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Author

Uddin, SJ, Bettadapura, J, Guillon, P, Grice I, D, Mahalingam, S, Tiralongo, E

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Research Article

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In-vitro Antiviral Activity of a Novel Phthalic Acid Ester Derivative Isolated from the Bangladeshi Mangrove Fern *Acrostichumaureum*

Shaikh J Uddin[1,2], Jayaram Bettadapura[3], Patrice Guillon[3], Darren Grice I[3,4], Suresh Mahalingam[3] and Evelin Tiralongo[1,5*]

[1]*School of Pharmacy, Griffith University, Australia*
[2]*Pharmacy Discipline, Khulna University, Bangladesh*
[3]*Institute for Glycomics, Griffith University, Australia*
[4]*School of Medical Science, Griffith University, Australia*
[5]*Griffith Health Institute, Griffith University, Australia*

Abstract

Background: Over the past century dengue (DENV2), chikungunya (CHIKV) and human parainfluenza (hPiV3) viruses have profoundly impacted on human morbidity, mortality and the economy worldwide. Current therapy options to treat infections of these viruses have severe limitations leading to a continued search for novel drug candidates. *Acrostichum aureum* L. (Pteridaceae) is a mangrove fern, that has been used as a traditional medicine in Bangladesh and other various countries for a variety of diseases including infection.

Objectives: Isolation and structural elucidation of novel antiviral secondary metabolites from the methanol extract of the aerial parts of *A. aureum.*

Materials and methods: The novel phthalate acid ester was isolated (HPLC) and structurally elucidated using 1D and 2D NMR, MS and other spectroscopic methods. The compound was tested for antiviral activity against DENV2 andhPiV3in Vero cells using the fluorescent focus (FFA) assay and against CHIKV virus in LLC-MK2 cells using the plaque-forming unit assay (PFU). The activity of the isolated compound was further compared with its known derivative.

Results: In this study, we report on the isolation of a novel phthalic acid ester, 2''-(methoxycarbonyl)-5''-methylpentyl 2'-methylhexyl phthalate from the aerial parts of the Bangladeshi mangrove fern *Acrostichumaureum* and its *in vitro* antiviral activity. The novel phthalate showed antiviral activity against dengue virus, human parainfluenza virus and chikungunya. The most potent activity was recorded against hPiV3 (EC_{50} 29.4 μM) and was slightly higher than the activity determined for the positive control BCX 2798 (EC_{50} 44 μM). Cellulose acetate phthalate was also evaluated for antiviral activity against these viruses for the first time and was found to be inactive. Both compounds were found to be non-toxic against Vero and LLC-MK2 cells.

Conclusion: This study shows that some selected phthalates have potent antiviral activity and should be further investigated as potential novel antiviral agents.

Keywords: *Acrostichumaureum*; 2''-(Methoxycarbonyl)-5''-methylpentyl 2'-methylhexyl phthalate; Antiviral activity; Dengue virus; Chikungunya virus; Human parainfluenza virus

Introduction

Infections account for about 15 million deaths annually worldwide and have ranked for centuries as a major challenge to human progress and survival [1]. They are a leading burden of largely avoidable morbidity and mortality worldwide, falling substantially on people in developing countries and particularly on infants and children [2]. Viral infections have challenged mankind's survival, and viruses' present recurrent socioeconomic and health problems worldwide. Preventive vaccines can only provide lifelong protection against a limited number of viruses. In addition, viruses often have the ability to mutate rapidly because of their low-fidelity replication process and resistance to conventional drug therapies [3]. There is an urgent need for drugs to treat the various viral diseases for which there are limited or no therapeutic options currently available.

Numerous natural products with selective potent antiviral activity have been identified worldwide, with some currently undergoing either pre-clinical or clinical trials [4]. A variety of medicinal plants have been identified that possess broad–spectrum antiviral activity of greater potency than conventional antiviral agents [5].

