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Design, Synthesis, and Biological Evaluation of Thiazoles Targeting Flavivirus Envelope Proteins

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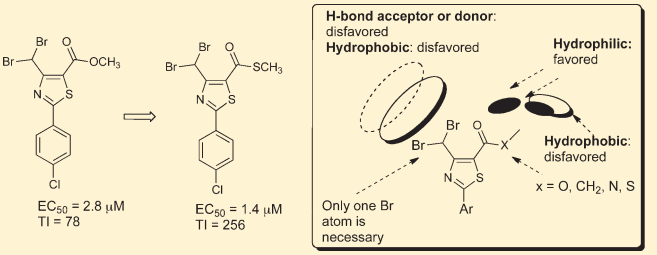
ABSTRACT: A series of third-generation analogues of methyl 4-(dibromomethyl)-2-(4-chlorophenyl)thiazole-5-carboxylate (**1**), which had the most potent antiviral activity among the first- and second-generation compounds, have been synthesized and tested against yellow fever virus using a cell-based assay. The compounds were designed with the objectives of improving metabolic stability, therapeutic index, and antiviral potency. The biological effects of C4 and C5 substitution were examined. The methylthio ester and the dihydroxypropylamide analogues had the best antiviral potencies and improved therapeutic indices and metabolic stabilities relative to the parent compound **1**.



INTRODUCTION

Flavivirus is a genus of the positive-sense single-stranded RNA family *Flaviviridae*, which includes many clinically important species such as dengue, Japanese encephalitis, and West Nile viruses. More than 50 million cases of dengue viral infections are reported per year in more than 80 countries in which the mosquito *Aedes aegypti* is endemic.[1] Of these cases, approximately 500 000 patients suffer the more severe and often lethal illnesses known as dengue hemorrhagic fever and dengue shock syndrome.[1] These diseases remain a significant problem in Africa, Europe, the Middle East, and West and Central Asia, where the infection is endemic, as well as in North America.[2] Although there are limited licensed vaccines against some flaviviruses such as yellow fever and Japanese encephalitis, there are no vaccines for other types such as dengue viruses nor effective therapy for treatment of the clinical cases.[3]

There are many flaviviral proteins that could be considered as targets for drug discovery such as helicase,[4,5] methyl transferase,[6,7] and serine protease.[8,9] In addition, the viral RNA is also reported to be a target for some antimicrobial agents.[10] The flaviviral E-protein plays a crucial role at the first step in viral infection, since it contains a receptor-binding site and also plays a role in fusion. It undergoes substantial conformational and translational changes through the virus replication cycle, thereby causing the native homodimer to change into a fusogenic homotrimer.[11] Moreover, crystallization of a dengue virus type 2 E protein (Figure 1a) in the presence and the absence of *n*-octyl-β-D-glucoside (β-OG)[12] paved the road for structure-based design of antiviral agents that could occupy the β-OG pocket. Since the β-OG-containing crystal structure revealed conformational changes relative to the unoccupied protein, it is believed that the β-OG pocket is an ideal target for designing new antiflaviviral agents.

Among the 39 molecules previously synthesized and tested for antiviral activity against yellow fever virus, compound **1** (Figure 1b) displayed the most potent antiviral activity.[13] This compound also exhibited potent inhibitory activity against dengue virus. These results have encouraged further structural optimization studies to search for more potent antiviral agents based on the phenylthiazole scaffold.[14]

The structural details of the binding of the lead compound **1** to its receptor are not known with certainty. It adopted two distinct docking poses having a very small calculated energy difference (Figure 2). A focused library of phenylthiazole structural analogues has therefore been synthesized so that SARs can be rigorously defined in order to enable the rational design of more effective antiviral agents. This article will focus on examination of the effects of C4 and C5 substitution (Figure 1c) on the antiviral activity.

In order to probe steric and electronic effects, the dibromomethyl and methyl ester moieties were replaced by a variety of substituents. Since the methyl ester moiety is expected to be metabolically labile to nonspecific esterases in blood plasma and the corresponding free acid analogue has already been established to be an inactive compound, C5 structural optimization included replacement of the methyl ester by bioisosteres that are more metabolically stable but still retain the antiviral potency. Fortunately, ester instability is a common problem in drug development that has been successfully dealt with multiple times before.[15] In the present case, the methyl ester is replaced by other more metabolically stable bioisosters such as amides, thioesters, ketones, and more bulky esters.

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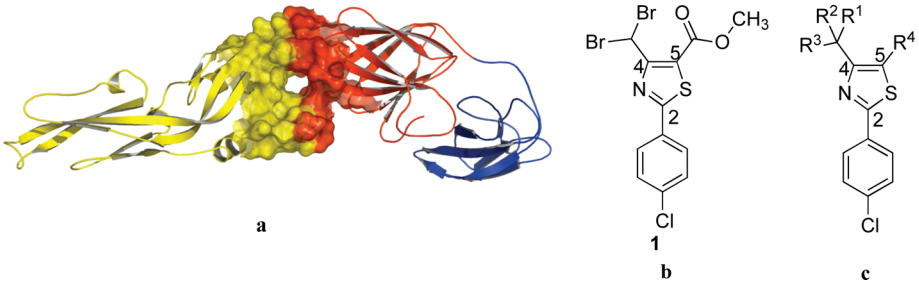

Figure 1. (a) Dengue viral 2 E protein: domain I, red; domain II, yellow; domain III, blue. The β-OG binding pocket is located between domains I and II.[13] (b) Structure of lead compound **1**. (c) Targeted lead modifications. R^1, R^2, and R^3 stand for bulky groups or aromatic systems, and R^4 represents ester bioisosteres.

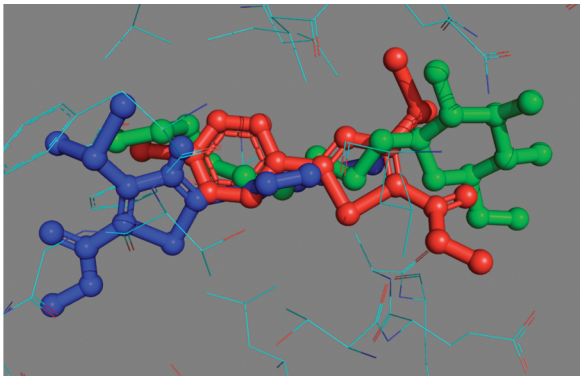

Figure 2. Hypothetical models of the lead compound **1** in the dengue viral 2 E protein β-OG binding pocket. Two different poses opposite each other are shown. Only protein residues within a 6 Å radius around the ligand are represented, and waters of crystallization were removed for the sake of clarity. Compound **1** parallel binding poses to β-OG is represented as red stick. Antiparallel is represented as blue stick. β-OG is represented as green stick, and the protein residues are represented as cyan blue lines (PDB code 1OKE).

