Antiviral Research 80 (2008) 135–142

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

In vitro inhibition of dengue virus entry by *p*-sulfoxy-cinnamic acid and structurally related combinatorial chemistries

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ARTICLE INFO

Article history:
Received 15 December 2007
Accepted 17 May 2008

Keywords:
Dengue
Anti-adhesion
Adhesion
Zosteric acid
Anti-viral
Entry

ABSTRACT

The anti-adhesive compound *p*-sulfoxy-cinnamic acid, zosteric acid (ZA), is derived from the temperate marine eelgrass, *Zostera marina*. ZA and five combinatorial chemistries based on ZA were evaluated for their anti-viral properties against dengue virus in a focus forming unit reduction assay. None of the compounds showed evidence of toxicity to the monkey kidney cell line LLCMK-2 over the concentration ranges tested. ZA showed a modest IC_{50} of approximately 2.3 mM against DENV-2. Three other compounds showed IC_{50} values of 2.5, 2.4, 0.3 mM, with a fourth not achieving a 50% inhibitory concentration against DENV-2. The most active compound, CF 238, showed IC_{50} values of 24, 46, 14 and 47 μM against DENV-1, DENV-2, DENV-3 and DENV-4, respectively. CF 238 showed evidence of inhibition at an entry step in the viral life cycle and enhanced virus:cell binding as evidenced by a quantitative RT-PCR assay system. CF 238 may promote inappropriate virus:cell attachments common to all DENV strains that interfere with receptor interactions required for viral entry. These and other related chemistries may be useful as reagents for studying DENV entry, capturing and detecting DENV, and development of pharmaceuticals.

1. Introduction

Biological attachments are ubiquitous and required for the existence of all multicellular life. However, these attachments are also used by parasites and pathogens. The impact of detrimental biological adhesion is broad and involves interactions at multiple levels, including macroscopic encrustation, biofilm formation, and microscopic pathogen–host recognition. As a result, organisms have evolved strategies to defend against harmful biological interactions. The temperate marine eelgrass, *Zostera marina*, produces an anti-adhesive chemical, *p*-sulfoxy-cinnamic acid, zosteric acid (ZA), that inhibits colonization of the leaf surfaces by encrusting bryozoans and other organisms (Todd et al., 1993). The mechanism of activity is thought to be mediated by binding to, or coating, the encrusting organisms, and subsequent release of the ZA and the organism from the leaf surface (Todd et al., 1993). In support of this, solutions of free ZA have been shown to have anti-fouling and anti-adhesion activities against algae, fungal spores and bacteria (Todd et al., 1993; Callow and Callow, 1998; Stanley et al., 2002; Xu et al., 2005a,b). Several groups have also reported anti-adhesive effects against crustacean larvae (barnacles), mollusks, algae, fungal spores, and bacteria by incorporation of ZA into slow-release surface coatings (Haslbeck et al., 1996; Shin et al., 2001; Hany et al., 2004; Barrios et al., 2005; Newby et al., 2006). This wide range of anti-adhesion activity displayed by ZA against such a variety of different organisms suggests a mechanism targeting chemical interactions that are highly conserved in many biological attachment processes.

Due to its anti-adhesive properties and low toxicity, ZA and other natural product anti-foulant coatings are being evaluated as replacements for highly toxic coatings currently used on ship hulls (Haslbeck et al., 1996; Callow and Callow, 1998; Shin et al., 2001; Hany et al., 2004; Barrios et al., 2005; Xu et al., 2005a,b; Newby et al., 2006), and as novel crop protection sprays to prevent adherence of pathogenic fungi (Stanley et al., 2002). These

Abbreviations: DENV, dengue virus; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; FFU, focus forming unit; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; ZA, zosteric acid, *p*-sulfoxy-cinnamic acid.

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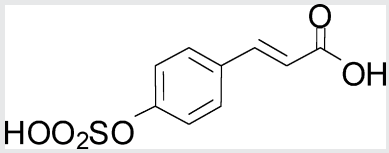
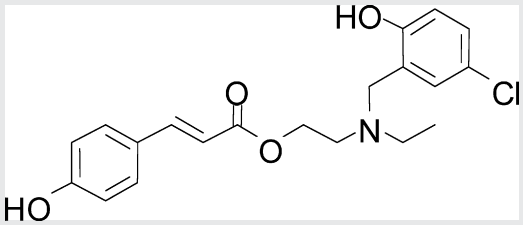
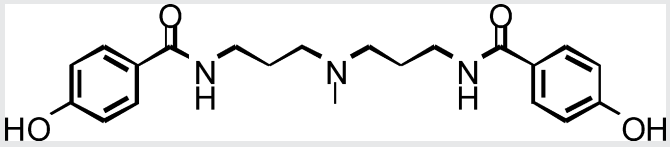
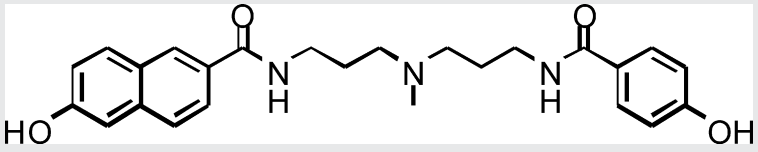
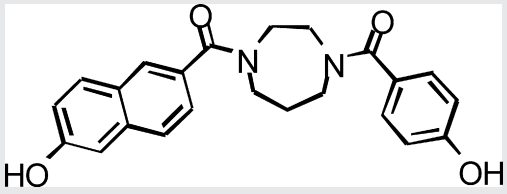
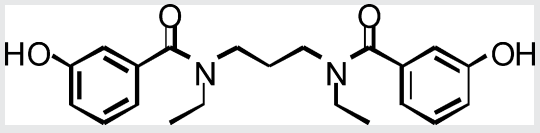
doi:10.1016/j.antiviral.2008.05.007