Esters of phthalic acid (1,2-benzenedicarboxylic acid) can be found in soils, sediments, terrestrial and marine waters, animals, microbes, plants and marine algae [6-8]. It has not been confirmed to date whether plants or other organisms produce these compounds or whether their presence is due to contamination during the separation process or from accumulation from the environment [6,8]. Of note though is the report indicating the formation of di-butyl phthalate by a soil bacterium [9]. Some phthalate esters are routinely used as industrial plasticizers [10] and some are reported to produce toxic effects in the reproductive system [11], be teratogenic [10] or hepatotoxic [12]. However, according to the FDA in the US, "some phthalates have demonstrated no appreciable toxicity" [13]. Moreover, some phthalates, such as bis(2-methylheptyl) phthalate isolated from the medicinal plant

***Corresponding author:** Dr. EvelinTiralongo, School of Pharmacy, Griffith University, Gold Coast campus, Queensland 4222, Australia, Tel: + 61 7 5552 7098; Fax: + 61 7 5552 8804; E-mail: e.tiralongo@griffith.edu.au

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Pongamiapinnata and a fraction from the root bark extract of the guava tree Psidiumguajava, which contains diethyl phthalate and phthalic acid, displayed antiviral activity against White Spot Syndrome Virus [14,15]. Another phthalate, cellulose acetate phthalate (CAP), which is commonly used as a pharmaceutical excipient for the enteric coating of tablets and capsules, has been shown to inactivate herpes simplex virus types 1 and 2 (HSV-1 and 2) and cytomegalovirus *in vitro* [16], as well as HSV-2 *in vivo* [16,17].

Recently, we reported on the isolation and cytotoxic activity of novel pterosins, flavonoids, tetracosanes and patriscarbatine from the methanolic extract of the aerial parts of the Bangladeshi medicinal plantAcrostichum aureum [18,19]. Here we report on the isolation of a novel phthalic acid ester from this mangrove fern and investigations into its cytotoxicity and also that of CAP against Vero and LLC-MK2 cells, as well as their antiviral activities against dengue virus (DENV-2), chikungunya virus (CHIKV) and human parainfluenza virus (hPiV3).

Materials and Methods

Isolation and characterization of a novel phthalic acid ester

General experimental procedures: Optical rotations were measured on a Jasco P-1010 polarimeter. IR spectra were recorded on a Bruker Optics alpha-QuickSnap FT-IR spectrophotometer. NMR spectra were recorded on a BrukerAvance 300 MHz spectrometer in $CDCl_3$.LR-MS and HR-ESI-MS were obtained on BrukerDaltonics esquire 3000 and BrukerDaltonics Apex III 4.7e mass spectrometers, respectively. Analytical HPLC was performed on a Varian Prostar instrument with a 335 DAD using a RP (Luna C18, 5 µm, 250 × 4.6 mm) column. Preparative HPLC was performed on a Waters instrument equipped with a Waters 600E pump, Rheodyne 7725i injector, using a RP (Luna C18, 5 µm, 150 × 21.2 mm) column. SPE cartridges (Alltech, 10 g, RP-C18) were used to fractionate the extract.

Plant material: The aerial parts of *A. aureum* were collected from tidal forests in the coastal Sundarbans (a swamp region in the Ganges delta) of Bangladesh in February, 2007. The plant material was identified by Dr. Momtaz Mahal Mirza, Principle Scientific Officer, Bangladesh National Herbarium, Dhaka, and shade-dried. A specimen was deposited in the Bangladesh National Herbarium, Dhaka (Voucher no.: DACB 31538).

Extraction and isolation: The dried and pulverized plant material of *A. aureum* (150 g) was extracted with methanol (1 L, 99.9% pure HPLC grade, Merck, Darmstadt, Germany) by soaking it overnight at room temperature with continuous stirring. The extract was filtered and the residue was further extracted with 1L of methanol three times for 1 h while sonicated. All of the resulting extracts were combined and concentrated under reduced pressure (rotary evaporator and freeze dryer) to give 7.57 g of extract (5.04% w/w). The resulting MeOH extract was partitioned between *n*-hexane (99.9% pure HPLC grade, Merck Darmstadt, Germany) and MeOH (1:1) using a separating funnel. The *n*-hexane fractions were further analysed with analytical RP-HPLC (Aqua, 5 µm, 250×4.6 mm). Semi-preparative RP-HPLC (Aqua, 5 µm, 250×10 mm) using a H_2O:MeOH gradient system of solvent A (10% MeOH in water with 0.05% TFA) and solvent B (90% MeOH in water with 0.05% TFA) was employed to separate the n-hexane fraction (0.33 g). It yielded CPHP (6.3 mg) using a linear gradient of 50-100% solvent B.

