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Bis(benzofuran–thiazolidinone)s and bis(benzofuran–thiazinanone)s as inhibiting agents for chikungunya virus

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ABSTRACT

There are currently still no approved antiviral drugs to treat or prevent chikungunya virus (CHIKV) infections despite the fact that this arbovirus continues to cause outbreaks in Africa, Asia, and South- and Central-America. Thus 20 new conjugated compounds in the families of bis(benzofuran–1,3-thiazolidin-4-one)s and bis(benzofuran–1,3-thiazinan-4-one)s were designed based on the structural features of suramin. These new compounds were synthesized by chemical methods and their structures were confirmed spectroscopically. In CPE reduction assays, six of these new bis-conjugates inhibited CHIKV replication in Vero E6 cells with EC_{50} in the range of 1.9–2.7 μM and selectivity index values of ~75 or higher. These results and compounds provide a starting point for further optimization, design, and synthesis of new antiviral agents for this (re)emerging disease.

1. Introduction

Chikungunya virus (CHIKV) is an arthrogenic alphavirus, which has infected millions of people since its re-emergence in 2005, when it caused large outbreaks in Asia and Africa. In 2013, CHIKV emerged in the Caribbean (Weaver, 2014; Weaver and Lecuit, 2015) and started another massive outbreak, which has thus far resulted in >1.2 million cases in the Americas alone. At the moment there are no approved vaccines or specific antiviral drugs to prevent or treat chikungunya disease (Hwu et al., 2017). Several molecules with inhibitory effects on CHIKV in cell culture, and in some cases in animal/mouse models, have been reported (Kuo et al., 2016), but none of them has advanced into clinical trials (Abdelnabi et al., 2017).

Our laboratories have previously developed several new compounds with activity against CHIKV (Hwu et al., 2015; Albulescu et al., 2015). The first class of these compounds consists of uracil–coumarin–arene conjugates and the second class concerns suramin (**1**) and its derivatives. Suramin inhibits CHIKV replication through multiple mechanisms (Kuo et al., 2016; Albulescu et al., 2015), mainly by interfering with an early step of the replication cycle, but with (minor) additional effects on later steps, like RNA synthesis. Recently, suramin has also been reported to inhibit Zika virus replication by interfering with virus attachment and release of infectious particles (Albulescu et al., 2017).

Suramin is a symmetric molecule with a urea (NH–CO–NH) group in the center as the “neck”, two benzamido moieties as the “shoulder”, two methylbenzamido moieties as the “arms”, two

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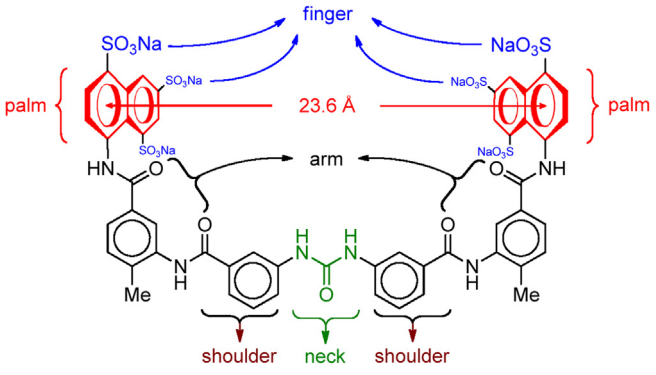
naphthalene rings as the “palms”, and six sulfonate groups as the “fingers”. A tetra-sulfonate derivative of suramin that lacks two sulfonate groups is less active against CHIKV compared with suramin (Albulescu et al., 2015), although it inhibits human and murine norovirus RNA-dependent RNA polymerase (RdRp) activity *in vitro* with an IC_{50} of 28 nM (Croci et al., 2014). Of a series of eleven suramin-related analogs that were tested for their ability to inhibit CHIKV RNA synthesis *in vitro* and to inhibit CHIKV replication in cell culture, only three exhibited inhibitory activity. All of these had features very similar to suramin (Albulescu et al., 2015).

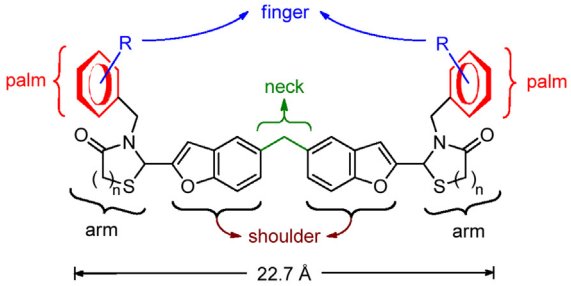
Several other synthetic compounds with a dimeric structure similar to suramin also show significant biological activities. Prominent examples include atracurium besilate (Hughes, 1986), cisatracurium besilate (Bryson and Faulds, 1997), cromoglicic acid (Penumutchu et al., 2014), daclatasvir (Press release, 2015), ombitasvir (Press release, 2014), and pentamidine (Nguewa et al., 2005). Among them, daclatasvir (trade name Daklinza) and ombitasvir (trade names Viekira Pak and Technivie) are antiviral drugs for the treatment of hepatitis C virus infection. The structural “widths” of these two dimeric antiviral compounds are around 22.4 and 24.5 Å, respectively.

