3pro expression and purification

The expression and purification of the WNV NS2BH-NS3pro containing the 5 amino acid spacerbetween the NS2B and NS3prodomains was previously described[20]. **(**(Niklaus *et al.,* 2007; Yusof *et al.,* 2000)

In vitro WNV NS2B-NS3pro assays and inhibition studies

The compounds were dissolved in dimethylsulfoxide (DMSO) to make 50 mM stock solutions. The compounds were screened at 25 µM in 1% v/v DMSO in the final reaction mixture. Protease assays were performed in triplicates in Greiner Black 96 well plates. Each assay consisted of the reaction mixture of 100 µL containing 200 mM Tris–HCl buffer, pH 9.5, 30% glycerol, 28 nM WNV NS2BH-NS3 protease and the compound. The enzyme and the compound were pre-incubated at room temperature prior to addition of the substrate (5 µM), **Bz-Nle-Lys-Arg-Arg-AMC**. The time course of the reaction at 37^0C was followed at every 90 s intervals for up to 30 min in a monochrometer-based spectrofluorometer (Molecular Devices, Sunnyvale, CA) at excitation and emission wavelengths of 380 and 460 nm, respectively. The percent inhibition for each compound at 25 µM was first determined. For determining

IC_{50} values, the range of 10 nM, 50 nM, 0.1, 1, 2, 5, 10, and 25 µM concentrations of selected compounds were used. IC_{50} values were calculated using the SigmaPlot software.

Molecular Modelling

The synthesized compound were modeled using the crystal structure of WNV NS2B-NS3 pro co-crystallized with the tetrapeptide aldehyde. (PDB ID 2FP7). Peptide coordinates were removed from the PDB file, and hydrogen were added using "Add hydrogen" in Discovery Studio 2.0, 2D structures of the compounds are arrived using chemdraw and then using ligand preparation module in Discovery studio 3D coordinates are generated for each compound nearly 4 enantiomers were generated, all the enantiomers were docked Using GOLD and the results are taken based on the best possible scores

RESULTS AND DISCUSSION

We tested the effects of several compounds on WNV NS3 protease activity along with aprotinin as the positive control. The results indicated that out of four mercaptobenzimidazole compounds analyzed the percent inhibition varied from lowest (13% by SN) to highest (98% MBZ-SN). The results indicated that out of four mercaptobenzimidazole derivatives analyzed the percent inhibition compound MBZ-SN (96%) approached the percent inhibition by aprotinin (95%). From the study we find mercaptobenzimidazole as new class of lead for WNV protease inhibitor and their derivatives MBZ-SN (IC_{50}= 2.86 µM) exhibited significant activity. Future work will be focused on optimization of MBZ-SN compounds based on this differential inhibition of this class of compounds. Molecular modelling studies indicate MBZ-SN bind with active site of WNV NS3 and suitable for further modifications. Protease. but inhibitory activity against Dengue protease activity not yet been reported and present work is first kind of report.

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Table 1. Inhibitory activity of Mercaptobenzimidazole against WNV NS3 Protease

COMPOUNDS	% INHIBITION	IC_{50} (μM)	CC_{50} (μM)
SN	7	…	…
MBZ-SN	98	2.86	
MBZ-SAC	…	26.67	26.67
MBZ-SDM	…	113.8	159
Aprotenin	95	0.01	….