Table 1
Structures of chemistries with IC_{50} values calculated from straight line extrapolations

Name	Chemical structure	$IC_{50} \pm$ S.E.M. (μM)
ZA		2380 ± 150
CF 238		24 ± 6, D-1 46 ± 4, D-2 14 ± 2, D-3 47 ± 5, D-4
CF 285		2516 ± 172
CF 290		294 ± 42
CF 296		N/A
CF 490		2378 ± 192

IC_{50} values are against DENV-2 unless otherwise indicated.

economic interests have led to the design and synthesis of novel, dimeric chemistries designed from ZA with two symmetrical or non-symmetrical phenolic groups, different length linkers, and modifications to the functional groups (see Table 1). Based on the possibility that viruses utilize interactions similar to other biological adhesives as they target new host cells for infection, we hypothesized that ZA or related chemistries may possess antiviral activities. Viruses are structurally much simpler than cellular microorganisms and, as such, may present useful systems to examine the interactions of ZA and other anti-fouling chemistries with biologically relevant surface molecules. Here, we present results demonstrating the inhibitory activity of ZA, and selected combinatorial chemistries based on ZA, against dengue viruses (DENVs) in cell culture systems.

Together, the four strains of DENV comprise the most common human arboviral infection and the most important public health threat from mosquito-borne viral pathogens, causing an estimated 50 million human infections per year (Mackenzie et al., 2004). Infection with any of the four DENV strains can cause variable outcomes, ranging from inapparent symptoms, to dengue fever, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Cross-reactive, but non-neutralizing antibodies can mediate entry of DENV into macrophages, dendritic cells and other viral target cells via Fc receptors, increasing virus titers and thus pathology, a phenomenon known as antibody-dependent enhancement (ADE). Because of this, infection with one DENV strain can increase the risk of DHF or DSS when the individual is subsequently exposed to a different serotype (Halstead, 1988). This has complicated the development of vaccine strategies. With no approved vaccine or specific therapy for prevention or treatment, the development of inhibitors with activity against all four strains of DENV is an important goal.

We show here that ZA and two other chemistries inhibit DENV-2 with 50% inhibitory concentration (IC_{50}) in the 2 mM range, another compound inhibits in the 300 μM range, and the most active compound shows IC_{50} values in the range of 14–47 μM against all of

the four strains of DENV. We show that this most active compound functions at an early entry step in the viral life cycle, prior to internalization and fusion, but that it does not prevent virion binding to the target host cell. This represents the first demonstration of an anti-viral effect of ZA and combinatorial chemistries based on ZA.

2. Materials and methods

2.1. Chemistries

Five novel chemistries related to ZA were designed and provided by CernoFina, LLC (Portland, ME) employing a combinatorial approach using phenol or naphthol rings in a symmetric or non-symmetric fashion attached together with amine-containing, variable linker regions (see Table 1) (Alberte and Smith, 2005). ZA was provided as the sodium salt and was dissolved in DMEM or PBS and further buffered with 10 mM HEPES pH 7.2. The other chemistries were dissolved in DMSO and diluted into PBS or DMEM to a final concentration of 1% (v/v) DMSO. The CF 285 solutions were also further buffered with 10 mM HEPES pH 7.2.

2.2. Viruses and cells

DENV-1 strain HI-1, DENV-2 strain NG-2, DENV-3 strain H-78, and DENV-4 strain H-42 were obtained from R. Tesh at the World Health Organization Arbovirus Reference Laboratory at the University of Texas at Galveston. Viruses were propagated in the African green monkey kidney epithelial cell line, LLCMK-2, a gift of K. Olsen at Colorado State University. LLCMK-2 cells were grown in DMEM with 10% (v/v) FBS, 2 mM Glutamax, 100 U/ml penicillin G, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B, at 37 °C with 5% (v/v) CO_2.