RESULTS AND DISCUSSION

Chemistry. The acid chloride **4** served as a key intermediate for the replacement of the metabolically labile ester with more stable bioisosteres. As shown in Scheme 1, two pathways were used to synthesize it. Hydrolysis of ester **1** gave a very low yield of the corresponding free acid because, under the reaction conditions, the carboxylate salt formed in situ underwent S_N2 reaction with the adjacent alkyl bromide, forming a cyclic lactone byproduct. On the other hand, bromination of the acid chloride **3**, utilizing NBS and UV light as a free radical initiator, gave the desired dibromo acid chloride derivative **4** in a good yield with no detectable mono or tribromo byproduct. The ^{1}H NMR spectrum of compound **4** revealed three signals, one singlet and two doublets, at δ 7.30, 7.49, and 8.01 ppm, corresponding to the methine and 1,4-disubstituted phenyl moieties, respectively.

To synthesize the desired amide derivatives **5**−**12**, the acid chloride **4** was allowed to react with primary or secondary amines in dichloromethane or with ammonia solution at room temperature for 5−10 min (Scheme 1). The esters were prepared by dissolving the acid chloride **4** in the required alcohols (Scheme 1). Similarly, treatment of acid chloride **4** with alkanethiols or their sodium salts afforded the corresponding thioesters as depicted in Scheme 1.

In order to synthesize a derivative **20** containing dibromomethyl and methylketo functionalities, the methyl ketone **19** was prepared by utilizing thioamide **18** and the appropriate diketo derivative **17** (Scheme 2). The only reaction that was found to brominate the desired methyl group of **19** efficiently without detectable bromoketone was one involving exactly 2 equiv NBS in CCl_4 with UV irradiation. Using other chemical-free radical initiators afforded mixtures of different brominated compounds. The bromination was assigned to be on the Ar-methyl carbon and not on the ketone-methyl carbon, based on the spectral data. For instance, the Ar-methyl carbon signal of **19** at 18.34 ppm was not present in the ^{13}C NMR spectrum of the product **20**, which revealed only two signals corresponding to dibromomethyl and acylmethyl groups at 32.00 and 31.28 ppm in the aliphatic region. In addition, the mass spectrum showed a base peak at m/z 395 corresponding to the acylium cation ($M^+ - CH_3$).

The amide derivatives with hydroxyalkyl or carbohydrate moieties were prepared by treatment of the acid chloride **4** with the appropriate amines in DMF (Scheme 3). To synthesize the desired thiazole-C4 derivatives, chlorination of the commercially available dicarbonyl compounds **26a**−**c** was performed using sulfuryl chloride to afford the corresponding α-chloro derivatives **27a**−**d** in high yields (Scheme 4). The ^{1}H NMR spectra of these compounds exhibited singlets at approximately δ 5 ppm due to the methine proton. Treatment of the α-chloro derivatives with 4-chlorobenzothioamide (**18**) in ethanol afforded, in each case, the corresponding 2-*p*-chlorophenylthiazole derivatives **28**−**31** (Scheme 4).

To prepare phenylthiazoles with hydrogen bond acceptor moieties at position 4, two reactions were adopted. The first one included treatment of the ether **33** with 1 equiv of NBS under free radical conditions to afford the corresponding aldehyde **34** in reasonable yield as reported by Markees (Scheme 5).[16] To prepare the methyl esters **40**, **43**, and **45** (Chart 1), a modified version of the Markees procedure was used, as previously reported.[17]

The aldehyde derivative **35** was treated with acetyl bromide and a catalytic amount of $AlCl_3$ to afford the desired dichloromethyl derivative **37**, which could not be obtained by treatment of **2** with NCS (Scheme 6). The nitrile derivative **36** was obtained from the reaction of the corresponding aldehyde **35** and ammonia solution. Oxidation of the imine intermediate in situ by elemental iodine provided the required nitrile in 99% yield (Scheme 6). The sulfone derivative **39** was obtained from the corresponding monobromo derivative **38** (Scheme 7).

Biological Results. All of the thiazole derivatives have been evaluated in a yellow fever virus luciferase cellular assay. Initially, optimization of R^4 at thiazole-C5 (Figure 1c) was

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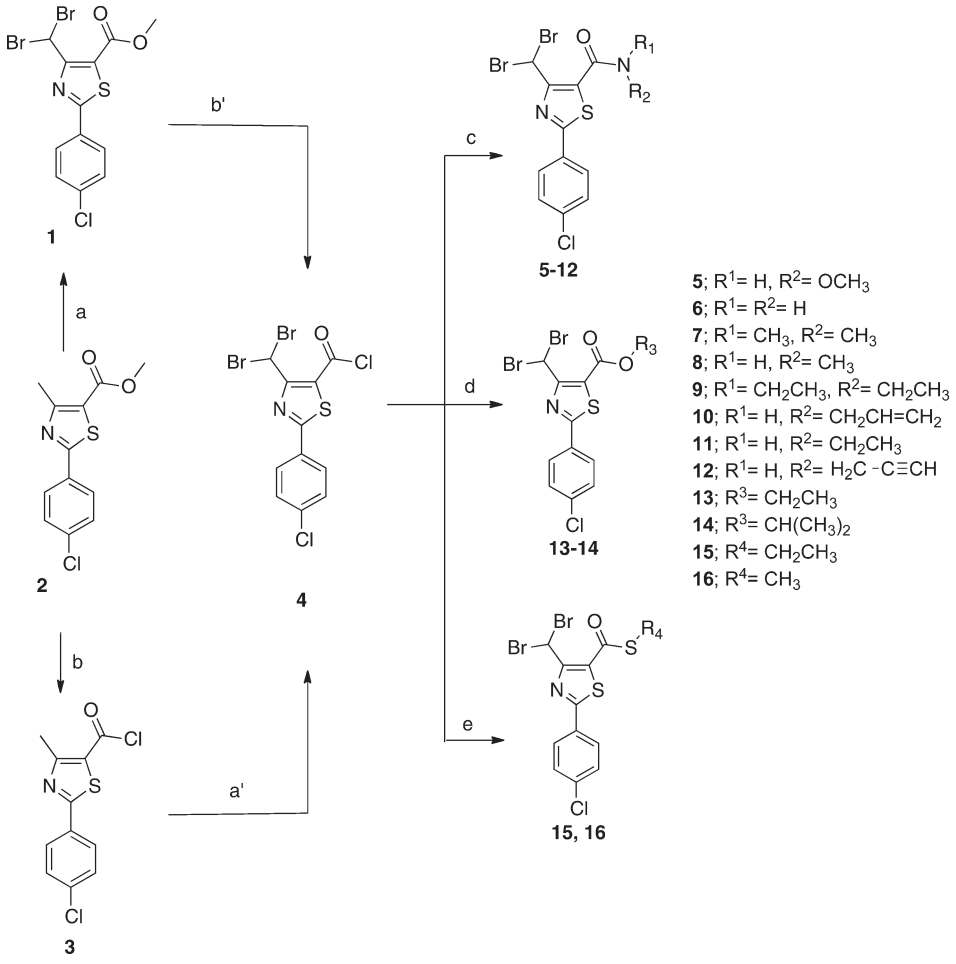
Scheme 1[a]