Fig 1. WNV NS3 Protease inhibitory activity of MBZ-SN

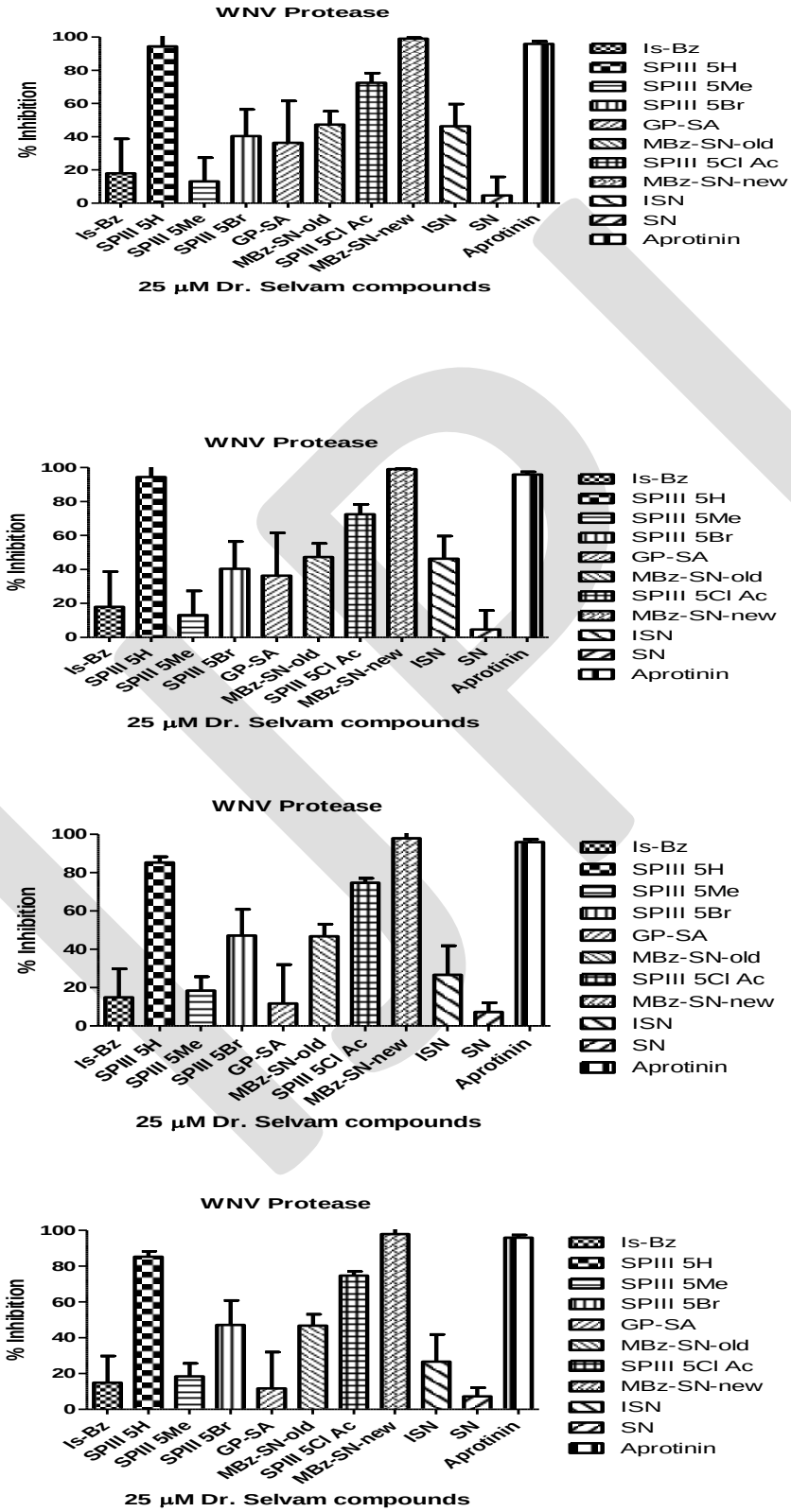
