Evaluation of novel phosphorylated derivatives of abacavir for antiviral activity in DF-1 cells infected with Newcastle Disease Virus (NDV).

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INTRODUCTION

- Newcastle disease is a contagious, fatal and severe viral disease which affects more than 240 avian species.

- Newcastle disease virus is negative sense, single stranded RNA virus, belongs to the family Paramyxoviridae and genus Avulavirus.

- Newcastle disease virus encodes six proteins Nucleoprotein, phosphoprotein, large polymerase protein, haemagglutinin neuraminidase, fusion and matrix protein.

- Based on the virulence Newcastle disease virus is classified into three types
    1. Lentogenic
    2. Mesogenic
    3. Velogenic

ABACAVIR

- Abacavir is a Nucleoside Reverse Transcriptase Inhibitor (NRTIs), chemical analogues of DNA blocks generally used as antiviral drug for RNA viruses.

- Abacavir is intracellularly converted to its active metabolite carbovir triphosphate by enzyme phosphotransferase to exert its activity.

- Carbovir triphosphate is incorporated into the viral DNA during replication and it lacks 3'-OH group which terminates the DNA chain elongation by preventing addition of new bases.

- currently available drugs are not effective due to their
    1. High cytotoxicity
    2. Low bioavailability
    3. Target specificity
    4. Low solubility

OBJECTIVES

- ✓ To design and carry out docking studies of abacavir derivatives against NDV fusion protein.
- ✓ Synthesis and characterization of best compounds from QSAR study.
- ✓ To determine cytotoxicity and antiviral activity of novel compounds against NDV on chick embryo fibroblast cells (DF-1).

MATERIALS AND METHODS

ABACAVIR

↓ **Hyperchem Software**

ABACAVIR DERIVATIVES

↓ **QSAR Studies**

IDENTIFICATION OF BEST LEAD MOLECULE

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SYNTHESIS AND CHARACTERIZATION OF LEAD MOLECULE

IN VITRO ANALYSIS

IN VIVO ANALYSIS

ANTI VIRAL ACTIVITY AGAINST NDV INFECTED DF-1 CELLS

- Cytotoxicity
- Plaque formation Assay
- Cytopathic inhibition Assay

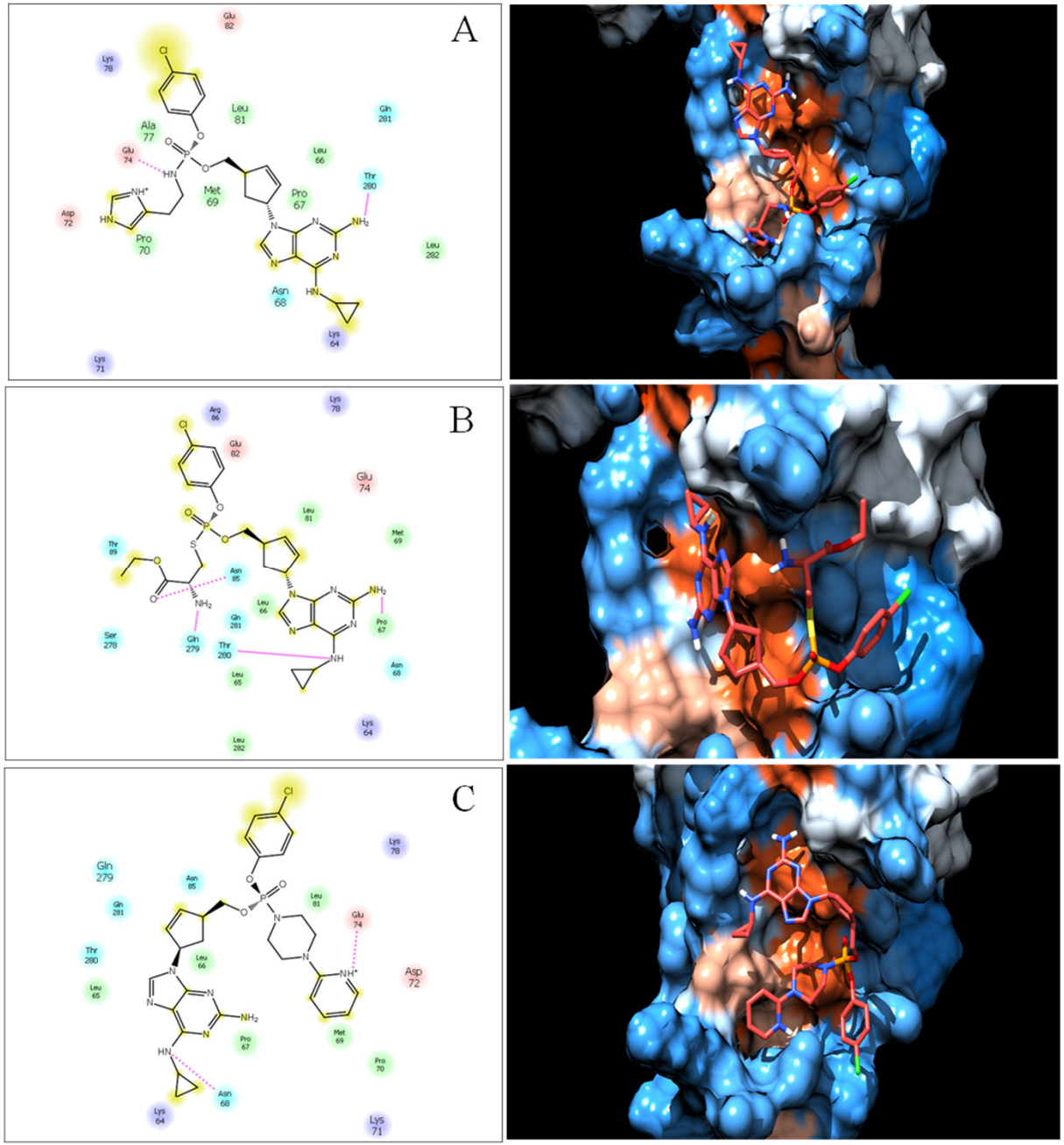
Docking scores and interactions of Novel phosphorylated analogues against NDV fusion protein.