[a] Reagents and conditions: (a, a′) NBS, UV irradiation, heat to reflux for 24 h, CCl_4, 87% for step a and 63% for step a′; (b, b′) (i) 80% methanol, NaOH, heat to reflux for 2 h, (ii) $SOCl_2$, heat to reflux for 2 h, 95% for step b and 53% for step b′. (c) CH_2Cl_2, alkylamine, 23 °C, 2−10 min, 10−86%, or NH_3 solution, 40 °C, 30 min, 84%; (d) absolute ethanol or 2-propanol, Et_3N, heat to reflux for 6 h, 49−81%; (e) ethanethiol heat to reflux for 4 h, or MeONa/CH_2Cl_2, 23 °C, 0.5 h, 73−91%.

performed in order to increase both the metabolic stability of the lead compound and its potency. Therefore, the ester group was replaced by more metabolically stable groups such as amides, thioesters, or ketones. Among the newly synthesized amides (Scheme 1), the unsubstituted amide **6** and the secondary methylamide derivative **8** revealed slightly better EC_{50} values than the lead compound **1** (Table 1). Increasing the alkyl chain length, from methyl to ethyl, retained the potency, but the toxicity was increased (Table 1, compound **11**). On the other hand, increasing the side chain polarity by replacement of the ethyl group in compound **11** by a methoxy group improved the antiviral activity (compound **5**). However, tertiary amides, such as **7** and **9**, showed 5- to 10-fold lower activity. Replacement of the methyl group of **8** with an allyl group (compound **10**) did not improve the potency. Further increasing the unsaturation led to an increase in the toxicity (compound **12**).

Comparison of the EC_{50} values for the secondary amide **8** (EC_{50} = 1.8 μM) vs the corresponding tertiary amide **7** (EC_{50} = 20 μM), as well as the secondary amide **11** (EC_{50} = 2.7 μM) vs the corresponding tertiary amide **9** (EC_{50} = 13.7 μM), suggests that abrogating the hydrogen-bond-donating ability of the amide and increasing its hydrophobicity and steric bulk decrease the antiviral activity. In order to explore this trend further, the ethyl ester **13** (EC_{50} = 8.7 μM) and the isopropyl ester **14** (EC_{50} = 19.9 μM) were synthesized and compared with the methyl ester **1** (EC_{50} = 2.8 μM). The results obtained with the esters also document a correlation of decreased antiviral activity with an increase in hydrophobicity. This would be consistent with a hydrophobically and sterically unfavorable region of the envelope protein surrounding the C-5 substituent of the ligand.

With this information in hand, the metabolically unstable methyl ester moiety was further replaced by a methyl ketone, as well as methyl and ethyl thioester analogues (structures **20**, **16**, and **15**). Bulkier derivatives were excluded because they were expected to have lower biological activities. Both the ketone **20** (EC_{50} = 1.3 μM) and the ethyl thioester **15** (EC_{50} = 1.6 μM) derivatives displayed approximately double the antiviral potency compared with the lead compound **1** (EC_{50} = 2.8 μM). More significantly, the methyl thioester **16** (EC_{50} = 1.4 μM, GI_{50} 368.7 μM) had higher antiviral potency and was also less cytotoxic in uninfected cells than **1** (GI_{50} = 222.1 μM), resulting in a therapeutic index (TI) for

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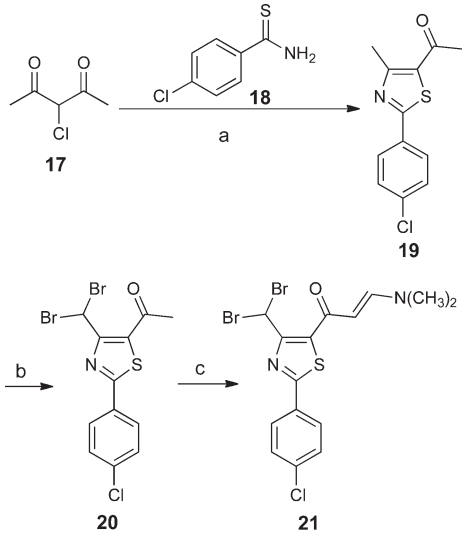
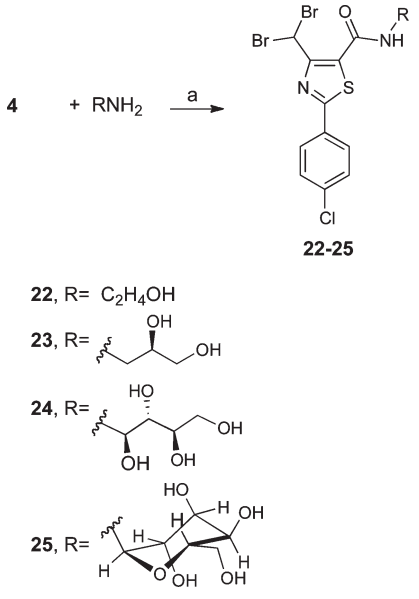
Scheme 2[a]



[a] Reagents and conditions: (a) absolute ethanol, heat to reflux for 24 h, 73%; (b) NBS, UV irradiation, heat to reflux for 12 h, CCl_4, 50%; (c) DMF-DMA, dry toluene, heat to reflux to 24 h, 66%.

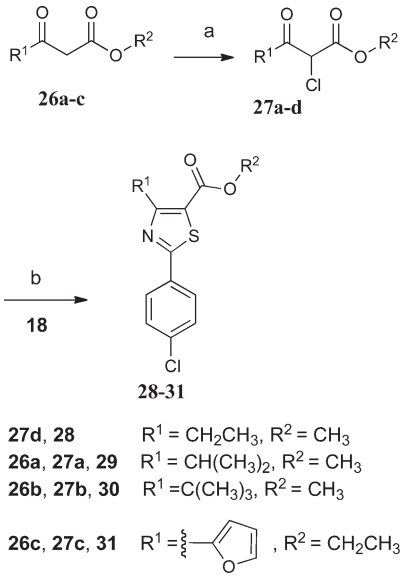
Scheme 3[a]



[a] Reagents and conditions: (a) DMF, 23 °C, 0.5−1 h, 12−86%.

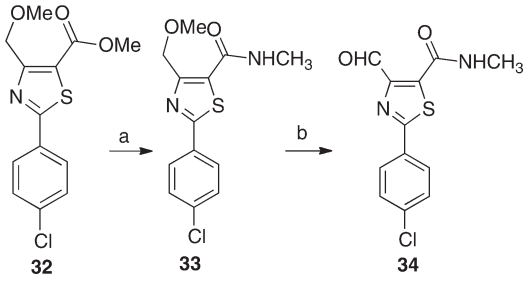
16 of 263, which compares favorably with that of the lead compound **1** (TI 79). The metabolic stability of a methyl thioester in the rat plasma in some cases has been found to be much higher than the corresponding ester.[18] Compound **16** was subjected to hydrolytic stability analysis utilizing lyophilized rat plasma. The half-life of compound **16** was found to be 24.725 h. This value is around 17 times higher than the lead compound **1**, which had a half-life of 1.415 h under the same experimental conditions. Therefore, compound **16** provided the desired increased metabolic stability, along with improved antiviral potency, lower cytotoxicity, and an improved TI.