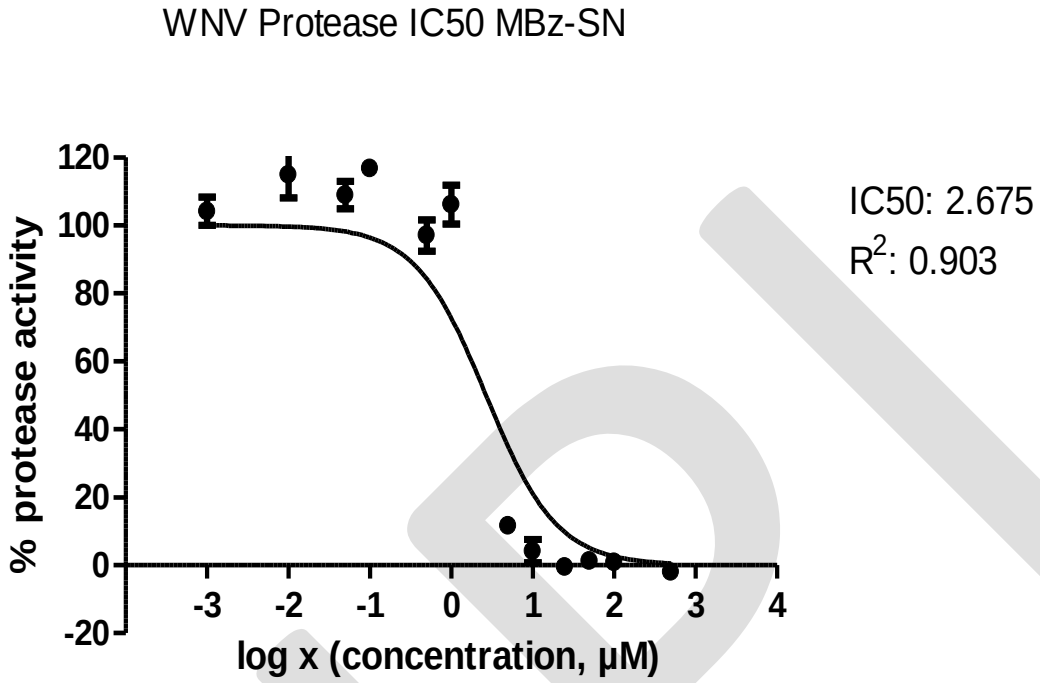
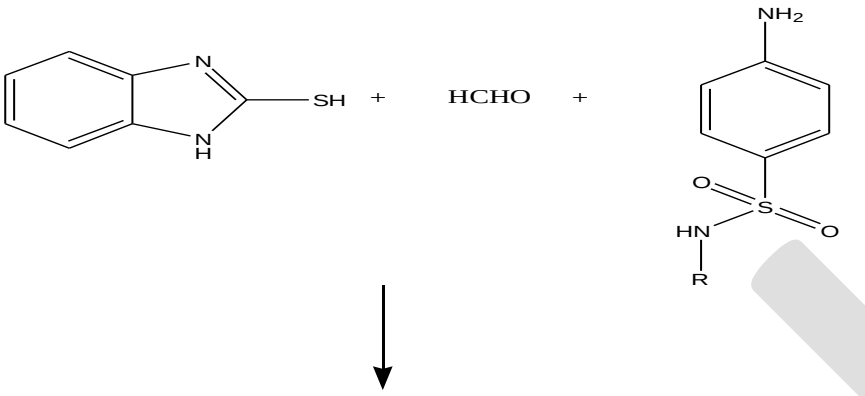
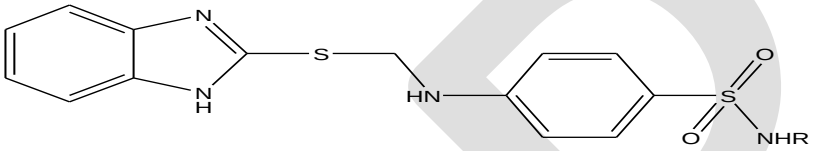