2.3. Focus forming unit (FFU) reduction assay

A modified version of the FFU reduction assay described by Hrobowski et al. (2005) was used. Twenty-four hours prior to use, LLCMK-2 target cells were seeded at a density of 1×10^5 cells in each well of a six-well plate. Approximately 200 FFU of virus were incubated with or without chemistries in serum-free DMEM for 1 h at r.t. Virus/chemistry or virus/control mixtures were allowed to infect confluent target cell monolayers for 1 h at 37 °C, with rocking every 15 m, after which time the medium was aspirated and overlaid with fresh DMEM/10% (v/v) FBS containing 0.85% (w/v) Sea-Plaque Agarose (Cambrex Bio Science, Rockland, ME). Cells with agarose overlays were incubated at 4 °C for 20 m to set the agar. Infected cells were then incubated at 37 °C with 5% CO_2 for 3 days (DENV-1, -3 and -4) or 5 days (DENV-2). Infected cultures were fixed with neutral buffered 10% formalin (Formalde-Fresh, Fisher Scientific, Waltham, MA) overnight at 4 °C, permeabilized with 70% (v/v) ethanol for 20 m at r.t., and rinsed with PBS prior to immunostaining. Virus foci were detected using supernatant from mouse anti-DENV hybridoma E60 (obtained from M. Diamond at Washington University) followed by horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Pierce, Rockford, IL) and developed using AEC chromogen substrate (Dako, Carpinteria, CA). Results were expressed as the average of at least two independent trials with three replicates in each trial.

2.4. Cytotoxicity assay

The cytotoxicity of the chemistries was measured by monitoring mitochondrial reductase activity using the TACS™ MTT cell proliferation assay (R&D Systems Inc., Minneapolis, MN) according to the manufacturer's instructions. Dilutions of chemistries in serum-free DMEM were added to confluent monolayers of LLCMK-2 cells in 96-well plates for 1 h at 37 °C, similar to the focus forming inhibition assays, and incubated at 37 °C with 5% (v/v) CO_2 for 24 h. Absorbance at 560 nm was measured using a Tecan GeniosPro plate reader (Tecan US, Durham, NC).

2.5. Mechanistic assays with CF 238

2.5.1. Post-entry focus-forming assay with CF 238 against DENV-2

To determine if the observed inhibitory effect was due to interference with post-entry steps in the viral life cycle, approximately 200 FFU of DENV-2 without CF 238 was allowed to bind and enter target cells for 1 h at 37 °C as described for the focus-forming assay. Unbound virus was then removed by rinsing with PBS and CF 238 was added to the cells post-entry for 1 h at 37 °C. Cultures were washed again in PBS and agarose overlays, incubation, and immunological detection was conducted as described for the focus-forming assay.

2.5.2. Pre-binding focus-forming assay with CF 238 against DENV-2

To determine if the observed inhibitory effect was due to interference caused by modifications to the target cell surface, CF 238 was incubated with the target cells for 1 h at 37 °C, the cells were rinsed with PBS, and approximately 200 FFU of DENV-2 was allowed to infect the cells for 1 h at 37 °C, and then rinsed with PBS. Agarose overlays, incubation, and immunological detection were conducted as described for the focus-forming assay.

2.5.3. qRT-PCR virus binding assay

Infection of LLCMK-2 target cells in six-well plates was performed in duplicate using 10^5 FFU of DENV-2 that had been pre-incubated for 45 m at 37 °C with CF 238 or pooled heterotypic anti-DENV human serum. After a 45 m infection, infected monolayers were washed with PBS and harvested with a cell scraper, added to a 1.5 ml microfuge tube containing 350 μl of AR-200 silicone oil (Sigma–Aldrich, St. Louis, MO) mixed with 150 μl of silicone fluid (Thomas, Swedesboro, NJ), and spun at 18,000 × *g* in a microfuge for 1 m to separate the unbound virus from the cell-bound virus in the pellets. The tubes were then submerged in liquid nitrogen for 30 s to freeze the contents. The cell pellets with bound virus were recovered by clipping off the bottoms of the tubes with small wire clippers into 15 ml conical tubes. Viral RNA was extracted from the cell pellets using the Qiagen Viral RNA Extraction kit (Qiagen Inc., Chatsworth, CA). Quantitative real time reverse transcription PCR (qRT-PCR) was performed on the extracted RNA using the Quantitect Sybr Green RT-PCR kit (Qiagen Inc., Chatsworth, CA), following the manufacturer's specifications and amplification protocols, using dengue-specific primers: (Den2F: catatgggtggaatctagtacg; Den2R: catatgggtggaatctagtacg). Each reaction was performed in 20 μl total volume (10 μl 2× SYBR green master mix, 0.5 μl of 10 μM of each primer, 0.2 μl reverse transcriptase, and 5 μl viral RNA) using a Lightcycler thermal cycler (Roche Diagnostics, Carlsbad, CA), and according to the following amplification protocol: 50 °C for 20 min to reverse transcribe the RNA; 95 °C for 15 min to activate the HotStart Taq DNA Polymerase; 45 PCR cycles: 94 °C for 15 s, 50 °C for 15 s, 72 °C for 30 s, the last step was also the fluorescence data acquisition step. Melting curve analysis was performed by a slow increase in temperature (0.1 °C/s) up to 95 °C. The threshold cycle, representing the number of cycles at which the fluorescence of the amplified product was significantly above background, was calculated using Lightcycler 5.3.2 software (Roche).

