results of analysis of commercial formulations significantly showed low values for standard deviation, standard error and coefficient of variation and thus show the precision of the methods. These values in each instance are compared with the theoretical value of 100 percent by means of unpaired students 't' test. (Table 2). As the calculated 't' values were less than theoretical 't' values, it is concluded that the results of analysis were in good agreement for each tablet. To test the accuracy and reproducibility of the proposed method, recovery experiments were performed by adding known amount of pure drug to previously analyzed samples, and these samples were reanalyzed by the proposed methods. The percentage recovery was close to 100% for both the methods. The results are summarized in the Table 3. The reproducibility, repeatability and accuracy of these methods were found to be good, which is evidenced by low standard deviation. The percent recovery obtained indicates non-interference from the excipients used in the formulations. Thus it can be concluded that the methods developed in the present investigation are simple, sensitive, accurate and precise. Hence these can be successfully applied in the estimation of clopidogrel in dosage forms.

TABLE 3: RECOVERY STUDIES

Method	Tablets	% Recovery ±S.D*.
A	ORAWIS	97.97±0.91
	NOKLOT	100.5±0.94
	DEPLATT	102.6±0.96
B	ORAWIS	102.8±0.96
	NOKLOT	101.9±0.97
	DEPLATT	98.35±0.93

*average of six determinations. SD is the standard deviation

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Microwave Assisted Synthesis, AntiHIV, and AntiYFV Activities of Schiff Bases of N-Hydroxy-N^1-Aminoguanidine Tosylate

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The microwave-assisted syntheses of N-hydroxy-N1-aminoguanidines (S1-S8) starting from thiosemicarbazide are reported herein. These derivatives were evaluated against infection by the

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human immunodeficiency virus type-1 using human T4 lymphocyte cell line and yellow fever virus strain 17D activity in Vero cells. Compound 1-(4'-chlorobenzylidene) amino-3-hydroxy guanidine (S3) showed maximum percentage protection of 43.68% against HIV-1 induced cytopathogenicity. Compound S3 was also found to be the most active compound against YFV with EC_{50} of 2.5 μM, and CC_{50} of more than 100 μM when compared to ribavirin.

Hydroxyurea[1], hydroxyguanidine[2], and thiosemicarbazone[3] derivatives have been reported to have antiviral as well as antitumour activities. N-Hydroxy-N^2-aminoguanidine (HAG) contains the major functional group hydroxyguanidine; they also combine the structural features of hydroxyl urea and thiosemicarbazone. Schiff bases of HAG had been reported to show antiviral (Corona virus, Adenovirus) and antitumour activities[4-6]. Inhibition of the enzymes reverse transcriptase and ribonucleotide reductase has been reported for HAG that account for their antiviral and antitumour activities[7]. Prompted by these reports, we have aimed to synthesize a series of HAG and investigate their *in vitro* antihuman immuno virus (antiHIV) and antiyellow fever virus (antiYFV) activities.

A domestic LG make microwave oven with the following specifications has been used: input- 220 V, ~50 Hz, 980 W, 4.7°A and frequency 2450 MHz. Melting points were determined in open capillary tubes in a Buchi melting point apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Jasco IR Report 100 (KBr) and Jeol Fx 90Q (Fourier transform) instruments, respectively. Elemental analysis (C, H, N) was performed using a Perkin–Elmer Model 240 C analyser.

N-hydroxy-N^1-aminoguanidine tosylate was synthesized from thiosemicarbazide in a similar manner as reported earlier[8]. Equimolar quantities (0.1 mol) of N-hydroxy-N^1-aminoguanidine tosylate and the appropriate substituted benzaldehyde were dissolved in ethanol (75 ml) containing glacial acetic acid (1 ml). The reaction mixture was irradiated in an unmodified domestic microwave oven at 60% power with 30 s/cycle. The number of cycle in turn depended on the completion of the reaction, which was monitored by TLC. The reaction time varied from 1.5-3 min. To the final reaction mixture ether was added to precipitate the target compounds (S1-S8) with the yields of 80-94%.

Candidate agents were dissolved in dimethylsulfoxide, and then diluted 1:100 in cell culture medium before preparing serial half- log10 dilutions. T4 lymphocytes (CEM cell-line) were added and after a brief interval HIV-1 was added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound served as a toxicity control, and infected and uninfected cells without the compound served as basic controls. Cultures were incubated at 37° in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XTT was added to all the wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitative formazan production, and in addition were viewed microscopically for detection of viable cells and confirmation of protective activity[9].