Fig 2. WNV NS3 Protease inhibitory activity of MBZ-SN



Scheme 1. Synthesis of N-Sulphanoamidomethyl-Mercaptobenzimidazole





Where	**R**
MBZ-SN	**H**
MBZ-SAC	**-COCH$_3$**
MBZ-SDM	**-2,6-Dimethylpyrimidine**

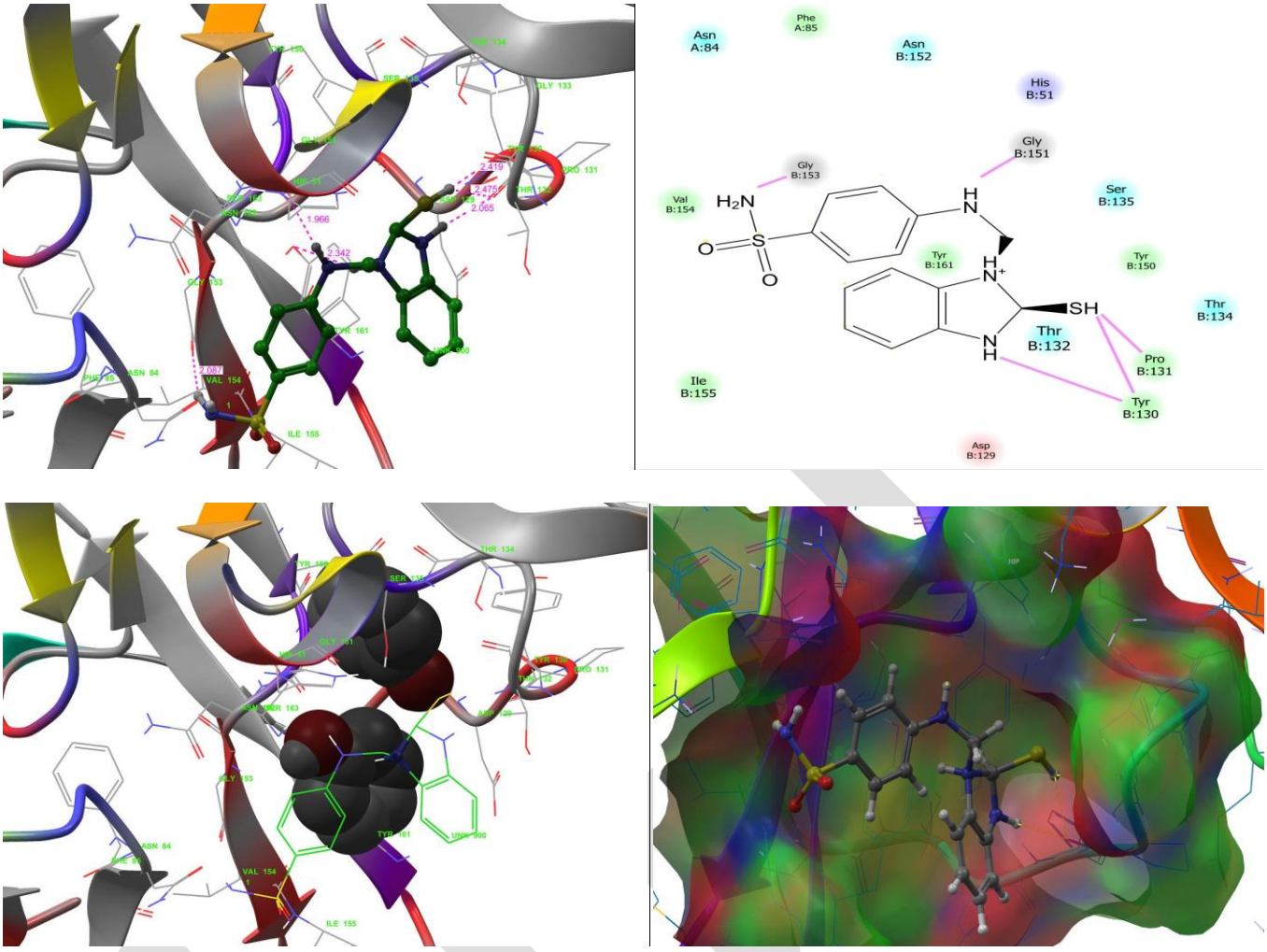


Fig 3. Molecular modeling studies of MBZ-SN with WNV NS3 PROTEASE

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Studies on anti-HIV activity, Cytotoxicity and HIV Integrase inhibitory activity of *Polyherbal formulation BH*

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Background:

The development of antiviral drugs has provided crucial new means to mitigate or relieve the debilitating effects of many viral pathogens. A rich source for the discovery of new HIV infection inhibitors has been and continues to be, the 'mining' of the large diversity of compounds from natural products. HIV integrase (IN) plays important roles at several steps, including reverse transcription, viral DNA nuclear import, targeting viral DNA to host chromatin and integration. Identification of novel inhibitors of HIV Integrase have emerged as promising new class of antiviral agents for the treatment of HIV/AIDS. Present work is to investigation of anti-HIV activity, cytotoxicity and HIV integrase inhibitory activity of various extracts of polyherbal formulation BH.

Method: Polyherbal extracts (BH) were tested for anti-HIV activity against HIV-1 and -2 in MT-4 cells and cytotoxicity also tested against uninfected MT-4 cells. BH extracts were investigated for inhibition of HIV integrase enzymatic activity to understand the mechanism of antiviral action. All the extracts were investigated for both 3'processing and strand transfer process of HIV-1 integrase enzymatic activity.