2″-(Methoxycarbonyl)-5″-methylpentyl 2′-methylhexyl phthalate (CPHP): A Whitish semi-solid (>98 % pure), $[\alpha]^{25}_{D}$ +34.4° (c 0.45, $CHCl_3$). UV($CHCl_3$) λ_{max} (log ε): 239.6 (3.08), 273.2 (2.51) nm. IR (film) ν_{max} cm^{-1}: 1627, 1400, 829, 702. For ^{1}H and ^{13}C NMR spectral data, Table S1. HR-ESI-MS *m/z* $[M+Na]^+$ 429.2347 (calculated for $C_{23}H_{34}O_6Na$ $[M+Na]^+$, 429.2253).

Antiviral activity study of CPHP and CAP

Compounds and reagents: CPHP was isolated from *A. aureum* and CAP was purchased from Sigma-Aldrich, USA. MTT dye and, Ribavirin were purchased from Sigma-Aldrich, Australia. Prof. Mark von Itzstein, Institute for Glycomics, Griffith University kindly provided the drug BCX 2798. All the compounds are dissolved in DMSO and used immediately in the assay.

Cell lines: Cell lines (Vero, ATCC #CCL-81; LLC-MK2, ATCC#CCl-7) were purchased from ATCC, Manassas, VA 20108, USA. Vero cells were cultured in OptiMem medium containing 3% HI-FCS, whereas LLC-MK2 cells were cultured in EMEM medium containing 2% HI-FCS. All cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Viruses and antibodies: Dengue virus (DENV-2) was obtained from Dr Linda Hueston, Arbovirus Emerging Disease Unit, CIDMLS-ICPMR, Westmead Hospital, Sydney, Australia. Chikungunya virus (CHIKV) La Reunion strain was obtained from Dr Pierre Roques, CEA, Institute of Emerging Diseases and Innovative Therapies, Division of Immuno-Virology, Laboratory of Virology, France. Human parainfluenza virus (mouse monoclonal IgG anti-hPIV-3HN (Fitzgerald, clone# M02122321, 2.0 mg/ml)was obtained from Prof. Mark von Itzstein, Institute for Glycomics, Griffith University, Gold Coast, Australia. Dengue virus stocks were prepared by infection of Vero cells at a multiplicity of infection (MOI) of 0.1 and culturing for 5-7 days before harvesting the supernatant. CHIKV stock was prepared using Vero cells. 3H5 antibody for DENV-2 was obtained from TropBio Limited, Townsville, Queensland. Goat anti-mouse HRP antibody was obtained from DAKO Laboratories, Netherlands, Europe.

MTT cytotoxicity assay : The cytotoxicity of CPHP and CAP were tested against monkey kidney cells using the MTT assay according to the method described previously [20]. The IC_{50} values were calculated with probit analysis software [21]. The negative control only contained vehicle (0.75% DMSO). Cycloheximide was used as a positive control.

Viral inhibition assay: Pre- and post-infection methods were used to determine the antiviral activity of the compounds. During the pre-infection test, CPHP and CAP were pre-incubated with virus for 30-60 min and then the mixture was added to the Vero or LLC-MK2 cells. After two hours of incubation, the virus inoculums were replaced with complete growth medium and incubated for 2 days. Any inhibition seen in this assay indicated that compounds in question inhibit the virus replication by inhibiting the entry process. During the post-infection test, the cells were treated with virus for 2 h, virus inoculums removed and replaced with compounds in complete growth medium. The cells were incubated with the compounds for 2 days at 37°C. Any inhibition of virus titer indicates that the compounds inhibit the replication of virus inside the cells. Culture supernatants were collected and assayed for viral titer by fluorescent focus assay (FFA) or a plaque assay [22,23] with minor modifications. All assays for determining the virus titers were carried out in triplicate.