LIGAND	DOCKING SCORE	NO.OF HYDROGEN BONDS	LIGAND HYDROGEN BOND	RECEPTOR HYDROGEN BOND	DISTANCE
Aba_1	-4.36	2	NH	Gl74 COOH	2.65
			NH	Thr280 COOH	2.85
Aba_2	-2.51	2	NH	Leu66	2.92
			NH	Gln279	3.2
Aba_3	-2.63	4	NH	Thr93 COH	2.94
			OH	Asn85 CONH2	3.11
			Cl	Arg86 NH2	3.29
			NH	Ser278 COH	2.84
Aba_4	-3.73	5	NH2	Pro67 COH	3.08
			NH	Thr280 COOH	2.89
			NH2	Gln279 COOH	2.75
			S	Asn85 CONH2	2.97
			COH	Asn85 CONH2	2.94
Aba_5	-0.48	3	NH2	Pro67 COH	2.99
			NH	Gln279 COOH	2.98
			PO	Asn85 CONH2	2.8
Aba_6	-2.06	1	NH2	Gln279 COOH	2.78
Aba_7	-2.00	2	NH	Asn85 CONH2	3.05
			NH	Glu82 COOH	2.97
Aba_8	-3.04	3	NH	Glu74 COOH	2.81
			NH	Glu74 COOH	2.67
			PO	Asn85 CONH2	3.05
Aba_9	-0.95	3	NH	Pro67 COH	3.21
			CF	Asn85 CONH2	3.31
			PO	Asn85 CONH2	2.86
Aba_10	-3.44	4	NH	Glu82 COOH	3.25
			NH	Glu74 COOH	2.78
			COH	Pro67 COH	2.74
			COH	Pro67 COH	2.68
Aba_11	-1.51	1	NH	Thr89 COH	3.18
Aba_12	-3.47	2	NH	Asn68 CONH2	3.01
			NH	Glu74 COOH	2.81
Aba_13	-2.09	4	NH	Glu74 COOH	2.85
			NH	Glu74 COOH	2.64
			PO	Asn85 CONH2	2.78
			PO	Asn85 CONH2	2.69
Aba_14	-2.36	2	NH	Asn85 CONH2	3.02
			NH	Glu82 COOH	2.78
Aba_15	-0.54	1	Cl	Asp72 NH2	3.13
Aba_16	-3.20	3	NH	Gln279 COOH	2.94
			PO	Asn85 CONH2	2.91
			NH	Pro67 COH	2.75
ABC	-3.47	2	NH	Glu74 COOH	3.01
			NH	Glu74 COOH	2.7