Results: All the extracts exhibited inhibitory activity against HIV-1 integrase enzyme (3'P IC_{50}: 8.8-63 µg/ml and ST IC_{50}: 4.9-65 µg/ml). The ethanolic extract (BH-H-ET) displayed significant inhibitory activity against both step of HIV In enzymatic activity (3'P IC_{50}: 8.8µg/ml and ST IC_{50}: 7.5 µg/ml). The ethanolic extract (BH-H-ET) also inhibits the HIV 1 replication at the concentration of 59.30 µg/ml and Cytotoxicity was found to be more than >125 µg/ml

Conclusion: All the extracts inhibit the HIV integrase enzymatic activity and ethanolic extract inhibit of HIV Virus and Integrase enzyme.

Keywords: *Wrightia tinctoria*, HIV Integrase activity, Aqueous extract

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a life threatening and debilitating disease state caused by retrovirus HIV. Three different classes of chemotherapeutic agents are generally combined to block the replication of human immunodeficiency virus type 1 (HIV-1) responsible for AIDS and to prevent the occurrence of resistance: reverse transcriptase inhibitors (RTI), protease inhibitors (PRI), and fusion inhibitors. This widespread triple combination therapy is referred to as HAART [highly active antiretroviral therapy] (Richman DD. 2001). HAART effectively inhibits HIV replication to such an extent that the virus becomes undetectable in the blood. However, it fails to eradicate viruses that are integrated in the host genome or that persist in cellular and anatomical "reservoirs". In addition, prolonged drug exposure led to HIV drug resistance, thus reducing patients' therapeutically available options (Cohen J. 2002). Based on above fact and the toxicity profile of available of antiretroviral agents have fueled the discovery of drugs against additional molecular targets. Among them, HIV integrase (IN), which has no cellular counterpart and crucial enzyme HIV replication, has been intensely studied over the past 15 years (Fesen *et al.*, 1993; Pommier, *et al.*, 2005; Dayam *et al.*, 2008).

Integration occurs via a sequence of reactions, which start with the IN-mediated cleavage of terminal dinucleotide from the 3'- end of the viral cDNA (termed "3'-processing", 3'-P) shortly after reverse transcriptions in the cytoplasm. Following transfer of the resulting processed viral cDNA into the nucleus, IN catalyzes the insertion of both ends into target cellular host DNA. That second reaction is referred as "strand transfer" (ST). In the past 15 years, a range of natural and synthetic compounds have been identified as inhibitors of recombinant IN enzyme in biochemical assays. Interestingly, polyhydroxylated aromatics and diketo compounds were among the first inhibitors identified. (Fesen *et al.,* 1993 & 1994). HIV integrase has recently been fully validated as a therapeutic target with the first FDA approved IN inhibitor Raltegravir (Evering T. H, Markowitz M.2007)

Review of literature revealed that herbal present in sidha formulation BH not reported for antiviral activity against the replication of HIV. The present study is designed to find the inhibitory activity of different extracts of BH against HIV 1 in MT-4 cells and HIV-1 integrase enzymatic activity.

Extraction: BH herbal powder of were subjected to hot continuous percolation using ether, chloroform, methanol and ethanol. The ether (BH.H.P.E), chloroform (BH.H.CH), methanol (BH.H.Me) and ethanol (BH.H.ET) extracts of BH have been evaluated for HIV Integrase enzyme inhibitory activity (Table BH were concentrated by distillation and dried under vaccum.Dried extracts were used for anti-HIV activity and HIV Integrase inhibitory activity

Anti-HIV assay

The compounds were tested for anti-HIV activity against the replication of HIV-1(III_B) and HIV-2(ROD) in MT-4 cells (Selvam *et al.,*2003). The cells were grown and maintained in RPMI 1640 Medium supplemented with 10% heat–inactivated Fetal Calf Serum (FCS), 2 mM- glutamine, 0.1% Sodium bicarbonate and 20 μgm/ml gentamicin (culture medium). HIV-1 (HTLV-IIIB/LAI) strain and HIV-2 ($LAV\text{-}2_{ROD}$) strain were used in the experiment. The virus strains were propagated in MT-4 cells. Titer of virus stock was determined in MT-4 cells and the virus stock was stored at - 70°C until used.