The effective concentration (EC_{50}) of the compounds were determined using the viral inhibition assay as described with concentrations of CPHP ranging from 500 to 7.5 µM and for CAP ranging from 100 to 6.2 µM. Antiviral activity of CPHP and CAP against DENV-2, CHIKV and hPiV3 were tested below their IC_{50} values for cytotoxicity. Viral inhibition assays were carried out in triplicates.

Fluorescent focus assay: The FFA assay was performed as per the method described by Payne et al. (2006) [21] with minor modifications. Briefly, Vero (for DENV-2) and LLC-MK2 (for hPiV3) cells were seeded in a 48-well plate at a concentration of 5×10^4 cells/well and incubated overnight at 37°C in 5% CO_2 to produce a 80-90% confluent monolayer. The viral samples were diluted appropriately in serum-free medium and added to the cell monolayer in a minimal volume. After allowing the viral adsorption by incubating for 1.5 h (DENV-2) and 1 hr (hPiV3) at 37°C with rocking the plates every 15 min, the virus inoculums were removed and replaced with 0.8% methylcellulose (DENV-2) or 1% avicell (hPiV3) overlay medium and incubated at 37°C in 5% CO_2 for 36-48 h. After 2.5 (DENV-2) and 1.5 (hPiV3) days of incubation the cells in each well were fixed for 20 min by adding 3.6% formaldehyde/PBS to each well. After fixation, the cells were washed with PBS and permeabilized with 1% Igepal for 20 min at room temperature. Cells were then washed again with PBS and incubated with primary antibody 3H5 (1:300) for DENV-2 and anti-hPiV3 (1:2000) for hPiV3 in PBS containing 4% non-fat dried milk at room temperature. The cells were incubated for 1 h in a humid chamber and washed with PBS/Tween-20 (0.02%). After washing, the cells were incubated for 1 h with Goat anti-mouse HRP secondary antibody in PBS/4% milk. A final wash of the cell was done with PBS/Tween-20 (0.02%) and then 100 µL true blue peroxidase substrate (KPL Biochemicals, USA) was added to develop the foci in the wells. The number of foci in each well was counted and expressed as FFU/mL.

Plaque assay: The plaque assay was performed as per method described by Libdury et al. [23] with minor modification to quantify the virus stain in titer for CHIKV virus. CHIKV virus stocks were prepared using Vero cells. Vero cells were infected with the virus at 0.1 MOI and harvested after 48 h and tittered by plaque assay. Briefly, Vero cells were seeded in a 24-well plate at a concentration of 1.5×10^5 cells/well and incubated overnight at 37°C in 5% CO_2 to produce a confluent monolayer. Following the cells attachment, the cells were inoculated with 2 days viral culture supernatants with a minimal volume. After allowing the viral adsorption by incubating for 1 h at 37°C with rocking the plates every 15 min, the virus inoculums were removed and replaced with 0.2% agarose overlay medium and incubated at 37°C in 5% CO_2 for 48 h. After 2 days incubation the overlay medium was removed from the cells and the cell monolayer was stained with 0.2% crystal violet in 20% ethanol. The plaques were counted and the viral titer was calculated and expressed as plaque forming units per mL (PFU/mL).

Results and Discussion

Identification of 2″-(methoxycarbonyl)-5″-methylpentyl 2′-methylhexyl phthalate (CPHP)