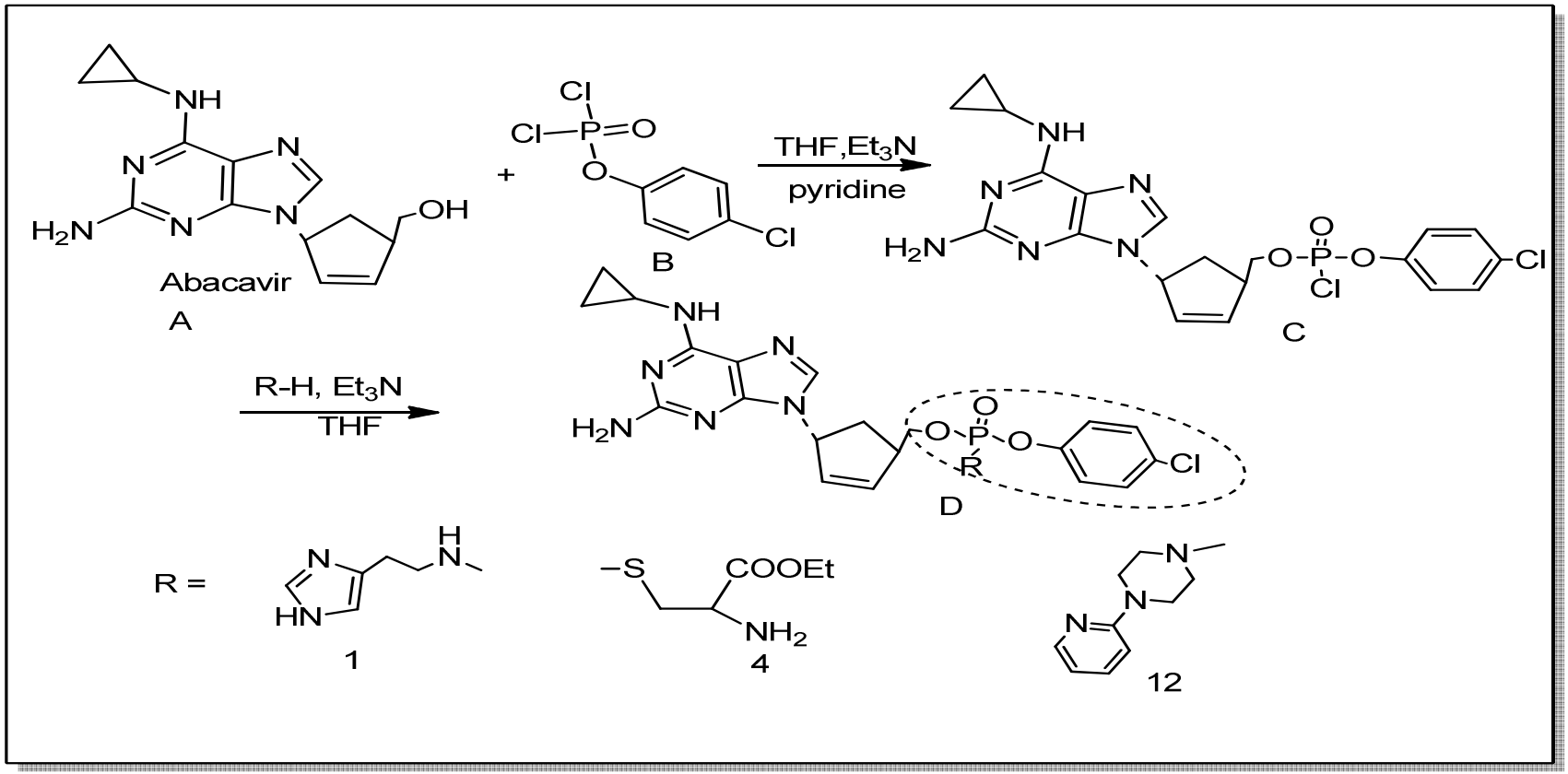
Molecular descriptors of the novel phosphorylated compounds derived from QSAR study.