Inhibitory effects of the compounds on HIV-1 and HIV-2 replications were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by MTT assay. Briefly, 50 μl of HIV-1 or HIV-2 (100-300 $CCID_{50}$) and MT-4 cells were added at a final concentration of 6x105 cells/ml were added to flat-bottomed MT–4 wells. After 5^{th} day of incubation, at 37°C the number of viable cells was determined by the 3 - (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. Cytostatic activity of the extracts for mock- infected MT-4 cells was also assessed by the

MTT method. Anti-HIV activity and cytotoxicity of standard AZT were also performed by a similar method in MT-4 cells. The anti-HIV data are presented in Table 1.

Anti-HIV Integrase assays.

Integrase Catalytic Assay (Marchand *et al.,* 2001): Integrase reactions were carried out by mixing 20 nM DNA with 400 nM IN in a buffer containing 50 mM MOPS, pH 7.2, 7.5 mM $MgCl_2$, 14 mM 2-mercaptoethanol, and the drug of interest or 10% DMSO. Reactions were incubated at 37°C for 1 h and quenched by addition of an equal volume of gel loading dye (formamide containing 1% SDS, 0.25% bromophenol blue, and xylene cyanol). Reaction products were separated in 20% polyacrylamide denaturing sequencing gels. Dried gels were visualized using the Typhoon 8600 (GE Healthcare, Piscataway, NJ). Densitometry analyses were performed using ImageQuant software from GE Healthcare

RESULTS AND DISCUSSION

The ether (BH.H.P.E), chloroform (BH.H.CH), methanol (BH.H.Me) and ethanol (BH.H.ET) extracts of BH have been evaluated for HIV Integrase enzyme inhibitory activity and also investigated for antiviral activity against HIV 1 (HTLV IIIB) replication in MT-4 cells (Table 1; Fig 1.). All the extracts were investigated for both 3' processing (3'P) and strands transfer reaction (ST) of HIV integrase enzymatic activity (Fig. 1). All the extracts exhibited inhibitory activity against HIV-1 integrase enzyme (3'P IC_{50}: 8.8-63 µg/ml and ST IC_{50}: 4.9-65 µg/ml). The ethanolic extract (BH-H-ET) displayed significant inhibitory activity against both step of HIV In enzymatic activity (3'P IC_{50}: 8.8µg/ml and ST IC_{50}: 7.5 µg/ml). The ethanolic extract (BH-H-ET) also inhibits the HIV 1 replication at the concentration of 59.30 µg/ml and Cytotoxicity was found to be more than >125 µg/ml. To our best knowledge, this is the first time to report the anti-HIV-1 Integrase activities of these Indian plants locally known as BH. The isolation of active compounds possessing anti-HIV-1 activities from BH are now in progress.

Acknowledgment: Author express thanks to Dr.Yves pommier, DTP, CCR, NCI, NIH, USA for HIV Integrase activity and Dr. E De Clercq, Rega Institute for Medical Research, Katholieke University, Leuven Belgium for anti-HIV activity.