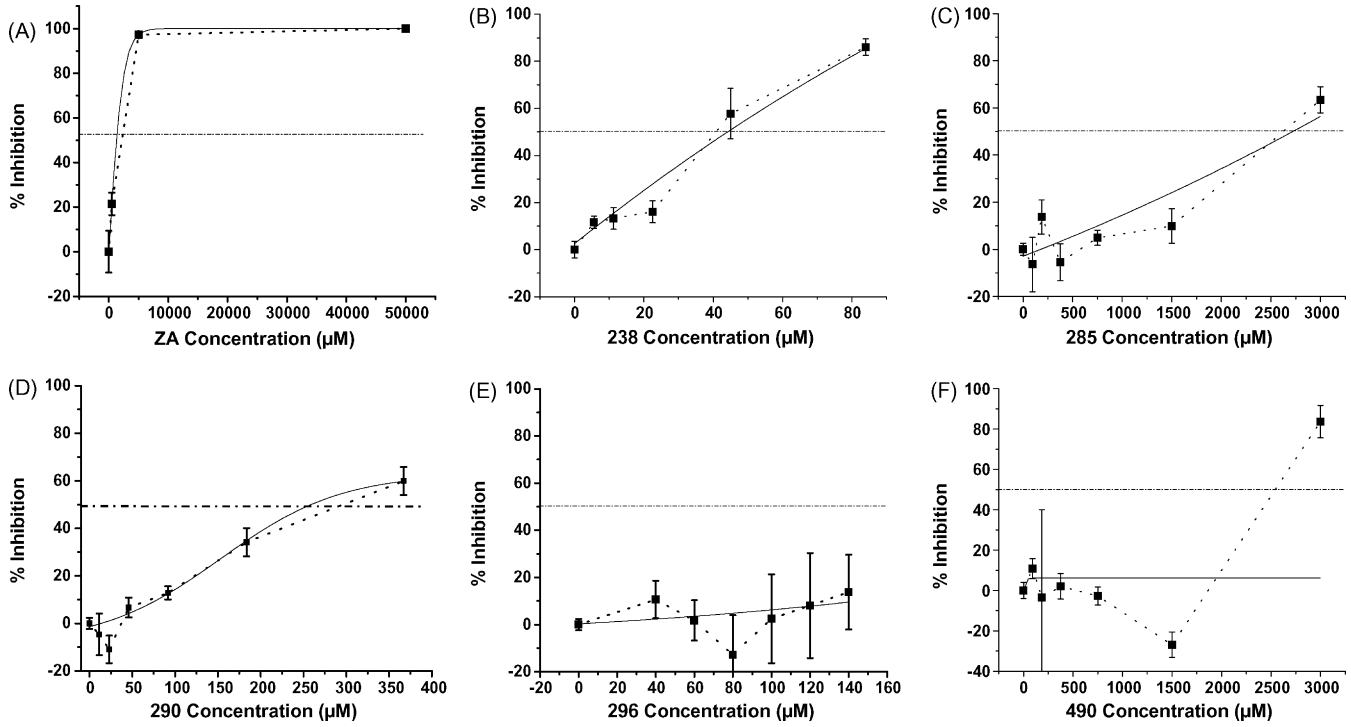


Fig. 1. Dose response inhibition graphs for ZA, CF 238, CF 285, CF 290, CF 296, and CF 490 against DENV-2 in focus-forming assays. The means of at least six trials are shown, ±S.E.M. Best fit sigmoidal curves are shown as solid lines. A dot-dash line is also shown at 50% inhibition for each compound. In these experiments, the compounds were co-incubated with virus prior to infection. ZA, CF 285, and CF 490 show IC_{50} values above 1 mM. CF 290 shows an IC_{50} value in the 300 μM range and CF 238 is the most active with an IC_{50} of approximately 50 μM.

