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バージョン：

Exploration of *in vitro* Antiherpetic Activity of Coumarin-sulphonamide Derivative against Genital Herpes

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Abstract: To develop the non-nucleoside antiviral agent for genital herpes caused by HSV-2, (Z)-4-((7-amino-4-methyl-2-oxo-2H-chromen-3-yl)diazenyl)-N-(5,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (ADPB) was synthesised by reaction of 6-amino-4-methyl-2H-Chromen-2-one and 4-amino-N-(5,6-di-methoxypyrimidinyl)benzene sulphonamide. The structure of ADPB (molar mass 496) was established using Infrared, ^{1}H and ^{13}C-NMR spectroscopy. During the assessment of *in-vitro* anti-herpetic activity of ADPB against HSV-2, this coumarin sulphonamide derivative was found as potent antiviral agent for HSV-2 indicated by its selectivity index greater than 10 and less cytotoxicity. The potential of compound to inhibit the replication of virus at post-infection was visualized by reduction of specific gene products[β-(ICP-6) and γ-(ICP-5 and gB) groups] in western blotting.

Keywords: Antiviral agent, genital herpes, Acyclovir, western blot analysis, coumarin-sulphonamide

1. Introduction

Genital herpes is a generic sexually disseminated disease conved by HSV-2 and signalized by lifelong infection in infected person.[1)] The HSV-2 is a double stranded DNA virus pertaining to the herpes viridae family.[2,3)] Since HSV-2 behave as intracellular parasite in neural ganglia and it is too much arduous to completely eliminate it.[4)] To remediated the infections caused by HSV-2 innumerable natural and artificial chemical substances are discovered.

In synthetic chemistry, during the treatment of HSV-2 Acyclovir and its nucleoside analogues viz Valaciclovir, Famiciclovir and Cidofovir are amply consumed. These drugs retard replication of virus by inhibiting DNA polymerase enzyme of virus and clog the synthesis of genetical material of virus in host cell.[5)] but the long tome use of these drugs as antiviral agent can manifest the possibility to develop the immunity by virus for these medicines due to tendency of virus to mutate.[6)] so these limitations require the need to develop new anti HSV agent for causative infection by HSV-2 based on alternative mechanism of action.

In synthetic chemistry α-hydroxytropolones derivatives were found active against HSV-2 by their lower cytotoxicity and ability to suppress virus replication. These compounds have tendency to suppress β-galactosidase protein expression and inhibit the replication of virus at early stages of infection.[7)]

Anita Toscani and *coworkers* scrutinized the anti HSV-2 activity of amidinourea derivatives and found active against Acyclovir resistant strain of HSV type 1 and 2. Their finding suggested that synthesized amindinourea derivatives show lower cytotoxicity for host cell and block the replication of virus in early stages of infection. Being non-nucleoside inhibitor of HSV-1 and 2 they act through a new antiviral mechanism and designated as analogues of moroxydine.[8)]

On investigation of antiherpetic nature of bezothiozole based pyrimidinesulfonamide derivatives, it was found that these compounds were potent anti HSV agent due to their inhibitory activity against Hsp90α protein.[9)]

Mariyam Zangi and coworker synthesized non-nucleosidic antiviral agent for genital herpes virus from antifungal drug ciclopirox (Fig :A). The ciclopirox derivatives possessing hydrophobic $-C_6H_5CH_2$ group at position 4 and 6 position show remarkable anti HSV activity. [10)]

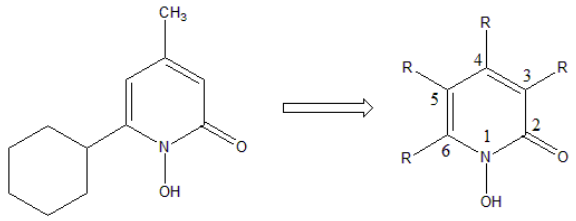


Figure-A: Ciclopirox

In the direction of development of antiviral agent, substituted benzo-pyron (coumarin) skeleton is contemplated as privileged structure for development of antivirucidal agent by virtue of their high affinity and specificity to target different molecular binding sites of virus.[11)]

Various derivatives of coumarin can be used as broad spectrum antiviral agent for HIV-1, Hepatitis C virus, Bovine viral diarrheoa virus and Herpes simplex virus type-1 and 2 due to their capacity to block intracellular activity affecting the synthesis viral proteins, genetic materials and viral polymerase.[12)]