Ligand	MW	HBD	HBA	AlogP	Molar refractivity (A°3)	SASA /Surface area (Ao2)	Volume (Ao3)	Polarizability (Ao3)	Minimised Energy	Strain Energy
Aba_1	569.98	4	7	2.25	151.52	733.31	1629.73	64.83	117.96	39.69
Aba_2	555.95	3	7	3.33	143.87	815.74	1620.79	63.10	114.06	44.79
Aba_3	619.05	4	6	4.47	168.73	833.42	1769.11	74.44	125.09	48.24
Aba_4	608.04	3	8	3.36	156.78	861.44	1766.43	65.53	93.12	56.31
Aba_5	610.98	3	6	4.27	160.92	924.48	1737.58	68.94	91.46	94.63
Aba_6	566.97	3	7	3.22	151.44	1003.3	1679.89	66.14	94.95	41.81
Aba_7	581.99	4	7	4.10	155.29	991.74	1706.73	68.03	92.77	50.83
Aba_8	633.98	3	6	5.31	159.57	998.06	1627.35	68.95	82.22	58.1
Aba_9	619.96	3	6	5.31	154.74	994.05	1725.60	67.12	100.53	48.47
Aba_10	612.01	5	8	4.16	161.74	745.58	1783.64	70.50	91.78	50.67
Aba_11	639.06	2	6	4.83	170.65	772.52	1860.30	73.38	107.07	56.7
Aba_12	622.05	2	7	4.01	168.59	863.47	1827.84	72.22	110.34	53
Aba_13	623.04	2	8	3.37	167.04	894.30	1822.36	70.97	107.63	110.72
Aba_14	666.06	2	6	4.52	177.76	958.88	1846.20	75.02	106.88	101.07
Aba_15	655.51	2	6	5.29	175.24	728.49	1883.91	75.44	107.17	53.52
Aba_16	583.98	3	6	4.58	153.82	855.62	1692.01	67.30	91.57	41.11
Aba	382.39	4	10	0.87	95.00	515.63	1015.68	34.10	86.1	59.82

(A)ABC-1, (B) ABC-4, (C) ABC-12 showing protein – ligand interactions with fusion protein of NDV.