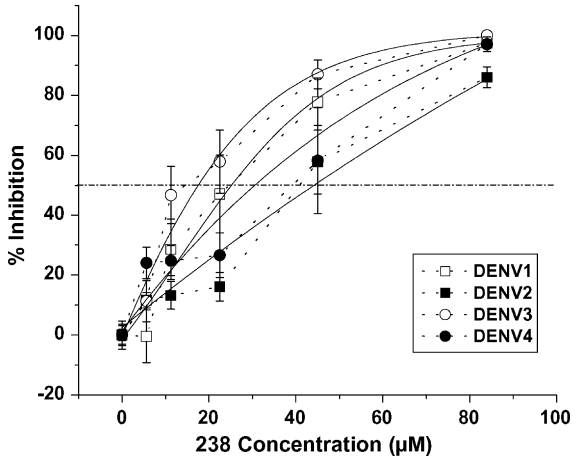


Fig. 2. Dose response inhibition graphs for CF 238 against DENV-1, -2, -3 and -4 in focus-forming assays. The means of at least six trials are shown, ±S.E.M. Best fit sigmoidal curves are shown as solid lines. A dot-dash line is shown at 50% inhibition. In these experiments, CF 238 was co-incubated with virus prior to infection.

2.6. Analysis

Figures, including sigmoidal curve fits for Figs. 1 and 2, were generated using Origin 6.0 graphing software (Northampton, MA). IC_{50} values were calculated from straight line extrapolations between the two closest concentration points on either side of the IC_{50}. Statistical analyses were performed as described using GraphPad Prism 4.0 software package (GraphPad Software, San Diego, CA) or KaleidaGraph (Synergy, Reading, PA). *p* values less than 0.05 were considered significant.

3. Results

3.1. Inhibition assays with different chemistries against DENV-2

Focus-forming assays were used to quantitate the inhibitory activities of each chemistry against DENV-2 as previously described (Hrobowski et al., 2005). Dose response data were generated over concentration ranges dictated by the solubilities of the chemistries in 1% DMSO/aqueous solution (Fig. 1). Control 1% DMSO/DMEM solutions showed no DENV inhibitory activity in this assay system (data not shown). Sigmoidal dose response curves fit the data well for all compounds, with the exception of CF 490. The natural product, ZA showed a dose response inhibition with an IC_{50} (±S.E.M.) of 2380 ± 150 μM, and at 5000 μM ZA showed 99% inhibition. CF 285 showed inhibition with an IC_{50} similar to ZA (2516 ± 172 μM) and a maximum inhibition of 63% at 3000 μM. Another compound, CF 290, showed inhibition with an IC_{50} of 294 ± 42 μM and a maximum inhibition of 60% at 367 μM. Although the data for CF 490 was not fit well using a sigmoidal dose response curve, this compound did show inhibition at the highest concentration tested, with an IC_{50} of 2378 ± 192 μM and a maximum inhibition of 84% at 3000 μM. The most active chemistry, CF 238, showed an IC_{50} of 46 ± 4 μM and achieved 86% inhibition at 84 μM. CF 296 did not show any evidence of dose-dependent inhibition against DENV-2. See Table 1 for a summary of structures and IC_{50} results.

3.2. Inhibition assays with CF 238 against DENV-1, -3 and -4

Dose response inhibition curves were generated for the most active chemistry, CF 238, against the other three strains of dengue virus, resulting in similar overall inhibitory effects against all four strains of dengue virus (Fig. 2). CF 238 showed IC_{50} values of 24 ± 6,