The aerial parts of *A. aureum* were collected from tidal forests in the coastal Sundarbans of Bangladesh and shade-dried. The dried and pulverized plant material of *A. aureum* was extracted with methanol and further fractionated with reverse-phase SPE columns for isolation of pure compounds. The novel compound, 2″-(methoxycarbonyl)-5″-methylpentyl 2′-methylhexyl phthalate (CPHP) (Figure 1) was isolated using HPLC and obtained as a whitish semi-solid (>98% pure). The structure of the compound was elucidated using 1D and 2D NMR and MS data. CPHP was obtained as a whitish semi-solid. HR-ESI-MS data showed a sodium adduct molecular ion peak at *m/z* 429.23472 $[M+Na]^+$ suggesting a molecular ion *m/z* 406.23554 and thus a molecular formula of $C_{23}H_{34}O_6$. The IR spectrum of CPHP indicated the presence of an aromatic system (1627, 1400, 829 and 702 cm^{-1}) and an ester moiety (1729 and 1262 cm^{-1}). The ^1H NMR spectrum (Table 1) indicated four aromatic protons resonating as two symmetrical 2H multiplets, at δ_H 7.70 (two protons at C-3/C-6) and δ_H 7.52 (two protons at C-4/C-5) which indicated a symmetrical substitution pattern. The presence of a doublet of doublets at δ_H 4.20 (2H, *J*=14.7, 5.7 Hz) and δ_H 4.19 ppm (2H, *J*=14.7, 6.0 Hz) indicated two oxymethylene groups adjacent to a methine group. A singlet (3H, $-OCH_3$) at δ_H 3.64 along with a methylene triplet at δ_H 2.30 (*J*=7.5 Hz) were evident as an indication of a methyl ester group on one side chain [24]. A 10 H multiplet at δ_H 1.28 - 1.64 was evident and was assigned to

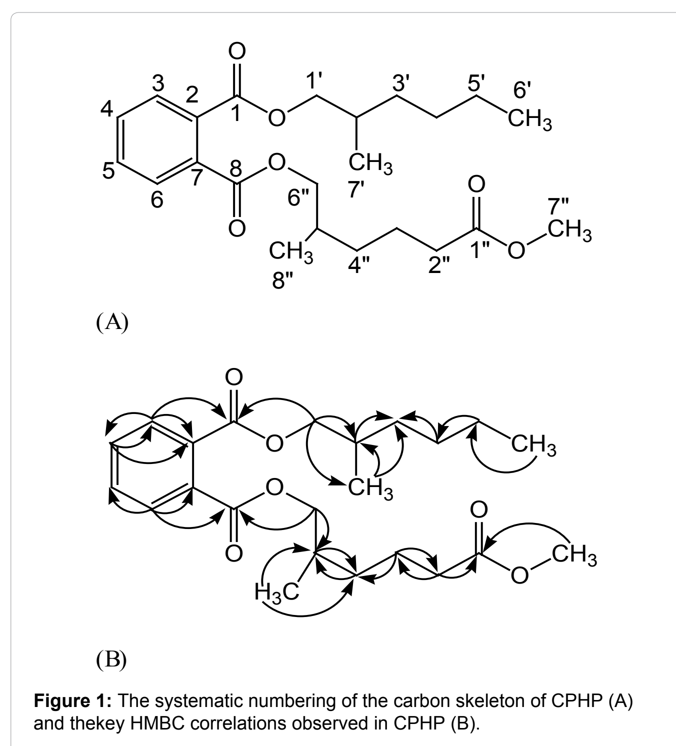

Figure 1: The systematic numbering of the carbon skeleton of CPHP (A) and thekey HMBC correlations observed in CPHP (B).

Position	δ_C	δ_H (*J*)
1/8	167.5	-
2/7	132.4	-
3/6	128.8	7.70 (2H, m)
4/5	130.9	7.52 (2H, m)
1'	68.7	4.20 (2H, dd, 14.7, 5.7)[a]
2'	38.7	1.68 (1H, m)
3'	23.7	1.44 (2H, m)
4'	31.9	1.28 (2H, m)
5'	22.7	1.32 (2H, m)
6'	14.0	0.89 (3H, t, 7.2)
7'	10.9	0.92 (3H, d, 7.2)
8'	-	-
1"	174.3	-
2"	34.1	2.30 (2H, t, 7.5)
3"	25.0	1.64 (2H, m)
4"	30.1	1.37 (2H, m)
5"	38.7	1.68 (1H, m)
6"	68.7	4.19 (2H, dd, 14.7, 6.0)[a]
7"	51.2	3.64 (3H, s)
8"	14.1	0.86 (3H, d, 7.2)

[a]Interchangeable assignment

Table 1: ^1H and ^{13}C NMR (*Jmod*) data of CPHPin $CDCl_3$ (300 MHz, δ in ppm, *J* in Hz).