Scheme 4[a]



[a] Reagents and conditions: (a) CH_2Cl_2, SO_2Cl_2, 23 °C, 2 h, 90−96%; (b) absolute ethanol, heat to reflux for 24 h, 63−96%.

Scheme 5[a]



[a] Reagents and conditions: (a) (i) 80% methanol, NaOH, heat to reflux for 2 h, 84%, (ii) $SOCl_2$, heat to reflux for 2 h, 96%, (iii) $MeNH_2 \cdot HCl$, K_2CO_3, CH_2Cl_2, 23 °C, 1 h, 91%; (b) NBS (1 equiv), UV irradiation, heat to reflux for 2 h, CCl_4, 56%.

In a summary, simple amide, ketone, and thioester derivative exhibited similar to better antiviral activity relative to the lead compound **1** with higher metabolic stability in rat blood plasma.

Since the N- and O-hydrophobic substitutions decreased the antiviral activity, it may be hypothesized that this part either lies in a hydrophilic region within the binding pocket or faces the solvent-accessible surface. Therefore, the next structural optimization efforts included adding hydrophilic moieties to the alkyl side chain. Among the ester isosteres that showed significant antiviral activity, the amide was chosen because of the commercial availability of hydroxy- and sugar-containing primary amines. To systematically examine this hypothesis, amides with a variable number of hydroxy groups were synthesized (Scheme 3). Compound **23**, which contains two hydroxyl groups, revealed an EC_{50} value 3-fold better than the lead compound. Removal of the terminal hydroxylmethyl group afforded compound **22**, which displayed a higher EC_{50} value. Compounds **24** and **25** revealed much lower biological activity than **23**.

The higher activity of compound **23** could be rationalized from the molecular modeling (Figure 3). The modeling was

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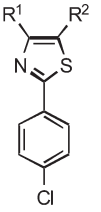
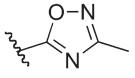
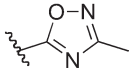
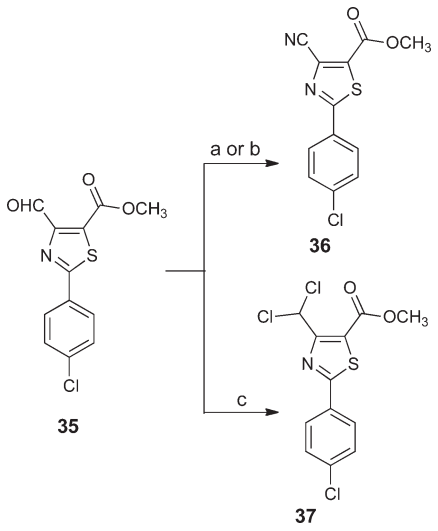
Chart 1. Polar Dibromomethyl Replacement



32, R^1= CH_2OCH_3, R^2= $COOCH_3$
33, R^1= CH_2OCH_3, R^2= $COOCH_3$
34, R^1= CHO, R^2= $CONHCH_3$
35, R^1= CHO, R^2= $COOCH_3$
36, R^1= CN, R^2= $COOCH_3$
40, R^1= $COOCH_3$, R^2= $COOCH_3$
41, R^1= CH_2OCH_3, R^2= $CON(CH_3)_2$
42, R^1= CHO, R^2= $CON(CH_3)_2$
43, R^1= $COOCH_3$, R^2= $CON(CH_3)_2$
44, R^1= CHO, R^2= 
45, R^1= $COOCH_3$, R^2= 

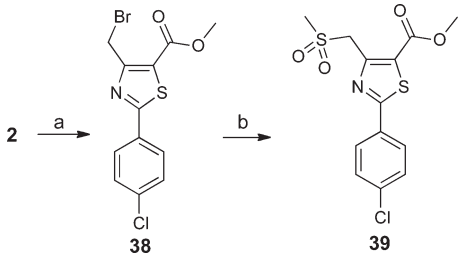
Scheme 6[a]



[a] Reagents and conditions: (a) anhydrous $AlCl_3$, THF, NaN_3, heat to gentle reflux for 24 h, 21%; (b) I_2, NH_3 solution, THF, 23 °C, 1 h, 99%; (c) AcCl (2 equiv), anhydrous $AlCl_3$, dichlorobenzene, heat to reflux for 24 h, 99%.

performed using the published structure of the dengue virus envelope protein, which is valid is this case because the sequence alignment of the dengue virus protein vs the yellow fever virus protein reveals that they have 45% identity and 61% similarity.[19] Among flaviviruses, the envelope proteins are predicted to be very similar in their overall fold and domain arrangement, including the hinge region, which is highly conserved. The terminal hydroxy group of the dihydroxypropyl moiety of **23** is calculated to have a binding pose that is very close to that of the sugar moiety of β-OG (Figure 3). It is calculated to form two hydrogen bonds with Ser274 and Gln271, which might improve the binding energy of **23** and consequently its inhibitory activity.

Scheme 7[a]



[a] Reagents and conditions: (a) NBS (1.1 equiv), Bz_2O_2, CCl_4, heat to gentle reflux for 24 h, 50%; (b) $MeSO_2Na$, absolute ethanol, heat to reflux for 12 h, 83%.

Although the central hydroxyl group is not calculated to interact directly with any residues in the binding pocket, it points out toward the solvent-accessible surface so that it might be hydrated and could also stabilize the molecule within the active site.

The second point of optimization of the lead compound **1** is at thiazole-C4. Since the dibromomethyl derivative **1** revealed potent antiviral activity and since its nonhalogenated and dichloromethyl analogues showed no or substantially reduced antiviral properties, it was concluded that the dibromomethyl moiety is an important region for the antiviral activity. Therefore, a series of C4-optimized derivatives were designed to test the steric and the electronic properties at the dibromomethyl position. First, the dibromomethyl moiety was replaced by different alkyl and aryl substituents (compounds **28**−**31**). All of the derivatives showed much lower activity than the lead compound.

Next, the dibromomethyl was replaced by more polar moieties (Chart 1). High EC_{50} values were observed for all of the compounds with the exception of compound **33** (Table 1, compounds **32**−**36**, **40**−**45**).