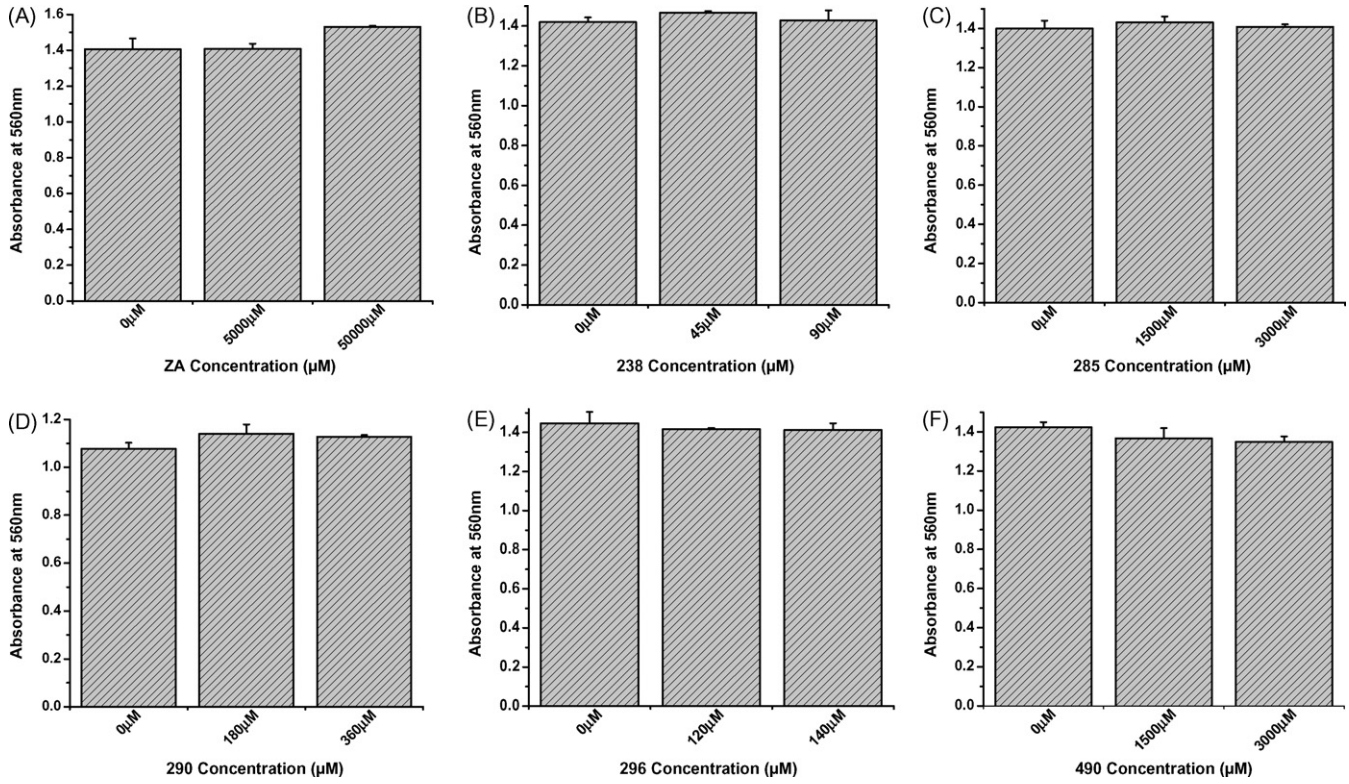


Fig. 3. MTT mitochondrial reductase toxicity assays. The means of three replicates are shown, ±S.D. Treatment of LLCMK-2 cells with ZA, CF 238, CF 285, CF 290, CF 296, or CF 490 showed no statistically significant decrease in mitochondrial reductase activity compared to media only controls, indicating that these compounds are not toxic to the target cells over the range of conditions used in the inhibition assays.

14 ± 2 and 47 ± 5 μM against DENV-1, -3 and -4, respectively. CF 238 showed consistent high-level inhibition, between 86 and 100%, of all dengue serotypes at 84 μM (Fig. 2).

3.3. Cytotoxicity

To determine if the observed DENV inhibition effects were due to cellular toxicity that impacted viral replication, the effect of each chemistry on the mitochondrial reductase activity of the target cells was measured over the concentration ranges that showed viral inhibition. In confluent cell monolayers that replicated the conditions in the focus-forming assays we observed no sign of toxicity with any compound compared to medium only controls ($p > 0.05$, ANOVA with Dunnett's post hoc test) (Fig. 3). Similarly, 1% DMSO controls have been previously shown to be not toxic in this cell line (Hrobowski et al., 2005). Medium only (no cells) gave a background of ~0.2 absorbance units.

3.4. Mechanistic assays

To investigate the mechanism of action of the inhibitory activity of the most active compound, we carried out a series of assays designed to identify the stage at which CF 238 exerts its effects against DENV-2.

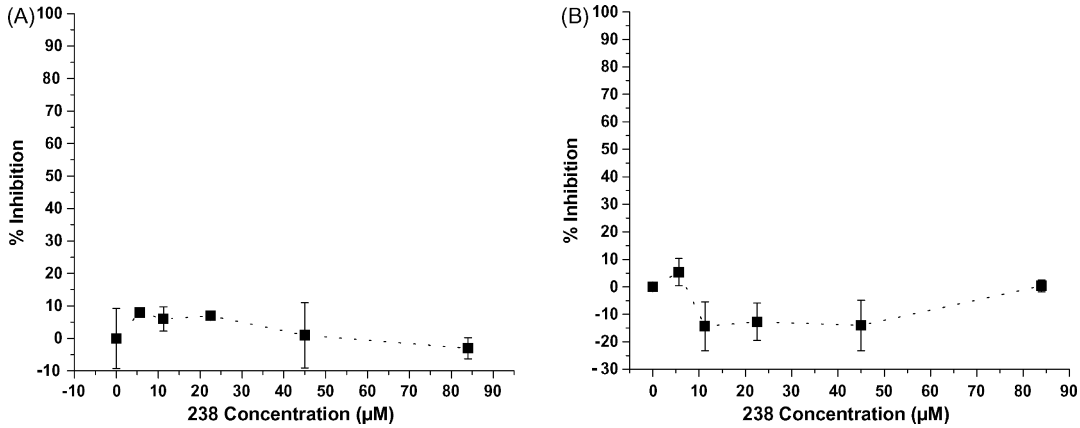


Fig. 4. Dose response inhibition graphs for CF 238 against DENV-2 in pre-binding (A) and post-entry (B) focus-forming assays. The means of at least six trials are shown, ±S.E.M. In the pre-binding assay, CF 238 was pre-incubated with the target cells and then rinsed prior to infection. In the post-entry assay, CF 238 was incubated with target cells after they had already been infected. Neither assay showed evidence of inhibition, indicating that CF 238 does not function when added only to the target cells, and that it does not function at a post-entry step in the virus life cycle.