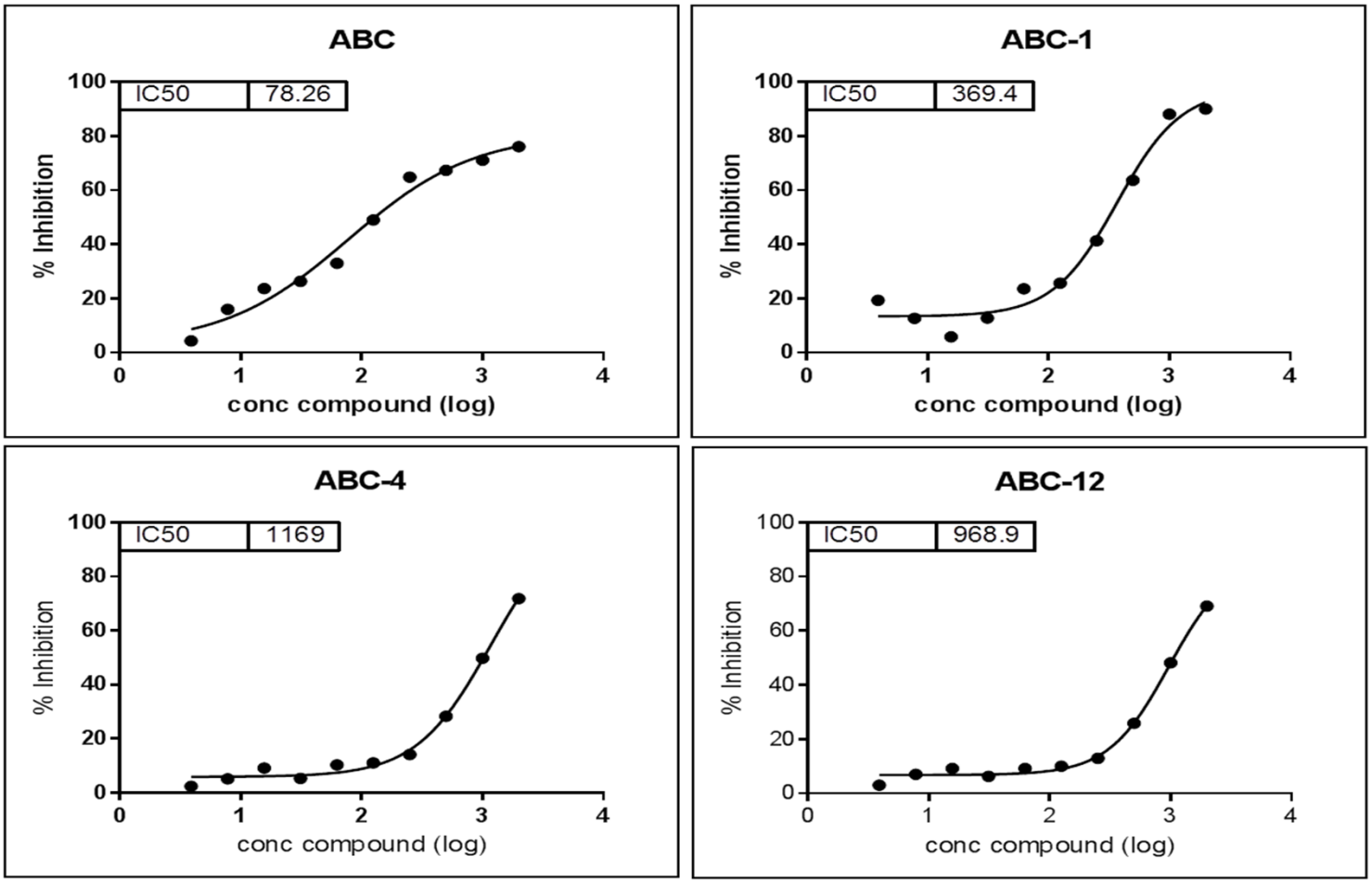


Synthetic route of Abacavir phosphorylated compounds



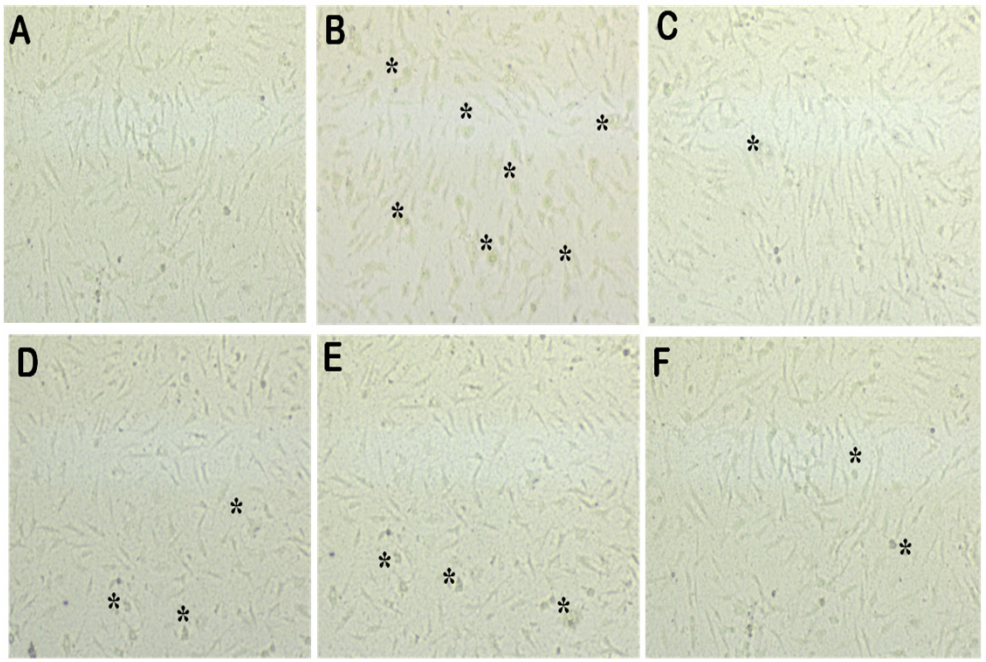
The structural properties of these compounds were elicited by IR, ^{1}H, ^{13}C, ^{31}P, CHN Analysis and Mass spectra.