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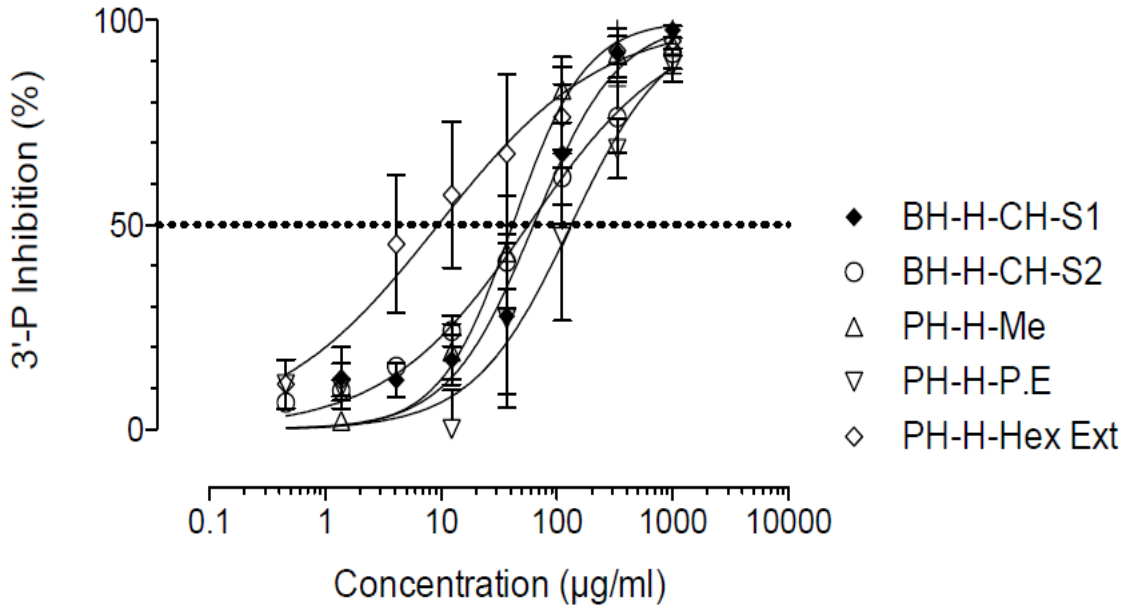
Fig 1. Anti-HIV activity and HIV Integrase Inhibitory activity of BH

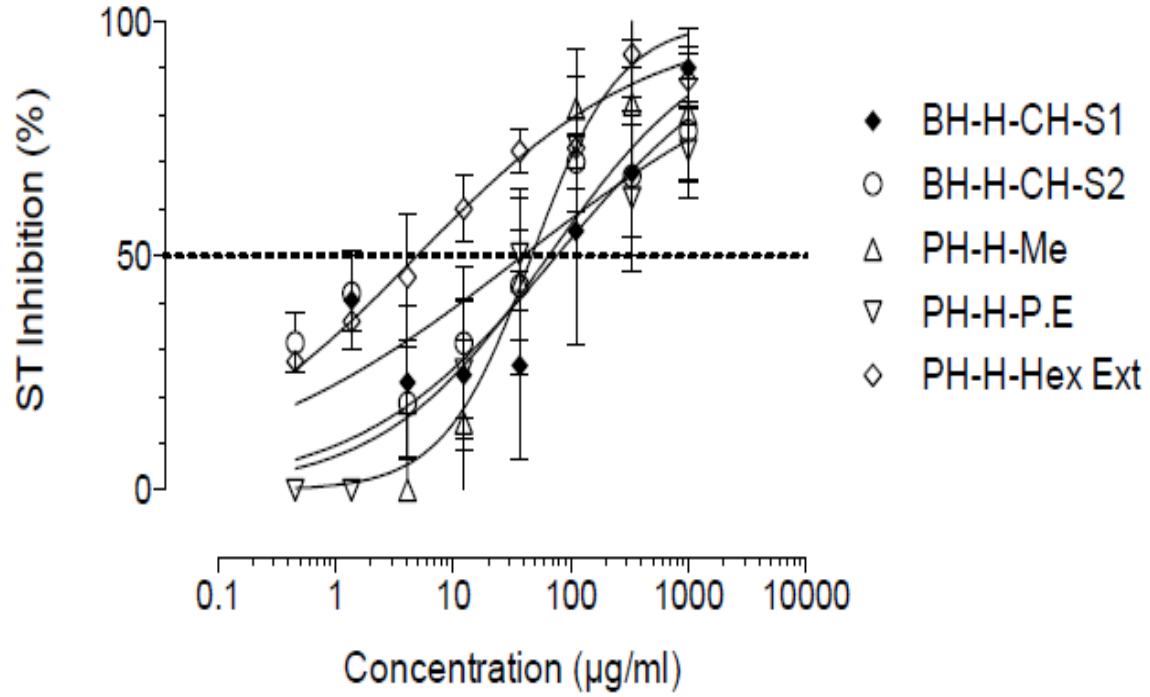
Extracts	HIV Integrase activity		Anti-HIV activity	
	IC_{50} 3'P [a], (μg/ml)	IC_{50} ST[b], (μg/ml)	IC_{50} [c](μg/ml)	CC_{50} [d] (μg/ml)
BH-H-ET	8.8 ± 1.5	7.3 ± 1.9	59.30±12.73	>125
BH-H-CH-S1	63 ± 15	75 ± 30	>147	147±6.56
BH-H-CH-S2	58 ± 8	39 ± 18	>145	145±4.24
BH-H-Me	42 ± 7	47 ± 13	>121	121±9.47
BH-H-P.E	>100	65± 23	>20.71	20.71±10.80
PH-H-HEX	9.5 ± 3.2	4.9 ± 1.2	>87.08	87.08 ±19.81