Benzofuran is one of the most important oxygen-containing heterocycles (Khanam and Shamsuzzaman, 2015). Many benzofuran derivatives display potent biological and pharmacological properties, such as β-adrenoceptor antagonistic (Narimatsu et al., 2003), analgesic, antiarrhythmic (Spaniol et al., 2001), antiAlzheimer's, antifeedantic, antihyperglycemic, antiinflammatory, antimicrobial, antipyretic, antitumor, immunosuppressive (Cheng et al., 2010; González–Gómez et al., 2005; Khanam and Shamsuzzaman, 2015; Kao and Chern, 2001), and especially antiviral activities (Naik et al., 2015; He et al., 2015). Moreover, the broad and potent activities of 1,3-thiazolidin-4-ones have established these compounds as biologically important scaffolds. Their biological properties include anthelmintic, antibacterial, anticancer, anticonvulsant, antidiabetic, antifungal, antihistaminic, antihyperlipidemic, antiinflammatory, antiproliferative, antitubercular, cardiovascular, follicle stimulating hormone receptor agonist, hypnotic (Verma and Saraf, 2008; Tripathi et al., 2014; Gouvea et al., 2016), and antiviral activities as well (Barreca et al., 2002; Rao et al., 2004; Rawal et al., 2007). Recently, thiazinanone derivatives have been reported with medicinally important roles. These compounds show potent HIV-RT inhibitory, cyclooxygenase (COX-2) inhibitory (Zebardast et al., 2009), antidyslipidemic, antihyperglycemic, antitumor (Kamel et al., 2010), antimalarial (Rudrapal et al., 2013), and antifungal activities (Verma et al., 2010; Qu et al., 2013).

Accordingly, using suramin (**1**) as a model, we designed bis(benzofuran−1,3-thiazolidin-4-one) derivatives **2** and bis(benzofuran−1,3-thiazinan-4-one) derivatives **3** as new types of dimeric compounds, of which the antiviral activities were tested. Suramin (**1**) and bis-benzofurans **2** and **3** have a width of ~23.6 Å (between the two naphthalene rings), 22.7 Å, and 21.7 Å (between the two benzene rings), respectively, on the basis of the conformations shown in structures **1**–**3**. Raj and co-workers (Raj et al., 1998) reported that the two naphthalene rings of suramin (**1**) either fold closer to each other with a distance of ~16–20 Å or stretch away from each other at a ~28–30 Å distance. We aimed to use the two new types of dimeric conjugates to develop leads against CHIKV. In total, 20 new conjugated compounds and one bis-aldehyde intermediate were synthesized, six of which exhibited significant inhibitory efficacy against CHIKV. The structure–activity relationships of these bis(benzofuran–thiazolidinone)s **2** and bis(benzofuran–thiazinanone)s **3** are discussed.



1 (suramin)



n =
2. 1 (benzofuran–thiazolidinone)
3. 2 (benzofuran–thiazinanone)

2. Materials and methods

A detailed description of the materials and methods is available in the supporting information file.

3. Results and discussion

3.1. Synthesis of new conjugated dimers and their structural identification

The target compounds with the scaffolds of **2** and **3** were obtained from the common intermediate bisbenzofuran-2-al **7**. Its preparation started with coupling bissalicyaldehyde **4** (Delogu et al., 2010) with commercially available bromoacetaldehyde diethyl ether (**5**) in the presence of $K_2CO_{3\,(s)}$ in dry DMF (Scheme 1). After the reaction mixture was heated to 120 °C, it gave the desired diacetal **6**. By use of acetic acid as the catalyst and also as the solvent, this diacetal **6** underwent sequential deacetylation, intramolecular aldol condensation, and acid-catalyzed dehydration at 110 °C. The desired benzofuran-2-al dimer **7** was generated in 88% yield and purified as yellow crystals with mp 150.2–151.6 °C. Then alkyl-, cycloalkyl-, aryl-, and aralkylamines **8a**–**j** were used to condense with bisbenzofuranal **7** in excess in dry methanol to give bisimines **9a**–**j** as solids in 83–94% yields.

Subsequent ring formation through condensation of bisimines **9a**–**j** with 2-mercaptoacetic acid (**10**) in benzene at 90 °C led to the desired targets **2a**–**j** as solids in 77–88% yields. The structures of all these new bis(benzofuran−1,3-thiazolidin-4-one)s were identified on the basis of their spectroscopic characteristics. For example, the exact mass of compound **2g** was measured as 534.1651 for M^+, which is very close to its theoretical value of 534.1647 for the