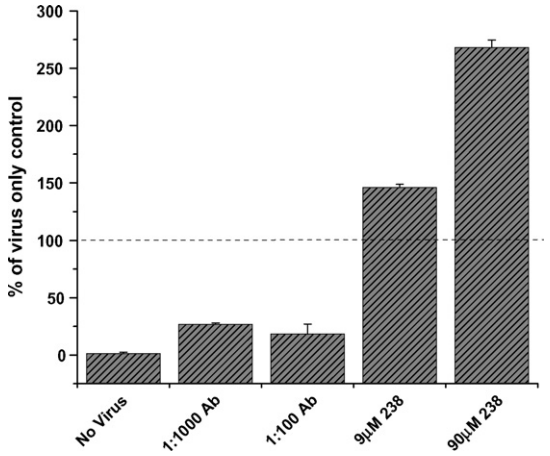


Fig. 5. qRT-PCR binding assay of DENV-2 to target cells. In this assay, virus was incubated with CF 238 or pooled anti-DEV antibody, allowed to bind to target cells, then separated through an oil-based gradient to remove unbound virus from the cells. Virus that remained bound to cells was quantified by qRT-PCR. Data are normalized to the virus only positive control as 100% binding (indicated by the dotted line). The means of three trials are shown, ±S.D.

3.4.1. Post-entry focus-forming assay with CF 238 against DENV-2

In this assay, CF 238 was added to target cells that had already been infected for 1 h with DENV-2 in order to determine if CF 238 functions during an entry or a post-entry step in the virus life cycle. Treatment of DENV-2 infected cells with CF 238 after viral entry resulted in no evidence of inhibition (Fig. 4). This indicates that CF 238 inhibits an entry step as opposed to a post-entry step.

3.4.2. Pre-binding focus-forming assay with CF 238 against DENV-2

In this assay, CF 238 was added to target cells for 1 h prior to infection with DENV-2 to determine if CF 238 inhibits entry through interaction directly with the target cells. Treatment of target cells with CF 238 prior to DENV-2 infection resulted in no evidence of inhibition (Fig. 4), indicating that CF 238 does not function by interacting with or modifying the target cell surface, and must be present along with the virus in order to inhibit entry.

3.4.3. qRT-PCR virus binding assay with CF 238 against DENV-2

In order to directly test if CF 238 interferes with virus binding to target cells, binding assays were conducted using qRT-PCR to monitor attachment of virus to target cells (Hrobowski, 2006). In these experiments, virus was co-incubated with CF 238 and used to infect target cells. The cells were then scraped off the plates and centrifuged through an oil mixture with a density that allowed passage of the cells, but not free virus, to the bottom of the tube. RNA was then extracted from the cell pellets and amplified with DENV-2-specific primers. Pre-incubation of DENV-2 with CF 238 increased virus binding in a dose-dependent manner, as measured by the qRT-PCR signal, whereas pre-incubation of DENV-2 with pooled human heterotypic anti-DENV-2 serum resulted in a dose-dependent decrease in the attachment of virus to target cells (Fig. 5). This indicates that CF 238 enhanced virus binding/attachment to target cells under the experimental condition tested.

4. Discussion

All organisms with a requirement for biological adhesion in their life cycles must identify and interact with target surfaces and actively distinguish between relevant surfaces and both biological and non-biological, non-relevant surfaces. Investigation of virus binding and entry events has led to a generalized multi-step model of "adhesion strengthening", where initial low affinity, high abundance interactions are followed by high affinity, low abundance, specific interactions that lead to target cell entry (Haywood, 1994). Some well-characterized examples include the initial interaction of herpes simplex virus with cell surface heparan sulfate (Williams and Straus, 1997) and reovirus and influenza virus with sialic acid (Weis et al., 1988; Barton et al., 2001). DENVs show a similar multi-step process during infection, using interactions with heparan sulfate on mammalian target cells for attachment (Chen et al., 1996; Hung et al., 1999; Thaisomboonsuk et al., 2005), although other carbohydrates may also be utilized in certain cells (Aoki et al., 2006), and infection in insect cells may occur by direct binding to a proteinacious receptor, bypassing interactions with heparin (Salas-Benito and del Angel, 1997; Hung et al., 2004). Direct DENV interactions with secondary protein receptors are apparently diverse between different mammalian cell lines (Marianneau et al., 1996; Ramos-Castaneda et al., 1997; Diamond et al., 2000; Bielefeldt-Ohmann et al., 2001; Martinez-Barragan and del Angel, 2001; Tassaneetrithep et al., 2003; Wei et al., 2003; Jindadamrongwech et al., 2004; Reyes-Del Valle et al., 2005; Thaisomboonsuk et al., 2005; Thepparit and Smith, 2004), between DENV types (Diamond et al., 2000; Bielefeldt-Ohmann et al., 2001), and between different DENV isolates within the same strain (Diamond et al., 2000). It is likely that DENV enters target cells by a multi-step binding/recognition mechanism potentially using several different carbohydrate and proteinacious receptors, perhaps in a redundant fashion that may differ between different cell types and DENV strains. Despite the diversity in receptor use, the DENV entry pathway has been identified as a promising target for the development of anti-virals (Dinglasan and Jacobs-Lorena, 2005).