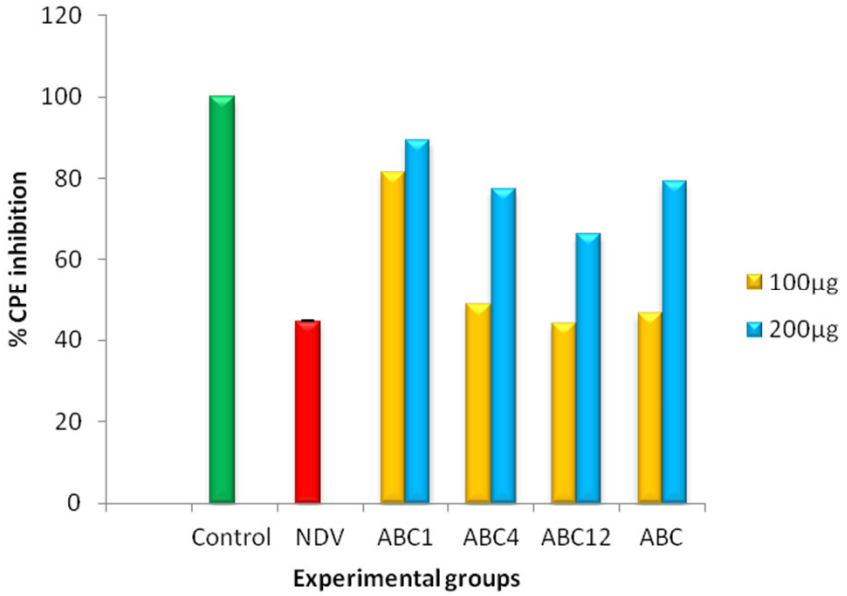
Cytotoxicity assay



Determination of IC_{50} values. Cells were treated for 48 h with various concentrations. MTT dye was then used to determine the cell viability (%). IC50 values were determined after plotting the absorbance values vs. various drug concentrations. IC_{50} values for ABC, ABC-1, ABC-4 and ABC 12 are 78.26µg/ml, 369.4 µg/ml, 1169 µg/ml and 968.9µg/ml respectively. The novel phosphorylated compounds showed lower Cytotoxicity than parent Abacavir (ABC).