A. Kumar and coworkers synthesized benzene sulfonamide derivative having tendency to inhibit replication of HSV-1 at post infection indicated by the reduction of specific gene expression which state the tendency of compound to block the various steps of virus DNA synthesis at late stage of infection.[13)]

To discover the new potent non-nucleosidic antiviral agent to treat the genital herpes present paper focus on the synthesis of coumarin-sulphonamide derivative by reaction with 6-amino-4-methyl-2H-chromen-2-one and (4-amino-N-(5,6-dimethoxy pyrimidinyl)benzenesulphonamide). The structure of synthesized compound was established by using Infrared, ^{1}H and ^{13}C-NMR. spectroscopic methods. *in-vitro* antiherpetic activity of compound was evaluated against HSV-2 (333 strain)

2. Material and Methods

Sodium nitrite, 6-amino-4-methyl coumarin, sodium hydroxide, hydrochloric acid, methanol, ethanol and Isopropanol, Phosphate Buffer Saline (PBS), Glutamine, Fetal Bovine Serum (FBS),Sodium pyruvate, Penicillin (standard drug) , Streptomycin (standard drug) and Dulbeco modified Eagle medium (DMEM), used in synthesis were acquired from Sigma Aldrich. All chemicals were 95 to 96.5 % pure.

Lymph Buffer solution, Phenyl methacophenol Fluoride, Triton Solution, Protein antibodies ICP-5,6,8, anti gb and β-actin were purchased from Merck-BDH. For cell culture Biobase Gray Co2 Incubator, Model Number BJPX-C50 temperature range 5°C to 75°C degree Celsius was used.

All solvents were purified by distillation method. Melting point was determined by open capillary method in sulphuric acid bath.

Elemental analysis for new synthesized compound was performed on a Perkin-Elmer series-II, 2400 elemental analyzer. Infrared spectral measurements were made on a JASCO-430 FT-IR spectrometer. The resolution was set to 2 cm^{-1} and the scanning range was 400 to 4000 cm^{-1}.The spectra was measured in the solid state as pressed KBr pellets (13 mm)

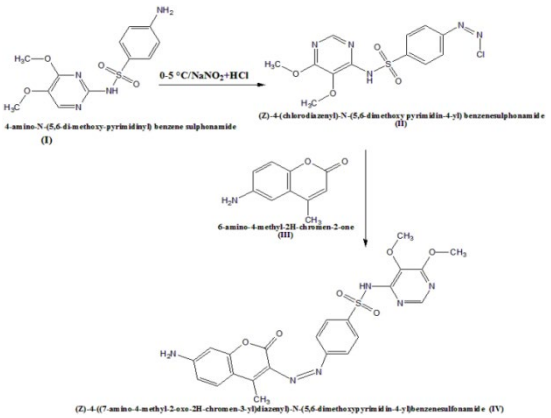
^{1}H and ^{13}C-NMR spectra of synthesized compound was measured on a BRUKER AC 300F, 300 MHz FT-NMR spectrometer. The spectra were recorded in $CDCl_3$ and DMSO with tetramethylsilane as internal standard. Experimental conditions for ^{1}HNMR : 300.134 MHz (spectral frequency), at 6024.096 (sweep width), 8.0 (pulse width), 1.0 second (relaxation delay), 63L CPD (decoupling power) and internally reference value (SR) was set at 4125.36 ppm for H_2O.

Experimental conditions for ^{13}CNMR : spectral frequency (SFt 75.469 MHz, sweep width (SW) at 22727.273, pulse width (PW) at 5.0, relaxation delay (RD) of 1.0(sec), acquisition time (AQ) was 0.360(sec), receiver gain (RG) 400, decoupling power (DP) was 14H CPD, filter to suppress noise (LB) 6.0, reference value (SR) was set at 701.89 ppm for DMSO at 39.5 ppm externally. The chemical shift value of synthesized compound was delineated on δ scale/ppm.

3. Synthesis of (Z)-4-((7-amino-4-methyl-2-oxo-2H-chromen-3-yl)diazenyl)-N-(5,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (ADPB)

(Z)-4-(chlorodiazenyl)-N-(5,6-dimethoxy pyrimidin-4-yl) benzenesulphonamide (II) was prepared by diazotization reaction by mixing 3 mMol (0.207 gram) cold aqueous solution of sodium nitrite dissolved in acidic solution of 3 mMol (0.801gram) 4-amino-N-(5,6-di-methoxy-pyrimidinyl) benzene sulphonamide (I) at 5°C in ice bath.