5 methylenes of the side chains. Also, a 2H multiplet was observed at δ_H 1.68 and assigned as two methine protons on the two side chains. The ^{13}C *Jmod* (Table 1) and DEPT experiments confirmed that there were 17 different carbons, including two carbonyl carbons, two aromatic methines, one aromatic quaternary, four methyls, seven methylenes and one methine. Comparison of ^1H and ^{13}C NMR data of isolated compound with published data indicated that the isolated compound was a phthalic acid ester derivative [14,25]. The chirality of the C-2′ and C-5′′ centres were assigned following close inspection of the ^1H NMR data [26]. Comparison of the coupling constants for the C-7′ and C-8′′ methyls (*J*=7.2 Hz) on isolated compound with the known compound di(2*S*-methyl heptyl)-1,2-benzenedicarboxylate(*J*=6.5 Hz, *S*,*S* configuration at C-2′/C-5′′) (also commonly referred to as di(2*S*-methyl heptyl)-1,2-benzenedicarboxylate) indicated the configuration on the isolated compound (CPHP) at C-2' and C-5" was either *S*,*S* or *R*,*R* [24] (Figure 1).

In the HMBC experiment (Figure 1), the aromatic protons at δ_H 7.7 (H-3/H-6) showed cross-peaks to δ_C 132.4 (C-2/C-7), 130.9 (C-4/C-5), 167.5 (C-1/C-8), indicating the attachment of the phthalate carbonyls (C=O) to C-2 and C-7 at 132.4 ppm. Both oxymethylene protons at δ_H 4.20/4.19 (C-1′/C-6′′) showed cross-peaks to δ_C 167.5 (C-1/C-8) and δ_C 38.7 (C-2′/C-5′′), which confirmed that both the ester chains started with methylene groups and are connected with a methine group. The methoxy methyl at δ_H 3.64 (H-7′′) showed a cross-peak to δ_C 174.3 (C-1") and confirmed the presence of a methyl ester in one side chain. Whereas, the methyl at δ_H 0.89 (H-6′) showed a cross-peak to δ_C 22.7 (C-5′) indicating that the other side chain contains a terminal methyl group (Figure 1). The above long-range correlations, along with comparison to reported literature on similar compounds indicated that the isolated compound was the novel structure 2′′-(methoxycarbonyl)-5′′-methylpentyl 2′-methylhexyl phthalate, having either the *R*,*R* or *S*,*S* configuration at the 2 and 5 positions.

Antiviral activity of CPHP and CAP

CPHP was investigated for its antiviral activity against DENV-2, hPiV3 and CHIKV given its structural similarity to di(2*S*-methylheptyl)-1,2-benzenedicarboxylate, which has reported potent antiviral activity [14]. The synthetic pharmaceutical excipient CAP was also investigated due to its previously reported antiviral activities [16,17,27]. The cytotoxicity of both compoundswas established against monkey kidney cells (Vero and LLC-MK2). CPHP and CAP showed no toxicity against both cell lines Vero and LLC-MK cells, at the highest concentration tested (500 µM and 100 µM, respectively). For BCX and Ribavirin literature data suggest that these compounds are not cytotoxic at concentrations of 100 µM and 4 mM, respectively [28,29]. Thus, CPHP is no more, and potentially even less, cytotoxic than BCX2798, given that at the highest concentration tested, 500 µM, it was shown to be not cytotoxic.

Both pre- and post-infection methods were employed to evaluate antiviral activity. CPHP and CAP were not effective against any of the three viruses tested in the pre-infection model. However, CPHP reduced virus titres in Vero and LLC-MK2 cells infected with DENV-2 and hPiV3 strains in the post-infection model in a concentration-dependent manner with EC_{50} values of 113.5 and 29.4 µM, respectively (Table 2), (Figure 2). Against parainfluenza strain hPiV3 the antiviral effect of CPHP in the post-infection model was slightly higher than that observed for the positive control BCX 2798 (EC_{50} 44 µM) (Figure 3). CPHP also showed antiviral activity against CHIKV strains in the post-infection model reducing the virus titre by 73% at a concentration of 500 µM (Figure 4). However, no post-infection antiviral activity was found for CAP against both DENV-2 and hPiV3 strains at the highest concentration (100 µM) tested (Figures 2 and 3).