Cytopathic effect inhibition assay

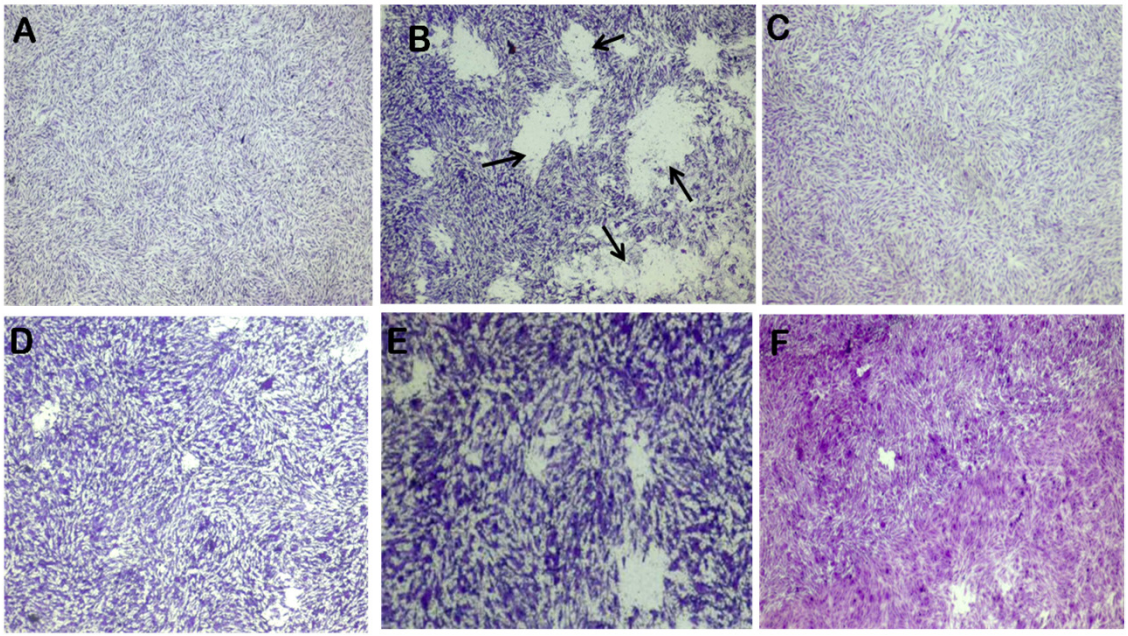




Cytopathic effects of NDV on DF-1 cells: cells were infected with NDV and treated with Novel phosphorylated compounds. A- Control, B-NDV control, C-ABC-1, D-ABC-4, E-ABC-12 and F-ABC.

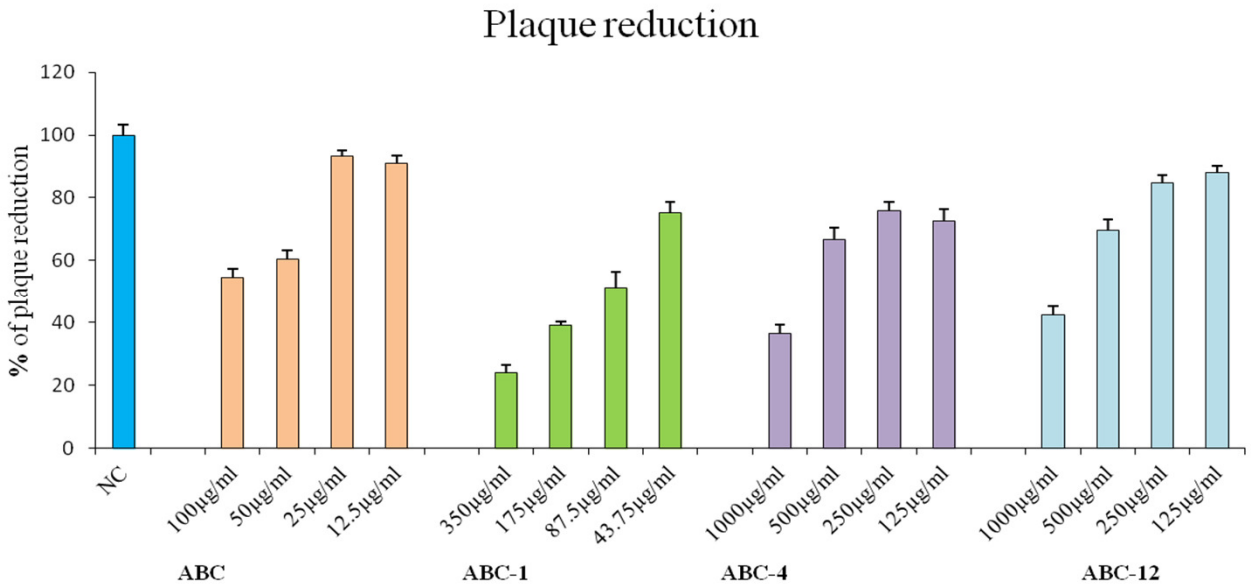
CPE inhibition: Percent of inhibition in CPE were calculated against uninfected DF-1 control cells. CPE inhibition results showed ABC-1 compound showed improved inhibition than the ABC. *=Cytopathic effects of NDV.

Plaque reduction assay



Plaque formation in NDV infected chicken embryo fibroblast (DF-1) cells. A-Control, B-NDV control, C-ABC-1, D-ABC-4, E-ABC-12 and F-ABC. ABC-1 showed more plaque inhibition compared to other compounds.

Antiviral activities of novel phosphorylated compounds were characterized by plaque assay method. Chicken embryo fibroblast (DF-1) cells was infected with NDV virus and treated with different concentrations of compounds ABC, ABC-1, ABC-4 and ABC-12, reduction in plaque number was calculated compared to controls.



CONCLUSION

✓From the designed 16 derivatives of abacavir analogues, 3 analogues with best binding affinity against fusion protein was synthesized and characterized.

✓Of the three compounds synthesized and tested in vitro ABC1 phosphorylated compound Showed enhanced antiviral activity against DF-1 cells infected with NDV than the Native Abacavir


THANK YOU