Further mix this solution in 20 mL volume of alkaline solution of 6-amino-4-methyl-2H-chromen-2-one (III) which was prepared by dissolving 3mMol (0.545 gram) 6-amino-4-methyl coumarin (III) in 20 mL of 15% lye solution. Whole mixture was placed in ice bath for 45 minute at 5°C and recrystallized by ethyl alcohol. Yellow crystal of (Z)-4-((7-amino-4-methyl-2-oxo-2H-chromen-3-yl)diazenyl)-N-(5,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (IV) was filtered.[14)] (Scheme-1)



Scheme 1 Synthetic route for preparation of ADPB

4. Evaluation of antiviral activity of ADPB

4.1Cell line and Virus

Vero Cells of Cercopithecus aethiops were multiplied in Dulbecco modified Eagle Medium (DMEB) comprising with 10% fetal bovine serum. This medium was supplemented in 2 milli-molar glutamine and 1 milli-molar sodium pyruvate along with antibiotic penicillin (100 unit per mL) and streptomycin (100 µg per mL). The viability of Vero cells were appraised by employing 0.02 % Trypan blue exclusion assay.[15)]

To induce the infection Vero cells were vaccinated by HSV-2 Viral stock solution (corresponding to MOI = 0.1). After infection all infected Vero cells were centrifuged to remove cellular debris and remaining viral titer was used to measure the plaques formed by using plaque number reduction method.[16)]

4.2 Assessment of cytotoxic effect of ADPB

The cytotoxic effect of ADPB was assessed by MTT assay.[17)] Vero cells of Cercopithecus aethiops were cultured in 96-well cultured plates. After the cultivation of Vero cells varied concentration (01-550 µgmL^{-1}) of DMSO solution of ADPB were added in Dulbecco's modified Eagle Medium for 3 days. 0.5 % solution of DMSO in DMEM was used as negative control. 150 µL volume of MTT reagent was added to each well before incubation for 4 hour. The former medium containing MTT was replaced by acidic solution of isopropanol (N/10 HCl dissolve in 20% isopropanol) to dissolve formazan crystals. All petri were placed in ELISA reader. The absorbance was recorded at test wavelength 570 nm with reference wavelength 690 nm. After Incubation, CC_{50} value of each one was calculated as minimum concentration of drug devouring ability to reduce the viability of cell up to 50% when compared to untreated control.

4.3 Assessment of half minimum inhibitory concentration of ADPB

The half minimum inhibitory concentration (IC_{50}) of ADPB against HSV-2 were appraised by MTT assay.[18)] Vero cells cultured in 96-well culture plates at 37 °C in 5% CO_2 atmosphere for 8 hour added by HSV-2 (MOI 0.1) and different concentration(0.50-40 µgmL^{-1}) of DMSO solution of ADPB. 0.5% DMSO in DMEM was used as negative while standard drug Acyclovir as positive control. Acyclovir is used as reference compound because this drug enters and acts on only those cells infected with HSV-2 and very less active for uninfected Vero cells

After 72 hours of incubation, the concentration of compound required for reduction of virus yield up to 50% (IC_{50}) was reckoned by regression analysis of the dose–response curves. Selectivity index value.[19)] of compound was calculated by using the formula Selectivity Index Value (SI Value) = CC_{50} /IC_{50} Value.

4.4 Cell protection Assay

To assess the efficiency of ADPB to block host cell receptors influenced by HSV-2 which averts adsorption and penetration of virus in host cell, cell protection assay was used.[20)]

Vero cells previously washed with phosphate buffer saline were incubated with 2.5 mL solution (160 µgmL^{-1}) of compound for 120 minutes. After 120 minute of incubation, infection of HSV-2 was induced by adding viral titer (corresponding to MOI = 0.1) using RPMI with 2% FBS as control. After 75 minute of incubation at 35°C, viral inoculum was segregated and infected Vero cells were covered with medium covering 1% methyl cellulose. Subsequently 48 hour of incubation, treated Vero cells were stained by 1.5% crystal violet solution. The percentage of viral inhibition was measured by counting plaques.