Serial dilutions of the test compounds were added to confluent Vero cell cultures in microtitre trays after which the cells were infected with 10 $CCID_{50}$ (cell culture infective dose 50%) of virus. Cultures were further incubated at temperature 37°, viral cytopathogenicity was recorded 7-8 days post infection. Cultures were fixed with 70% ethanol, stained with Giemsa solution (50-fold dilution; 2 h staining), washed and air-dried. The antiviral activity of the compound was expressed as the effective concentration required to inhibit the viral cytopathic effect by 50% (EC_{50}). Inhibition of uninfected host cell growth was assessed as follows: the cells were seeded at a rate of $3'10^3$ cells per well in a volume of 0.1 ml into 96-well microtitre plates and allowed to proliferate for 24 h in MEM containing 20% FCS, 1% L-glutamine and 0.3% sodium bicarbonate. After 24 h, 0.1 ml MEM (with 2% FCS, 1% L-glutamine and 0.3% sodium bicarbonate) containing different concentrations of the test compounds were added to each well. After 3 days of incubation at temperature 37° in 5% carbon dioxide, the cell number was determined with Coulter counter. The minimum cytotoxic concentration was expressed as CC_{50}, the concentration required to reduce cell growth by 50%.

N-Hydroxy-N^1-aminoguanidine tosylate was prepared by refluxing thiosemicarbazíde in methanol with methyl-p-toluene sulfonate to form S-methylisothiosemicarbazide tosylate, which in turn was reacted with hydroxylamine to give N-hydroxy-N^1-aminoguanidine tosylate. Microwave irradiation of the appropriate benzaldehyde with N-hydroxy-N^1-aminoguanidine tosylate in acidified ethanol delivered the title compounds S1-S8 (Table 1) via Schiff's base reaction

TABLE 1: PHYSICAL CONSTANTS OF THE TITLE COMPOUNDS

Compound	R	Yield %	mp °	Molecular formula	Molecular weight
S1	3-NO_2	92	205	$C_{15}H_{17}N_5O_6S$	363
S2	4-NO_2	94	223	$C_{15}H_{17}N_5O_6S$	363
S3	4-Cl	83	203	$C_{15}H_{17}N_4O_4SCl$	353
S4	2-OH	80	177	$C_{15}H_{18}N_4O_5S$	334
S5	4-N(CH_3)2	87	199	$C_{17}H_{23}N_5O_4S$	361
S6	4-CH_3	90	153	$C_{16}H_{20}N_4O_4S$	332
S7	4-OCH_3	82	173	$C_{16}H_{20}N_4O_5S$	348
S8	4-Br	81	198	$C_{15}H_{17}N_4O_4SBr$	397

mp refers to melting point in degree centigrade.

(fig. 1). Unlike conventional methods[2] (duration-3h; yields-58-70%), microwave assisted Schiff reactions were very facile (2-3 min), the product did not required any further purification and provided very good yields (80-94%). The assignment of the structure of the compounds was based on IR and ^{1}H-NMR data. IR data showed the imine peak at 1620 cm^{-1} which was characteristic for Schiff bases. The NMR data indicated a deuterium exchangeable peak at δ 8.96 which was assigned to the oxime proton and δ values of 8.05-8.33, 8.53, 9.48, and 10.46 for –CH=, NNH-C, C-NH, and OH protons. Elemental analyses were within 0.4% of the theoretical values for C, H, and N.

Some of the synthesized Schiff bases (S3-S8) were evaluated for their *in vitro* antiHIV activity in T4 lymphocytes using XTT assay method (Table 2). Among them, compound S3 was found to be the most active compound with percentage protection of 43.68% while the other compounds did not showed marked antiHIV activity at a concentration below their toxicity threshold.