Given that CPHP is active against dengue virus (*Flaviviridae, flavivirus*), CHIKV (*Togaviridae, alphavirus*) and hPiV3 (*Paramyxoviridae*) in post rather than pre-infection, it suggests that the compound inhibits the replication rather than the entry process of the viruses.

Both alphavirus and flavivirus are single-stranded RNA viruses which enter into host cells via receptor mediated endocytosis. For

Compound	Effective concentration 50 % (EC_{50}) (µM)	
	Anti-DENV2 activity	Anti-hPiV3 activity
CPHP	113.5	29.4
CAP	NAA*	NAA*
Ribavirin	61	NA**
BCX 2798	NA**	44

*NAA: no antiviral activity at highest concentration tested (100 µM);
**NA: not applicable

Table 2: Antiviral activity (EC_{50}) of CPHP and CAP.

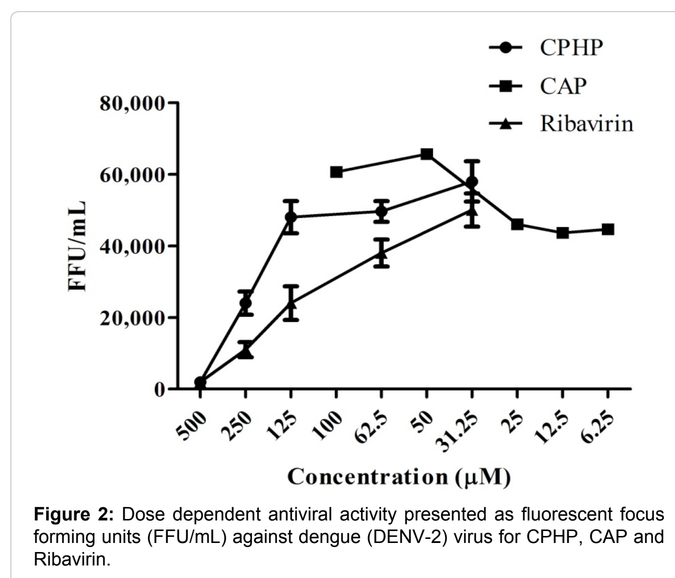

Figure 2: Dose dependent antiviral activity presented as fluorescent focus forming units (FFU/mL) against dengue (DENV-2) virus for CPHP, CAP and Ribavirin.

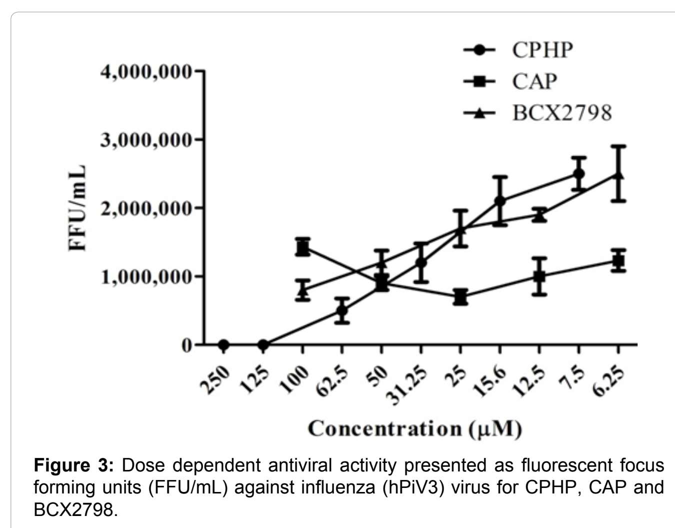

Figure 3: Dose dependent antiviral activity presented as fluorescent focus forming units (FFU/mL) against influenza (hPiV3) virus for CPHP, CAP and BCX2798.