4.4 Evaluation of combined effect of ADPB with Acyclovir

To observe the synergistic activity of ADPB and Acyclovir at post infection (p.i.), infected Vero cells were incubated with ADPB (MIC value = 2.5 µgmL^{-1}) and Acyclovir (MIC value = 0.5 µgmL^{-1}) for 60 minute at 37°C temperature. After centrifugation supernatants of infected Vero cells were separate out and used to observe the plaques while non treated infected Vero cells (CTR) were used as reference to compare plaques form by the virus between infected cells and treated cells.[21)]

4.5 Pharmacokinetics of ADPB at pre stages of HSV-2 infection

The effectivity of ADPB at various time gap upto 24 hour, time dependent pharmacokinetics assay was used. [22)]

Vero cells were grown on 96-well plate at 37°C in the atmosphere of 5% CO_2. Infection in Vero cells were induced by viral titer (corresponding to MOI = 0.1). Further these infected Vero cells were incubated with solution of ADPB having concentration 27.98 µgmL^{-1} at (i) during the adsorption period (iii) Immediate after the adsorption of virus.

In both steps Vero cells were incubated in 1% PBS containing RPMI medium for 24 hour at 35°C and supernatants of ADPB infected cells were used to identify the affectivity of compound for the infection caused by HSV-2.[23)]

4.6 Computation of anti-viral nature of ADPB at early stages of post infection

Vero cells matured in 24-well plates induced an infection by HSV-2 (corresponding to MOI = 0.1) and incubated for 2 hour at 4°C. Further infected Vero cells were washed by phosphate buffered saline to remove viral debaris. 27.98 μgmL^{-1} concentration of ADPB was supplemented with infected Vero cells at various times of post infection for 0–1 hour, 1–3 hour, 4–6 hour and 6–8 hour. After this specified time lap the supernatants of untreated infected Vero cells and ADPB treated infected cells were recovered and to assess anti herpetic behavior of ADPB by plaque number reduction assay. [24)]

4.7 Computation of antiviral nature of ADPB at late stages of post-infection

To evaluate the conseiences ADPB on viral replication at post infection, firstly Vero-cells were incubated with HSV-2 at 37°C temperature for 5-6 minute to allow virus to penetrate Vero cell membrane and replication. Infected Vero cells were treated with 27.98 μgmL^{-1} concentration of ADPB for 0-24 hour, 15 minute-24 hour, 45 minute-24 hour, 2-24 hour, 3-24 hour and 4-24 hour while untreated Infected Vero cells (CTR) were used as control.[25)]

4.8 Western Blot Analysis

To unearth the idiosyncracy of compound to interrupt protein expression of HSV-2 western blotting technique was applied.[26)]

In this experiment Vero cells were lysed by using 2% Triton-X-100 buffer solution. The protein contents were aloofed by SDS-PAGE and electro-blotted on to nitro-cellulose membrane. For recognition of reduced protein expression in HSV-2, anti-gb and anti β-actin antibodies were used. ECL plus detection system was used for visualization of protein by following instruction manualed by manufacturer and densitometry data for the blots were analysed with help of imagJ software.

5. Result and Discussion

Microanalysis of ADPB

Yellow crystal, Melting point 235-241°C, Yield (82%), Analysis for $C_{22}H_{20}N_6O_6S$ (molar mass 496) Calculated % C = 53.22, H = 4.06, N = 16.93, S = 6.46, O = 19.33, Found % C = 53.26, H = 4.06, N = 16.93, S = 6.47, O = 19.36, Soluble in DMSO, Acetone, m/z = 496.12(100%), 497.12 (25.2%), 498.12 (4.7%) **IR Spectral data**: 2751 cm^{-1} (free amino group), 3483 cm^{-1} (-NH group), 1690cm^{-1} (-C=O of Lactone carbonyl), 1235 cm^{-1}(O-C=O of coumarin moiety), 1574 cm^{-1} (-N=N-), 1172- 13011 cm^{-1} (symmetric and asymmetric vibration of SO_2 group), 1574 cm^{-1}, 689 cm^{-1}, 1198 cm^{-1} and 3683 cm^{-1} (benzenesulphonamide ring), 1172 cm^{-1}, 1235 cm^{-1}, 1678 cm^{-1} (di-methoxy pyrimidine ring)