Although both polar and nonpolar dibromomethyl replacements failed to improve the antiviral activity of the lead compound **1**, one more attempt was made to further investigate the possibility of increasing the antiviral activity of the lead compound **1** by adding a hydrogen bond acceptor moiety at the thiazole-C4 position. Starting from the molecular modeling study of the lead compound **1** and its amide analogues, two glutamine residues have been observed to be possible targets for C4-thiazole structural modification. In the case of the lead compound **1**, the binding pose that is parallel to the β-OG molecule indicated the possibility of hydrogen bond formation between the dibromomethyl moiety and both Gln200 and Gln271 residues (Figure 4). The same observation was found in the case of amide analogue **24**. Therefore, replacement of the dibromomethyl by a sulfinyl (SO_2) moiety was considered in which the two oxygen atoms are proposed to make two distinct hydrogen bonds with the glutamine residues. However, when this hypothetical compound was docked in the β-OG site, it was far away from the desired residues. In contrast, adding one CH_2 unit between the sulfinyl moiety and thiazole ring (compound **39**) was calculated to make the two oxygen atoms closer to the desired residues. Surprisingly, compound **39** stimulated the viral replication instead of inhibiting it (Table 1).

As all of the above-mentioned C4-optimization attempts failed to replace the dibromomethyl with a more potent moiety, other halogens were tried. The dichloromethyl analogue **37** had very weak antiviral activity, but the monobromo derivative **38** showed approximately 1-fold lower EC_{50} value than the lead compound.

Table 1. Antiviral Activities and Cytotoxicities of Compounds vs Yellow Fever Virus

compd	% inhibition[a]	GI_{50}[b] (μM)	EC_{50}[c] (μM)
1	99.6	222.1 ± 51.4	2.8 ± 1.0
5	93.6	81.4 ± 39.1	1.7 ± 0.6
6	99.8	99.7 ± 37.0	1.7 ± 0.5
7	93.2	162.9 ± 23.3	20.0 ± 2.5
8	99.7	74.6 ± 5.1	1.8 ± 0.7
9	99.8	63.5 ± 14.9	13.7 ± 0.6
10	99.9	40.8 ± 0.9	1.6 ± 0.4
11	99.9	33.3 ± 5.4	2.7 ± 0.8
12	99.7	23.9 ± 3.9	1.7 ± 0.2
13	98.1	94.6 ± 28.9	8.7 ± 2.9
14	84.4	372.3 ± 22.3	19.9 ± 2.5
15	99.7	81.4 ± 39.1	1.6 ± 0.1
16	99.6	368.7 ± 7.5	1.4 ± 0.1
20	99.9	83.5 ± 10.9	1.3 ± 0.5
21	99.8	38.2 ± 9.8	11.4 ± 6.4
22	99.8	40.8 ± 0.9	2.9 ± 0.5
23	99.9	158.0 ± 20.4	1.1 ± 0.1
24	53.2	NA[d]	NA[d]
25	43.4	NA[d]	NA[d]
28	−56.7	NA[d]	NA[d]
29	14.7	49.6 ± 3.6	58.7 ± 17.0
30	20.9	NA[d]	NA[d]
31	50.3	465 ± 40	311.0 ± 25.0
32	38.1	387.9 ± 25.0	158.3 ± 157.6
33	99.7	50.8 ± 4.2	2.9 ± 0.5
34	37.5	NA[d]	NA[d]
35	99.9	36.4 ± 2.1	42.8 ± 17.4
36	7.8	NA[d]	NA[d]
37	99.9	34.1 ± 1.4	52.8 ± 18.1
38	99.9	74.6 ± 5.1	1.6 ± 0.5
39	−74.7	NA[d]	NA[d]
40	30.1	NA[d]	NA[d]
41	11.3	NA[d]	NA[d]
42	5.5	NA[d]	NA[d]
43	74.0	99.7 ± 37.0	51.0 ± 18.0
44	99.6	162.9 ± 23.3	26.5 ± 1.8
45	1.5	NA[d]	NA[d]

[a] Measured as a reduction in luciferase activity of BHK cells infected with YF-IRES-Luc at 50 μM in comparison to the control. [b] The GI_{50} is the concentration of the compound causing a 50% growth inhibition of uninfected BHK cells. [c] The EC_{50} is the concentration of the compound resulting in a 50% inhibition in virus production. [d] NA indicates that the value was not determined.

Interestingly, the cytotoxicity of compound **38** was in the acceptable range (GI_{50} > 40 μM). Although the monobromo derivative **38** was the only C4-optimized structure that exceeds the antiviral activity of the lead compound, it is expected to be more vulnerable to attack by endogenous nucleophiles than the dibromo analogue because of steric factors.

CONCLUSION

The main aims of this study were to define the SARs at C4 and C5 of the 4-chlorophenylthiazole scaffold as well as to improve on the metabolically unstable methyl ester. The SARs at C5 were defined to a great extent, and the SARs at C4 were investigated. In addition, more metabolically stable methyl ester analogues were successfully prepared that had no decrease in the antiviral potency, which will increase the potential utility of this series of compounds as antiflaviviral agents.

Developing selective antiflaviviral agents is still a challenging field. Of the vast number of articles on the discovery and development of antiviral agents, relatively few deal with dengue, West Nile, or other flaviviruses. The current report of an antiflaviviral agent having a therapeutic index of 256 with enhanced metabolic stability is a significant achievement that will hopefully stimulate further research in this field.

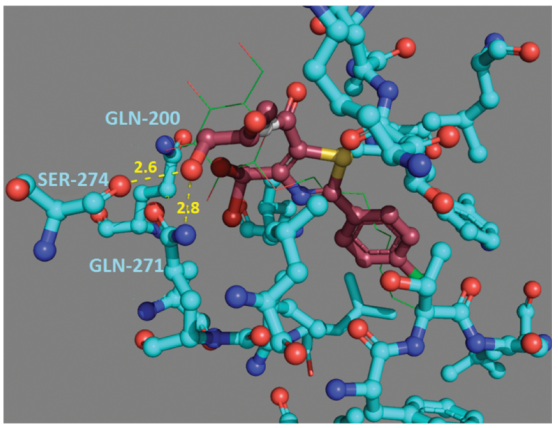


Figure 3. Hypothetical model of compound **23** in the dengue viral 2 E protein β-OG binding pocket (PDB code 1OKE).

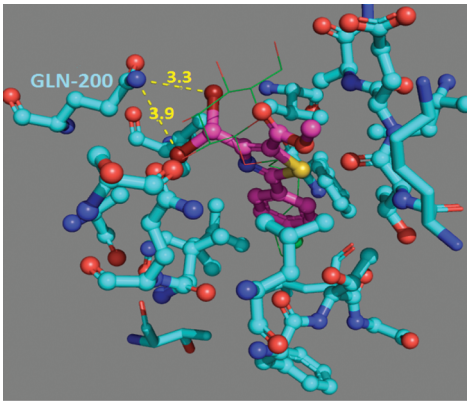


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Figure 4. Hypothetical model of the lead compound **1** in the dengue viral 2 E protein β-OG binding pocket.