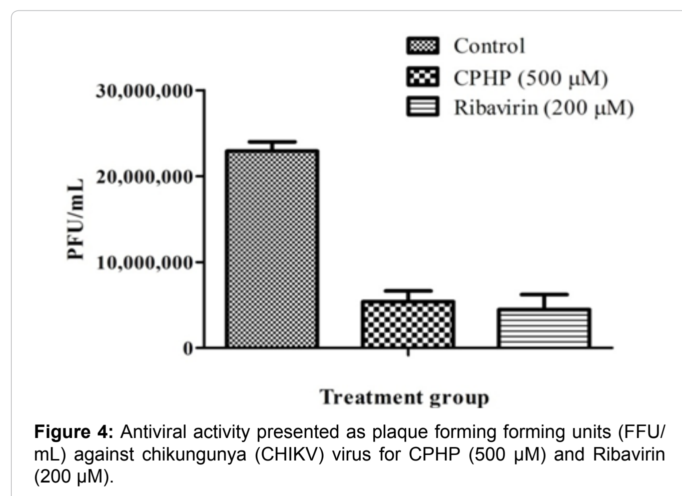

Figure 4: Antiviral activity presented as plaque forming forming units (FFU/mL) against chikungunya (CHIKV) virus for CPHP (500 µM) and Ribavirin (200 µM).

flavivirus, some short peptides compete with the regions of flaviviral E proteins which mediate the low pH-induced rearrangement of virus surface structures. Recent studies demonstrated that palmatine acts as a protease inhibitor of flavivirus whereas, ribavirin-5′-triphosphate and paclitaxel inhibited flavivirus NS3 protein-associated NTPase [30]. Neplanocin, another naturally occurring carbocyclic nucleoside, inhibits the flaviviral NS5 protein-associated methyltransferease [31]. Most alphavirus inhibitors, which inhibit virus replication, are nucleoside analogues e.g. ribavirin. Apart from anti-alphavirus nucleoside analogues, only a limited number of organic small molecules have been found to inhibit alphavirus replication. Betulin-derived compounds (lupine-type triterpene) were recently reported to inhibit alphavirus replication [32]. Another report showed natural products with a 5,7-dihydroxyflavone structure (such as apigenin, chrysin, naringenin and silybin) inhibit CHIKV replication rather than virus entry [33].

CAP binds to the envelope glycoprotein gp120 thus blocking and diminishing its activity [34]. Entry of enveloped viruses into their host cells requires fusion of the viral and cellular membranes via viral envelope glycoprotein [35]. Envelope glycoprotein GP120 (gp120) is a unique glycoprotein exposed on the surface of HIV and essential for virus entry [36]. The protein gp120 is specific for HIV. Although alpha- and flaviviruses express envelope glycoproteins, they are not the same as HIV gp120 [37]. Not surprising therefore, in our study CAP has no appreciable antiviral activity for DENV-2 and hPiV3 viruses.

Infection by parainfluenza virus requires the hemagglutinin-neuraminidase (HN) protein, a major surface glycoprotein containing haemagglutinin (HA) and neuraminidase (NA) activity. HN facilitates cell attachment to host cells, promotes virus spreading and the activity of fusion protein thereby allowing the virus to attach and penetrate cell surfaces [37]. BCX 2798 is a 4-azido compound that was designed based on the 3D structure of the HN protein. It is reported that BCX 2798 has potent anti-hPiV3 activity by inhibiting HA and NA activities, with an EC_{50} of 4.8 µM and 20 µM, respectively [28]. In our study, CPHP showed slightly higher activity than BCX 2798 against hPiV3 virus with EC_{50} values of 29.4 µM and 44 µM, respectively (Figure 2). In comparison, recent reports identified a small molecule which selectively inhibits influenza A and B virus (IC_{50} 12.5 µM) through PA-PB1 interaction (RNA polymerase protein) [38] and another compound with an IC_{50} of 3-14 µM, which is active against different influenza virus strains and acts through the induction of IFNβ [39].

There exist previous research reports exclusively on the antiviral activity of phthalates against DNA viruses. For example, bis (2-methylheptyl) phthalate, isolated from the plant species *Pongamiapinnata*, is active against the DNA virus WSSV [14,15] and similarly CAP was reported active against HSV and cytomegalovirus, both DNA viruses [18,19]. Thus, this study is the first to report on antiviral activity of phthalates against RNA(+) and (-) viruses. Further studies are needed to elucidate the mechanism of action for the antiviral activity of some phthalates and their Structure-Activity-Relationship (SAR).

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Conflict of Interest

The authors have no conflict of interest.

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