The ^{1}H-N.M.R spectroscopic data of ADPB which numbered as Figure-1, show the presence of one broad singlet at downfield value 4.0 ppm for one proton adjacent with nitrogen atom of –SO_2NH- moiety present in ADPB. The doublet of doublet appeared in aromatic region at 7.88 (2H, J = 6.6, 1.6, 0.8 Hz) ascribed to H-16 and H-18 show meta coupling with each other, likewise a new doublet of doublet appeared at 7.34 (2H, J = 6.6, 1.8, 0.48 Hz) due to H-15 exhibiting meta coupling with H-19. Moreover two sharp singlet of three protons embarked at downfield value 3.71 ppm and 3.58 ppm assigned to two methoxy group existent in compound and the existence of all these characteristic proton signals reveals the presence of sulphadoxine in ADPB. The compound exhibit three doublet of doublet appeared at 6.86 ppm (J = 1.5,0.5 Hz), 6.67 ppm (J = 7.7, 1.5 Hz) and 8.0 ppm (J=7.7,0.5 Hz) suggested the presence of coumarin ring while the appearance of one sharp singlet of three identical proton at 2.44 ppm and another sharp singlet of two identical proton at 4.0 ppm suggested the presence of methyl and amino group respectively. (Figure-2)

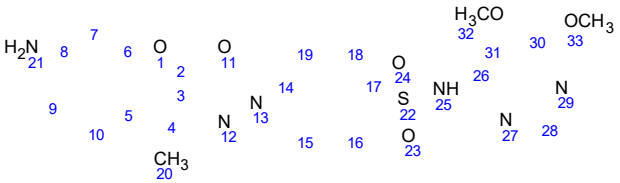


Fig.1: Numbering of ADPB

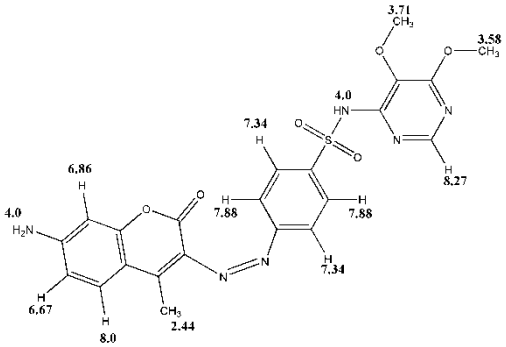


Fig.2:^{1}H-NMR chemical shift (δ/ppm) of ADPB

The ^{13}C-NMR spectra of ADPB (Figure-3) show sharp signal of 24 carbon atom. Out of these twenty carbon atom signal nine signal appeared at 156.2, 110, 155.5,111.1,151,106.7,148,113 and 127.6 ppm ascribed to C-2 (lactonyl carbon), C-3, C-4 and C-5,C-6, C-7,C-8,C-9 and C-10 atom of coumarin moiety. The appearance of downfield carbon signal at 12.1 ppm suggests the substitution of methyl group at C-4 atom of coumarin ring. Six aromatic carbon signal appeared at

131.9 ppm (C-14), 127.3 ppm (identical C-16 and C-18 atom) and 139.7 ppm (C-17) along with carbon signal appeared at 157.3 ppm (C-26), 145.7ppm (C-28), 158.3 (C-30), 130.8 ppm (C-31) and 56.2 ppm (identical C-33 and C-35) commulatively suggested the existence of 4-amino-N-(5,6-dimethoxy pyrimidin-4-yl)benzene sulphonamide moiety present in compound which was further confirmed by **^{1}H-^{13}C-HSQC** spectrum

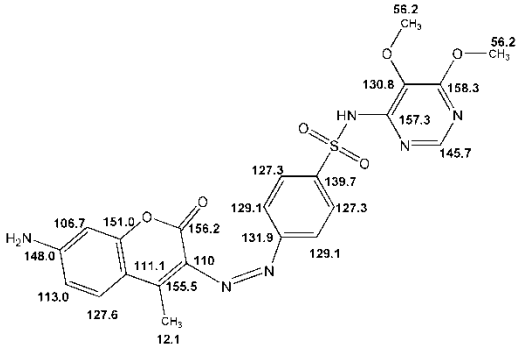


Fig.3:^{13}C-NMR chemical shift (δ/ppm) of ADPB

^{1}H-^{1}H COSY spectra of compound show all ^{1}H-^{1}H scalar coupling which is more significant being H-7↔H-9, H-9↔H-10, H-15↔H-19 and H-16↔H-18 and all protons of –CH_3 and –OCH_3 group (Figure-4)