The results are IC50 ±S.D, *n* = 3 for HIV-1 IN inhibitory activity

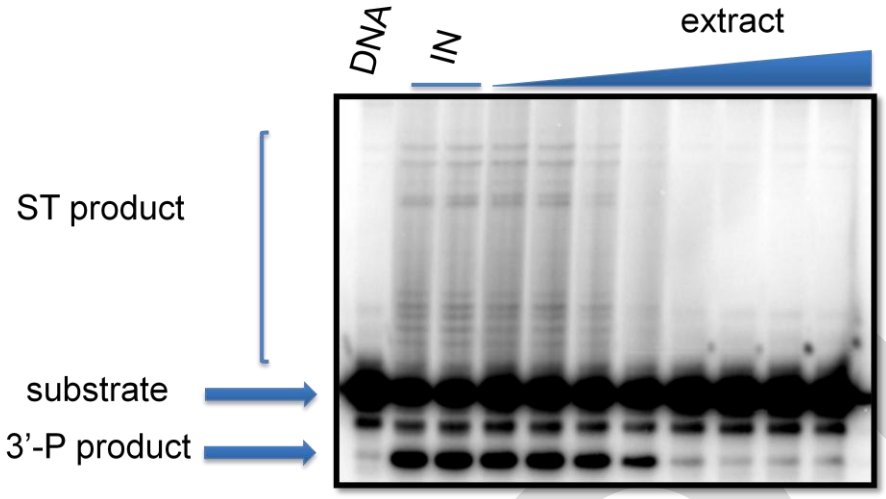
[a]Concentration required to inhibits 3' processing reaction (3'P), [b]Concentration required to inhibits 3' processing reaction (ST).[c]Effective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV. [d]50% Cytotoxic concentration of compound, required to reduce the viability of mock infected MT-4 cells by 50%.

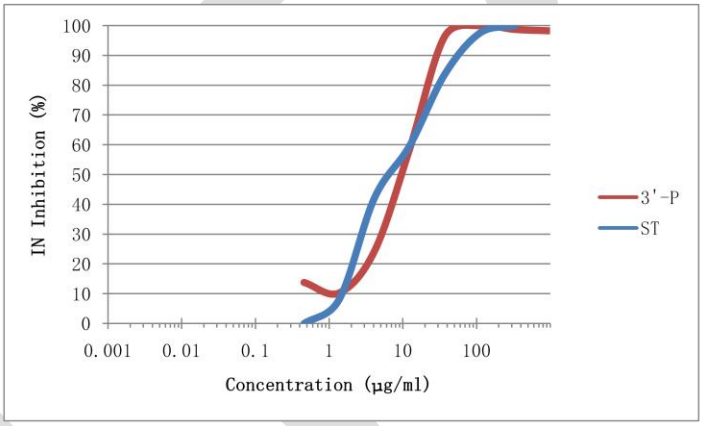
HIV I (HTLV IIIB) USED FOR ANTI-HIV ACTIVITY IN MT-4 CELLS


100
50
0
3'-P Inhibition (%)
0.1
1
10
100
1000
10000
Concentration (µg/ml)
BH-H-CH-S1
BH-H-CH-S2
PH-H-Me
PH-H-P.E
PH-H-Hex Ext


100
50
0
ST Inhibition (%)
0.1
1
10
100
1000
10000
Concentration (µg/ml)
BH-H-CH-S1
BH-H-CH-S2
PH-H-Me
PH-H-P.E
PH-H-Hex Ext

HIV integrase inhibitory activity of Herbal Formulation (BH)





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