EXPERIMENTAL SECTION

All biologically tested compounds had purity of ≥95% as established by HPLC. ^{1}H NMR spectra were run at 300 MHz, and ^{13}C spectra were determined at 75.46 MHz in deuterated chloroform ($CDCl_3$), dimethyl sulfoxide (DMSO-d_6), methanol (CD_3OD), or acetone (CD_3COCD_3). Chemical shifts are given in parts per million (ppm) on the δ scale. Chemical shifts were calibrated relative to those of the solvents. Mass spectra were recorded at 70 eV. All reactions were conducted under argon or nitrogen atmosphere, unless otherwise specified. Compounds **2**,[13] **3**,[20] **27a**,[21] **27b**,[22] **22**, **32**, **35**, and **40**−**45**[17] were prepared according to the reported procedures.

4-(Dibromomethyl)-5-chlorocarbonyl-2-(4-chlorophenyl)thiazole (4). *Method A.* 5-Chlorocarbonyl-(4-chlorophenyl)-4-methylthiazole (**3**) (3.538 g, 13 mmol) and NBS (20.7 g, 78 mmol) were added to CCl_4 (75 mL). The reaction mixture was heated at reflux for 48 h and irradiated with UV light (produced from a sun-lamp) 10 times (5 min every 30 min). The yellowish-white precipitate was purified by silica gel chromatography (ethyl acetate−hexanes 1:1) to provide the desired compound as a white solid (3.55 g, 63.7%).

Method B. NaOH (80 mg, 2 mmol) was added to a solution of methyl ester **1** (423 mg, 1 mmol) in methanol (20 mL) and water (5 mL). The reaction mixture was heated at reflux for 6 h and then allowed to cool to room temperature. The reaction mixture was filtered, and the pH of the liquid phase was adjusted to 2 with hydrochloride acid. The solid was filtered and dried and purified by silica gel chromatography (hexanes−ethyl acetate−glacial acetic acid 50:49:1) to provide the carboxylic acid. The free acid (411 mg, 1 mmol) was heated at reflux with thionyl chloride (7 mL) for 2 h. The solvent was evaporated under pressure. The pale yellow residue was collected and recrystallized from chloroform to yield a white solid product (231.7 mg, 53%): mp 131−132 °C. ^{1}H NMR ($CDCl_3$) δ 8.01 (d, J = 8.7 Hz, 2 H), 7.49 (d, J = 8.7 Hz, 2 H), 7.30 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 167.43, 155.31, 136.57, 131.21, 128.83, 127.67, 122.44; CIMS m/z (rel intensity) 432/430/428 (MH^+, 40/45/41), 352/350/348 (M^+ − Br, 26/100/38); HRMS (CI), m/z 347.8652 (M − HBr)$^+$, calcd for $C_{11}H_5BrCl_2NOS$ 347.8647.

Preparation of Amide Derivatives 5 and 7−12. The acid chloride **4** (430 mg, 1 mmol) was suspended in dry CH_2Cl_2 (5 mL) under argon. Amine (2 mmol) was added, and the mixture was stirred at room temperature for 2−10 min. Aqueous HCl (0.5 M, 15 mL) was added, and the solution was extracted with ethyl acetate (30 mL). The crude residue obtained after evaporation of solvent was purified by silica gel flash chromatography using hexane−ethyl acetate (1:1) to afford the desired product.

4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*-methoxythiazole-5-carboxamide (5). Off-white solid (7.1 mg, 11%), mp 85 °C. IR (film) 3584, 3047, 2960, 1715, 1593 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.98 (d, J = 7.5 Hz, 2 H), 7.69 (s, 1 H), 7.45 (d, J = 8.7 Hz, 2 H), 3.94 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 170.09, 161.35, 158.90, 137.85, 130.60, 129.36, 128.27, 52.93, 31.01; MS m/z (rel intensity) 443/441/439 (MH^+, 51/100/31), 394 (36); HRMS (ESI) m/z 438.8514 MH^+, calcd for $C_{12}H_{10}Br_2ClN_2O_2S$ 438.8513; HPLC purity 98.36%.

4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*,*N*-dimethylthiazole-5-carboxamide (7). White solid (51.1 mg, 83%), mp 63−64 °C. ^{1}H NMR ($CDCl_3$) δ 7.93 (d, J = 8.4 Hz, 2 H), 7.43 (d, J = 8.4 Hz, 2 H), 6.87 (s, 1 H), 3.11 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 166.50, 161.42, 153.57, 137.42, 130.60, 129.28. 128.06, 123.76, 39.72, 31.14, 31.27; MS m/z (rel intensity) 441/439/437 (MH^+, 11.2/15.4/9.6), 394 (100); HRMS (ESI), m/z 436.8728 M^+, calcd for $C_{13}H_{12}Br_2ClN_2OS$ 436.8720; HPLC purity 95.24%.

4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*-methylthiazole-5-carboxamide (8). Colorless solid (9.5 mg, 20%), mp 165−167 °C. ^{1}H NMR ($CDCl_3$) δ 7.93 (d, J = 8.4 Hz, 2 H), 7.73 (s, 1 H), 7.43 (d, J = 8.4 Hz, 2 H), 5.98 (brs, 1 H), 3.02 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 166.56, 160.35, 156.80, 137.65, 130.46, 129.34, 128.18, 123.35, 31.85, 29.59; CIMS m/z (rel intensity) 426/424/422 (MH^+, 15/71/22); HRMS (ESI), m/z 422.8568 MH^+, calcd for $C_{12}H_{10}Br_2ClN_2OS$ 422.8564; HPLC purity 95.13%.

4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*,*N*-diethylthiazole-5-carboxamide (9). Colorless solid (37.2 mg, 86%), mp 63 °C. ^{1}H NMR ($CDCl_3$) δ 7.92 (d, J = 8.4 Hz, 2 H), 7.72 (s, 1 H), 7.44 (d, J = 8.4 Hz, 2 H), 5.94 (brs, 1 H), 3.47 (q, J = 7.2 Hz, 2 H), 1.26 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 166.47, 159.63, 156.67, 137.62, 130.46, 129.33, 128.15, 123.71, 44.21, 41.02, 31.93, 13.55; MS m/z (rel intensity) 491/489/487 (MNa^+, 8.1/3.3/6.0), 394 (100); HRMS (ESI), m/z 486.8860 MNa^+, calcd for $C_{15}H_{15}Br_2ClN_2OSNa$ 486.8852; HPLC purity 99.42%.

***N*-Allyl-4-(dibromomethyl)-2-(4-chlorophenyl)thiazole-5-carboxamide (10).** Colorless solid (8 mg, 18%), mp 135−137 °C. ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 8.4 Hz, 2 H), 7.73 (s, 1 H), 7.45 (d, J = 8.4 Hz, 2 H), 5.95 (m, 2 H), 5.32 (brs, 1 H), 5.26 (m, 1 H), 4.07 (d, J = 1.2 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 166.63, 159.55, 157.03, 137.70, 132.89, 130.45, 129.36, 128.20, 123.21, 117.66, 42.67, 31.79; MS m/z (rel intensity) 452/450/448 (M^+, 70/100/26); HRMS (ESI), m/z 447.8652 M^+, calcd for $C_{14}H_{11}Br_2ClN_2OS$ 447.8647; HPLC purity 98.17%.