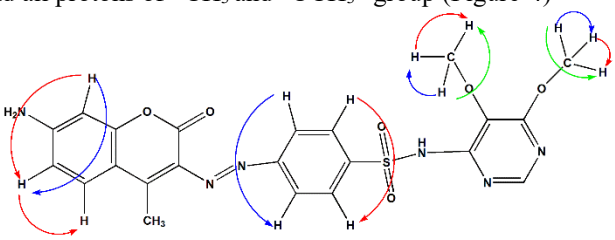


Fig.4:^{1}H-^{1}H coupling in ADPB

The **^{1}H-^{13}C HSQC** spectra of ADPB show that out of twenty two carbon atom, ten carbon atom were associated with substituted coumarin ring, six were associated with benzene sulphonamide moiety and other six were of 4,5-dimethy pyrimidine moiety present in compound. This ^{1}H-^{13}C-HSQC spectra show coupling of H-7 (δ_H 6.86) with C-7 (δ_C106.7), H-9 (δ_H 6.67) with C-9 (δ_C 113), H-10 (δ_H 8.0) with C-10 (δ_C 127.6), H-15 (δ_H 7.88) with C-15 (δ_C 106.7), H-19 (δ_H 7.91) with C-19 (δ_C 127.3) in 7-amino-4-methyl-2H-chromen-2-one moiety while in coupling in H-18 (δ_H 7.88) with C-18 (δ_C 127.3), H-16 (δ_H 7.34) with C-16 (δ_C 127.3), H-28 (δ_H 8.27) with C-28 (δ_C 145.7), H-20$_a$, H-20$_b$, H-20$_c$ (δ_H 3.58) with C-20 (δ_C 12.1), H-33$_a$, H-33$_b$, H-33$_c$ (δ_H 3.58) with C-33 (δ_C 56.2), H-35$_a$, H-35$_b$, H-35$_c$ (δ_H 3.7$_1$) with C-35 (δ_C 56.2), in 4-amino-N-(5,6-di-methyl pyrimidinyl)benzene sulphonamide moiety present in compound, which was further confirmed by broad range correlation of C-H shown in Figure-5. On analyzing the **^{1}H-^{13}C HMBC** correlation (Figure-5) observe that H-7 (δ_H 6.86) show cross peak with C-2 (δ_C 156.2) and C-4 (δ_C 110), H-9 (δ_H 6.67) show cross peak with C-6 (δ_C 151), H-10 (δ_H 8.0) cross peak with C-3 (δ_C 110), H-20$_a$, H-20$_b$, H-20$_c$ (δ_H 2.44) with C-10 (δ_C 127.6), H-21$_a$, H-21$_b$, H-21$_c$ (δ_H 4.0) with C-5 (δ_C 111.1) in 7-amino-4-methyl-2H-chromen-2-one moiety present in compound, other long rang correlation of H-15 (δ_H 7.34) with C-16 (δ_C 127.3) and C-18 (δ_C 127.3), H-16 (δ_H 7.34) with C-19 (δ_C 129.1), H-18 (δ_H 7.88) & H-19 (δ_H 7.34) with C-26 (δ_C 157.3), H-25 (δ_H 4.0) with C-16 (δ_C 127.3), H-35$_a$, H-35$_b$, H-35$_c$ (δ_H 3.71) with C-31 (δ_C 158.3) and H-33$_a$, H-33$_b$, H-33$_c$ (δ_H 3.58) with C-30 (δ_C 158.3) confirm the existence of 4-amino-N-(5,6-di-methoxy pyrimidinyl)benzene-sulphonamide moiety in compound.[27,28)]

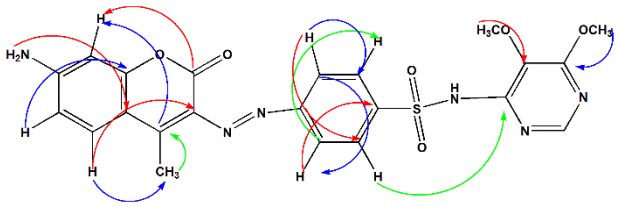


Fig.5:^{1}H-^{13}C HMBC coupling in ADPB

The dose response curve shown in Figure 6 represent the 50% cytotoxic concentration (CC_{50}) value of ADPB.

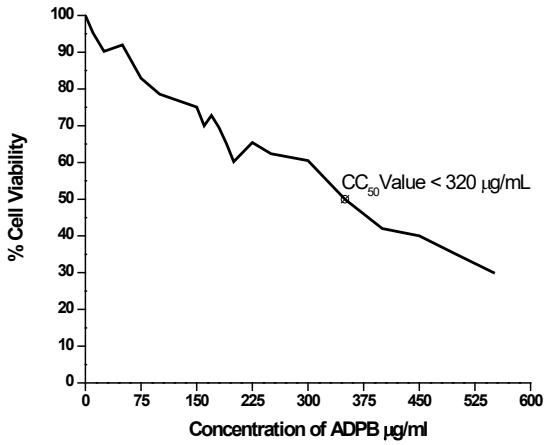


Fig.6: Dose response curve to show CC_{50} value of ADPB

This cytotoxic study revealed that upto 320 µgmL^{-1} compound not decrease the viability of Vero cells but on increasing the concentration of compound viability of cells starts to decreased till the highest concentration.