The title compounds were also evaluated for their inhibitory effects on the replication of YFV, by means of a cytopathic effect reduction assay. The EC_{50} values of the different compounds required for inhibition of YFV-induced cy-

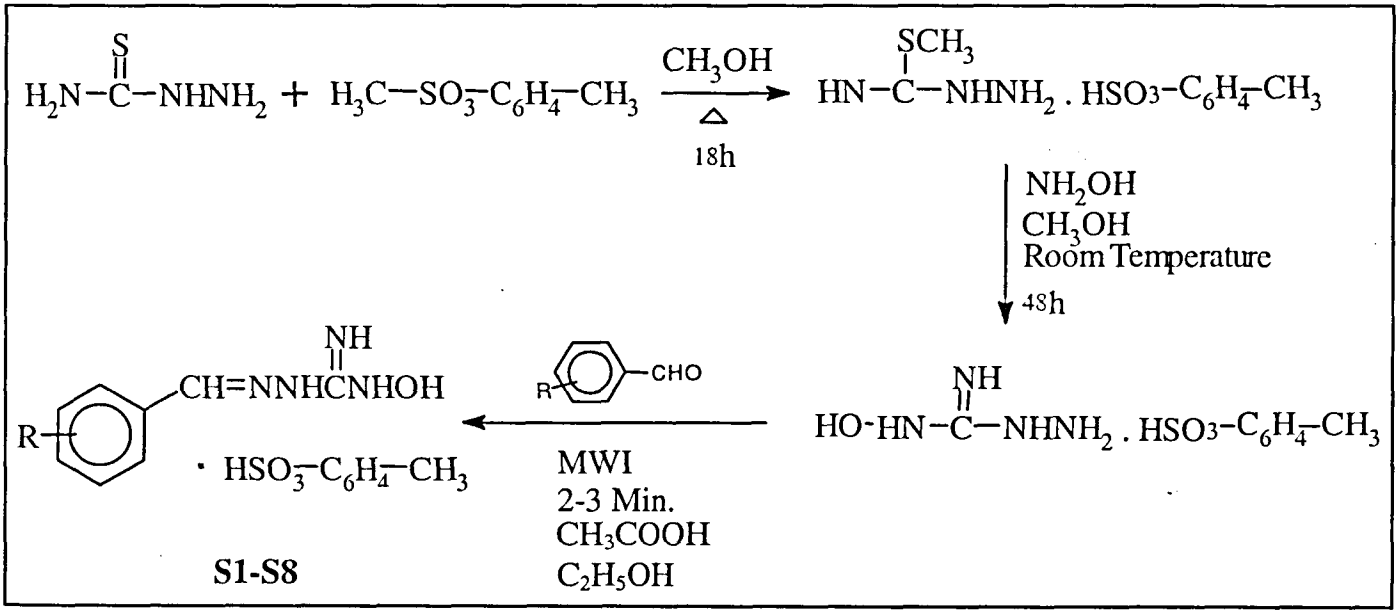

Fig. 1: Scheme for synthetic route to Schiff bases of N-hydroxy-N'-aminoguanindine tosylate

TABLE 2: ANTIHIV AND ANTIYFV ACTIVITIES OF TITLE COMPOUNDS

Compound	AntiHIV activity (μM)			AntiYFV activity (μM)	
	EC_{50}	CC_{50}	%Protection	EC_{50}	CC_{50}
S1	NT	NT	-	>100	100
S2	NT	NT	-	>100	100
S3	>104	104	43.68	2.5	>100
S4	>154	154	21.38	>20	20
S5	>132	132	24.20	>20	20
S6	>52.0	52.0	16.32	>20	20
S7	>98.8	98.8	15.87	15	>100
S8	>16.7	16.7	16.29	>4	4

EC_{50} refers to the effective concentration in 50% population and CC_{50} refers to the cytotoxic concentration in 50% population and NT indicates not tested.

topathic effect are presented in Table 2. Among the compounds tested, two compounds S3 and S7 showed promising activity with EC_{50} of 2.5 μM and 15 μM, respectively, CC_{50} of >100 μM and selectivity index of >40 and >6.6 respectively. These compounds were more potent than ribavirin (EC_{50}=49 μM and CC_{50} =>100 μM). From these results, compound 1-(4'-chlorobezylidene) amino-3-hydroxy guanidine (S3) emerged as a potent compound with both antiHIV and antiYFV activities.

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Microwave-Assisted Synthesis, and AntiHIV Activity of 2,3-Diaryl-1,3-Thiazolidin-4-Ones

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Several 1,3-thaizolidin-4-ones bearing variously substituted diaryl ring at C-2 and N-3 positions have been synthesized utilizing microwave irradiation and evaluated for their antiHIV and antiYFV activities. The results of the *in vitro* antiHIV evaluation showed that compound DS13 proved to be

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