4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*-ethylthiazole-5-carboxamide (11). Colorless solid (24 mg, 48%), mp 140−141 °C. ^{1}H NMR ($CDCl_3$) δ 7.94 (d, J = 8.4 Hz, 2 H), 7.43 (d, J = 8.4 Hz, 2 H), 6.84 (s, 1 H), 3.48 (q, J = 7.2 Hz, 4 H), 1.24 (t, J = 7.2 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 166.72, 160.65, 153.39, 137.18, 130.66, 129.27, 128.06, 124.45, 43.20, 31.18, 15.81; APCIMS m/z (rel intensity) 441/439/437 (MH^+, 42/100/48); HRMS (CI) m/z 436.8723 MH^+, calcd for $C_{13}H_{12}Br_2ClN_2OS$ 436.8720; HPLC purity 98.27%.

4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*-(prop-2-ynyl)thiazole-5-carboxamide (12). White solid (9.6 mg, 20%), mp 177−178 °C. ^{1}H NMR ($CDCl_3$) δ 7.94 (d, J = 8.7 Hz, 2 H), 7.70 (s, 1 H), 7.46 (d, J = 8.7 Hz, 2 H), 6.01 (brs, 1 H), 4.23 (s, 2 H), 2.3 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 167.03, 159.38, 157.52, 137.83, 130.37, 129.39, 128.23, 122.37, 72.68, 31.60, 30.00, 29.59; MS m/z (rel intensity) 453/451/499/477 (MH^+, 92/100/47/41.1); HRMS (ESI), m/z 446.8567 MH^+, calcd for $C_{14}H_{10}Br_2ClN_2OS$ 446.8569; HPLC purity 100%.

4-(Dibromomethyl)-2-(4-chlorophenyl)thiazole-5-carboxamide (6). Excess saturated ammonium hydroxide solution (30 mL) was added gradually to acid chloride **4** (85 mg, 2 mmol) with continuous stirring at 40−45 °C. The suspension was heated at 90 °C for 30 min, and the solid was filtered, washed with water several times, and purified by crystallization from EtOH to afford the product as a white solid (78.3 mg, 84%): mp 219−220 °C. ^{1}H NMR (DMSO) δ 8.00 (d, J = 8.4 Hz, 2 H), 7.80 (s, 1 H), 7.64 (d, J = 8.4 Hz, 2 H); ^{13}C NMR (DMSO) δ 166.35, 158.20, 157.11, 137.24, 131.44, 130.63, 129.21, 125.00, 33.61; CIMS m/z (rel intensity) 412/410/408 (MH^+, 13/100/38); HRMS (ESI) m/z 430.8238 MNa^+, calcd for $C_{11}H_7Br_2ClN_2OSNa$ 430.8227; HPLC purity 95.51%.

Alkyl 4-(Dibromomethyl)-2-(4-chlorophenyl)thiazole-5-carboxylates 13 and 14. The acid chloride **4** (30 mg, 0.07 mmol) was heated under reflux with the alcohol (2 mL) for 6 h. The reaction mixture was allowed to cool, the solvent was evaporated under reduced pressure, and the solid residue was purified by silica gel flash chromatography using hexane−ethyl acetate (9:1) to afford the products. The physical properties and spectral data are listed below.

Ethyl 4-(Dibromomethyl)-2-(4-chlorophenyl)thiazole-5-carboxylate (13). White solid (20 mg, 81%), mp 185−187 °C. ^{1}H NMR ($CDCl_3$) δ 7.98 (d, J = 8.4 Hz, 2 H), 7.69 (s, 1 H), 7.45 (d, J = 8.4 Hz, 2 H), 4.40 (q, J = 7.2 Hz, 2 H), 1.38 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 169.88, 160.44, 158.68, 137.77, 130.63, 129.33, 128.23, 120.01, 62.29, 31.15, 14.11; CIMS m/z (rel intensity) 441/439/437 (MH^+, 20/67/55), 393 (100); HRMS (EI), m/z 437.8562 MH^+, calcd for $C_{13}H_{11}Br_2ClNO_2S$ 437.8560; HPLC purity 96.98%.

Isopropyl 4-(Dibromomethyl)-2-(4-chlorophenyl)thiazole-5-carboxylate (14). White solid (15.3 mg, 49%), mp 176−178 °C. ^{1}H NMR ($CDCl_3$) δ 7.99 (d, J = 8.4 Hz, 2 H), 7.70 (s, 1 H), 7.45 (d, J = 8.4 Hz, 2 H), 5.25 (m, J = 7.2 Hz, 1 H), 1.39 (d, J = 7.2 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 169.02, 159.98, 158.47, 139.64, 137.74, 130.69, 129.33, 128.22, 70.43, 46.87, 31.21, 21.75; CIMS m/z (rel intensity) 456/454/452 (MH^+, 10/47/23), 393 (100); HRMS (EI), m/z 450.8647 M^+, calcd for $C_{14}H_{12}Br_2ClNO_2S$ 450.8644; HPLC purity 100%.

***S*-Ethyl 4-(Dibromomethyl)-2-(4-chlorophenyl)thiazole-5-carbothioate (15).** The acid chloride **4** (38 mg, 0.09 mmol) was

heated under reflux with ethanethiol (0.1 mL, 1.9 mmol) under solvent-free conditions for 4 h. The reaction mixture was allowed to cool. The solvent was taken off under reduced pressure, and the solid residue was purified by silica gel flash chromatography using hexane−ethyl acetate (7:3) to afford the product together with a disulfide byproduct, which was removed by another silica gel flash chromatography column using hexane−ethyl acetate (97.5:2.5) to yield a white solid (5.5 mg, 10%): mp 90−91 °C. ^{1}H NMR ($CDCl_3$) δ 8.00 (d, J = 8.7 Hz, 2 H), 7.56 (s, 1 H), 7.46 (d, J = 8.7 Hz, 2 H), 3.13 (q, J = 7.5 Hz, 2 H), 1.38 (t, J = 7.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 181.01, 156.11, 149.58, 144.87, 138.00, 130.89, 129.39, 128.36; APCIMS m/z (rel intensity) 458/456/454 (MH^+, 7/29/24), 393 (100); HRMS (ESI), m/z 453.8337 MH^+, calcd for $C_{13}H_{11}Br_2ClNOS_2$ 453.8332; HPLC purity 99.04%.