Further the half maximal inhibitory concentration of ADPB by dose response curve was found 27.95 µgmL^{-1} which was required concentration to inhibit the 50% growth of HSV-2. This dose response curve (Figure 7) suggested that atleast 4.53 µgmL^{-1} concentration was essentially required to inhibit the replication of virus. The Selectivity Index (SI) value 11.44 evidenced compound as potential antiHSV-2 agent.

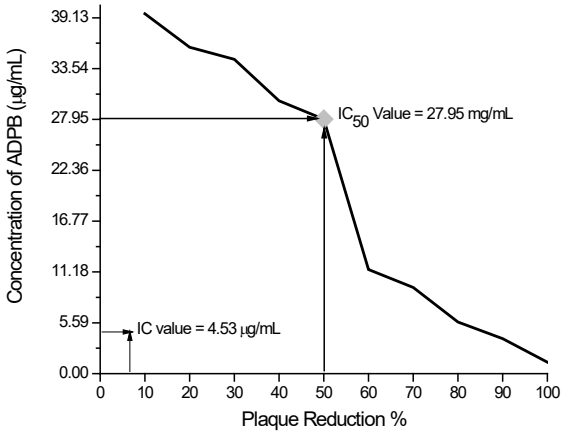


Fig. 7: Determination of IC_{50} value of ADPB

Synthesized Compound was combined with standard first line antiviral agent Acyclovir to assess the combined effect on rate of replication of HSV-2. The CC_{50} and IC_{50} values of Acyclovir was found 3000 μgmL^{-1} and 0.12 μgmL^{-1} respectively, with Selectivity index > 25000. Individually 0.285 μgmL^{-1} concentration of Acyclovir was able to inhibit HSV-2 replications nearly 68 percent as compared with CTR and almost alike inhibition was monitored in Vero cells treated with 3.48 μgmL^{-1} concentration of ADPB. The combined form of Acyclovir and compound need only 1.67 μgmL^{-1} concentrations to inhibit replication of virus upto 82%. (Figure 8)

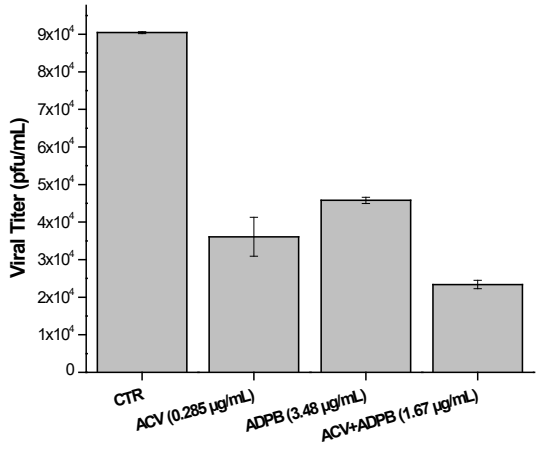


Fig.8:.Synergistic antiviral activity of ADPB and Standard drug Acyclovir

During the assessment of potency of ADPB to inhibit the infection induced by HSV-2, it was analysed that during the adsorption of virus in host cell synthesized compound do not show any inhibitory effect meanwhile after the entry of virus in host cell compound show a significant inhibitory effect which proportionate with time. (Figure 8) Such inhibitory effect of compound at early stage of infection suggested the capability of it to act as potential antiherpetic agent for infection caused by HSV-2.

The efficacy of ADPB to inhibit the replication of virus at post infection was increased with time (for 0-24 hour, 15minute-24 hour, 45minute-24 hour, 1-24 hour, 3-24 hour and 5-24 hour). At post infection ADPB reduces the HSV-2 population upto 82% which was visualized by Figure.