Here we have identified the anti-DENV activity of the sea grass-derived natural product, ZA, and several other novel chemistries designed from ZA. The anti-DENV activities of ZA and two of the combinatorial compounds are modest, with IC_{50} values of approximately 2 mM. We initially expected that the sulfoxy group on ZA might play an important role in DENV inhibition, by analogy with heparan sulfate and other sulfated polysaccharides as DENV entry factors and entry inhibitors (Chen et al., 1996; Hung et al., 1999; Talarico et al., 2005; Thaisomboonsuk et al., 2005). However, two other compounds without sulfoxy groups, CF 290 and CF 238, are substantially more inhibitory than ZA, with IC_{50} values of 294 and 46 μM against DENV-2, respectively. The highest concentrations tested were constrained by the aqueous solubility of the compounds and none of the compounds were toxic to cultures of target epithelial cells over the range of concentrations where viral inhibition was observed. CF 238 also showed similar inhibitory activity against the other three DENV types with IC_{50} values between 14 and 47 μM.

Post-infection treatment of cells with CF 238 does not produce inhibition, indicating that CF 238 inhibits DENV at a viral entry step as opposed to a later step in replication. CF 238 also does not inhibit virus infection when pre-incubated with target cells, indicating an activity that is dependent on interaction with the virions. qRT-PCR analysis of virus:cell binding shows that CF 238 does not interfere with virus binding to target cells, but instead enhances virus:cell binding. This result is curious because one would expect that preventing virus:cell binding would cause inhibition and that promoting virus:cell binding would cause increased infection. This is clearly not the case here, where we observe inhibition of infection associated with enhanced virus:cell binding. Based on this, we hypothesize that in order to initiate a productive infection, viruses must bind to target cells in a permissive

manner and that alternative, non-permissive binding modes exist. We speculate that CF 238 may therefore function by tethering or trapping the virus in some inappropriate manner on the target cell surface (Alberte and Smith, 2005). This may include causing the virus to attach to the cells in such a way that it no longer has access to primary or secondary receptor molecules that are required for productive entry. These chemistries may thus be useful reagents for probing the interactions between DENV and entry receptor molecules. However, it is also possible that CF 238 may inhibit entry by interfering with some step in the fusion process as has been proposed for some DENV inhibitory peptides (Hrobowski et al., 2005), although other explanations are possible. A mechanism of action where CF 238 interferes with entry by promoting inappropriate virus:cell contacts would lend support to the hypothesis that these compounds function through binding to attachment domains on adherent organisms and subsequent release from the protected surface. There are interesting structural similarities between several of the compounds used in this study and the modified amino acid 3,4-dihydroxyphenyl-L-alanine, responsible for the adhesion characteristics in mussel adhesive proteins and related synthetic mimics (Dalsin et al., 2003; Lee et al., 2006). Adhesion and anti-adhesion properties are likely to be related functions distinguished by relative affinities for the two potential binding surfaces as well as whether the active chemistries are surface tethered or diffusible from the surface. Some dimeric (or multimeric) chemistries, like those studied here, may have both single surface adherent and multi-surface tethering activities. These chemistries might then be useful in certain tethered configurations for trapping pathogens to make them non-infectious or for detection purposes. In this regard, CF 238 may also be a useful reagent for the study of DENV entry mechanisms because it may prevent interactions with virus receptors. Further experiments with other combinatorial chemistries may identify molecules with even greater inhibitory activities against DENV or other viruses.

Acknowledgements

We would like to thank R.F. Garry for the virus:cell binding assay system, R.S. Alberte and M.W. Mittelman for helpful discussions, B. Roschek Jr. and K.B. Williams for technical assistance, and M.S. Diamond, K.E. Olsen, and R.B. Tesh for generously providing virus and cell stocks. Funding for this project was provided by CernoFina, LLC and DoD awards N00173-06-1-G901 and N00173-07-1-G015.

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