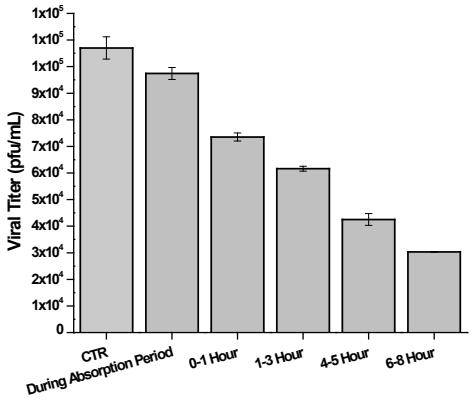


Fig. 9: Antivirucidal effect ADPB on Viral replication during adsorption period and at initial and different time of post infection (p.i.) at 0-1 hour, 1-3 hour, 4-6 hour and 6-8 hour

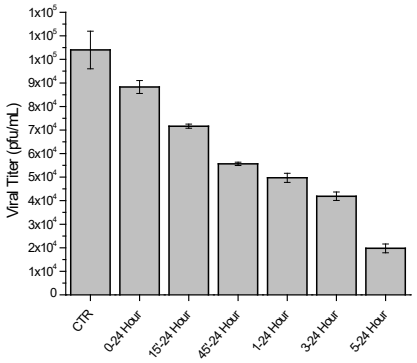


Fig.10: Antivirucidal effect of ADPB on adsorption period and different time of post infection .) at 0-24 hour, 15'-24 hour, 45'-24hour, 1-24 hour, 3-24 hour & 5-24 hour

The western blot analysis (Figure-10a, 10b and 10c) indicates at bot early and late stages of post infection these protein expression β-(ICP-6), γ-(ICP-5 and gB) and α-(ICP-8) of HSV-2 was reduced remarkably in Vero cells which was treated with ADPB.

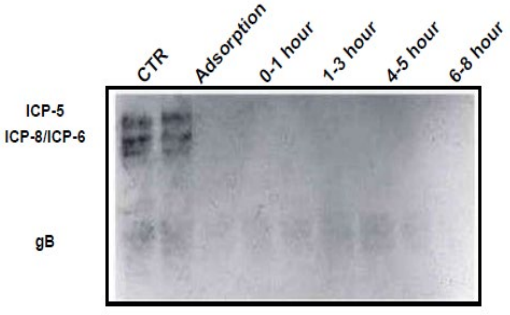


Fig.10a:western blot analysis of ADPB

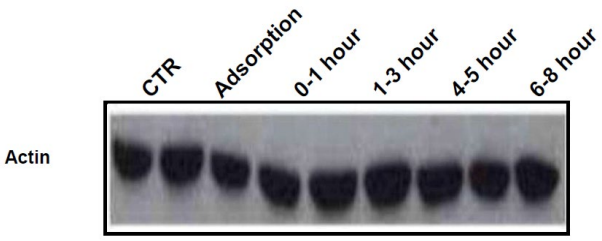


Fig.10b:western blot analysis of ADPB

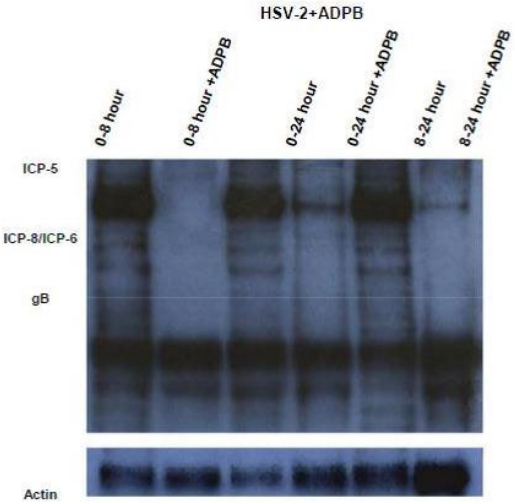


Fig.10c:western blot analysis of ADPB

6. Conclusion

Our disquisition explicated that synthesized compound ADPB can behave as antivirucidal agent for *HSV-2* which was authenticated by its lower cytotoxicity and good selectivity index value (SI < 10). On assessment of antiviral behavior of ADPB It was observed that during adsorption period, it was less able to inhibit the entry of virus in host cell but at late stage of post infection it was showing the capability to reduce replication of virus. this behavior of ADPB was evidenced in western blotting by the reduction of specific gene product β-(ICP-6), γ-(ICP-5 and gB) and α-(ICP-8) of HSV-2. The reduction of this protein expression strongly recommended that ADPB can inhibit HSV-2 replication in host cell by blocking the function of ribonucleotide reductase and growth of multifunctional single stranded DNA binding protein.

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Nomenclature

ACV	Acyclovir
CC_{50}	50% cytotoxic concentration
HSV-2	herpes simplex virus type-2
IC_{50}	half minimum effective concentration
MOI	multiplicity of infection
Pfu	plaque forming unit
SI	selectivity index
CTR	untreated infected Vero cells

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