***S*-Methyl 4-(Dibromomethyl)-2-(4-chlorophenyl)thiazole-5-carbothioate (16).** The acid chloride **4** (50 mg, 0.12 mmol) was stirred with sodium methanthiol (12 mg, 1.7 mmol) in dry dichloromethane (10 mL) for 30 min. The solvent was taken off under reduced pressure, and the solid residue was partitioned between EtOAc (10 mL) and water (10 mL). The organic layer was separated, dried, and evaporated. The yellow precipitate was further purified by crystallization from MeOH to afford the product as pale yellow needles (36 mg, 91%): mp 180−182 °C. ^{1}H NMR ($CDCl_3$) δ 7.99 (d, J = 8.1 Hz, 2 H), 7.56 (s, 1 H), 7.45 (d, J = 8.1 Hz, 2 H), 2.55 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 183.20, 169.11, 156.20, 138.11, 130.46, 129.45, 128.44, 127.34, 31.59, 12.97; APCIMS m/z (rel intensity) 444/442/440 (MH^+, 19/41/33), 395 ($MH^+ - CH_3$, 100); HRMS (ESI), m/z 439.8185 MH^+, calcd for $C_{12}H_9Br_2ClNOS_2$ 439.8175; HPLC purity 100%.

1-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)ethanone (19). 4-Chlorothiobenzamide (**18**, 1.71 g, 10 mmol) and 3-chloropentane-2,4-dione (1.604 g, 12 mmol) were added to absolute ethanol (50 mL). The reaction mixture was heated at reflux for 24 h. After removal of solvent under reduced pressure, the residue was crystallized from ethanol−methanol−ethyl acetate (10:85:5) to provide the desired compound as a white needles (1.852 g, 73%): mp 114−115 °C. ^{1}H NMR ($CDCl_3$) δ 7.91 (d, J = 8.7 Hz, 2 H), 7.44 (d, J = 8.7 Hz, 2 H), 2.77 (s, 3 H), 2.57 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 190.33, 167.86, 159.44, 137.16, 131.17, 129.24, 127.97, 30.67, 18.34; EIMS m/z (rel intensity) 253/251 (MH^+, 26/70), 236 ($M^+ - CH_3$, 100); HRMS (EI), m/z 251.0172 M^+, calcd for $C_{12}H_{10}ClNOS$ 251.0173; HPLC purity 98.98%.

1-(4-(Dibromomethyl)-2-(4-chlorophenyl)thiazol-5-yl)ethanone (20). Compound **19** (251 mg, 1 mmol) and NBS (358 mg, 2 mmol) were added to CCl_4 (5 mL). The reaction mixture was irradiated for 5 min frequently (15 min intervals) by an ultraviolet sunlamp (GE, 215 W) and heated at reflux for 12 h. After removal of solvent under reduced pressure, the residual NBS was removed by adding saturated aqueous NaOH (3 mL), and the mixture was filtered and washed with distilled water. The collected brown solid was purified by silica gel chromatography using ethyl acetate−hexanes (1:1) to provide the desired compound as a white solid (204 mg, 50%): mp 193−195 °C. ^{1}H NMR ($CDCl_3$) δ 8.00 (d, J = 8.7 Hz, 2 H), 7.67 (s, 1 H), 7.47 (d, J = 8.7 Hz, 2 H), 2.59 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 189.56, 168.67, 160.61, 158.02, 138.03, 130.40, 129.41, 128.34, 32.00, 31.28; CIMS m/z (rel intensity) 412/410/408 (MH^+, 30/78/32); HRMS (ESI), m/z 406.8378 MH^+, calcd for $C_{12}H_8Br_2ClNOS$ 406.8382; HPLC purity 95.13%.

(*E*)-1-(4-(Dibromomethyl)-2-(4-chlorophenyl)thiazol-5-yl)-3-(dimethylamino)prop-2-en-1-one (21). A mixture of thiazole derivative **20** (1 mmol) and DMF-DMA (0.357 mL, 3 mmol) was taken in dry toluene (20 mL), and the mixture was heated at reflux for 24 h and then left to cool at room temperature. The solvent was evaporated under reduced pressure. The reddish-yellow precipitated product was washed with petroleum ether (60/80 °C) and dried. Recrystallization from benzene afforded the desired compound as an orange solid (303 mg, 66%): mp 148−149 °C. ^{1}H NMR ($CDCl_3$) δ 7.96 (d, J = 8.3 Hz, 2 H), 7.93, (s, 1 H), 7.76 (d, J = 12 Hz, 2 H), 7.40 (d, J = 8.3 Hz, 2 H), 5.34 (d, J = 12 Hz, 2 H), 3.17 (s, 3 H), 2.93 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 178.70, 166.27, 154.92, 137.04, 131.26, 131.07, 130.40, 129.16, 128.09, 127.63, 94.83, 45.39, 37.52, 33.65; ESIMS m/z (rel intensity) 467/465/563 (MH^+, 65/100/42); HRMS (ESI), m/z 462.8880 MH^+, calcd for $C_{15}H_{14}Br_2ClN_2OS$ 462.8877; HPLC purity 100%.

Preparation of Hydroxyalkyl and Carbohydrate Amide Derivatives. General Procedure. Primary amine (1 mmol) was added to acid chloride **4** (41 mg, 1 mmol) in dry dichloromethane (2 mL). The reaction mixture was stirred at room temperature for 0.5−1 h. The solvent was evaporated under reduced pressure. The solid residue was purified by crystallization from methanol. The physical and spectral data of the obtained compounds are listed below.

4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*-(2-hydroxyethyl)thiazole-5-carboxamide (22). White solid (11 mg, 27%), mp 132−133 °C. ^{1}H NMR ($CDCl_3$) δ 7.93 (d, J = 8.4 Hz, 2 H), 7.72 (s, 1 H), 7.45 (d, J = 8.4 Hz, 2 H), 6.45 (brs, 1 H), 3.87 (t, J = 7 Hz, 2 H), 3.64 (t, J = 7 Hz, 2 H), 2.2 (brs, 1 H); ^{13}C NMR ($CDCl_3$) δ 166.89, 165.66, 157.10, 137.77, 130.48, 129.42, 128.26, 123.27, 61.51, 42.58, 31.88; CIMS m/z (rel intensity) 457/455/453 (MH^+, 65/79/17); HRMS (ESI), m/z 451.8594 M^+, calcd for $C_{13}H_{11}Br_2ClN_2OS$ 451.8596; HPLC purity 96.21%.

(*S*)-4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*-(2,3-dihydroxypropyl)thiazole-5-carboxamide (23). White solid (13 mg, 12%), mp 144−146 °C. ^{1}H NMR (CD_3OD) δ 8.03 (d, J = 8.7 Hz, 2 H), 7.80 (s, 1 H), 7.53 (d, J = 7.8 Hz, 2 H), 3.54 (m, J = 4.9 Hz, 1 H), 3.29 (dd, J = 1.5 and 4.9 Hz, 2 H), 3.21 (dd, J = 1.5 and 4.9 Hz, 2 H); ^{13}C NMR (CD_3OD) δ 166.28, 159.07, 158.18, 143.36, 138.65, 132.21, 130.59, 129.39, 71.70, 65.23, 44.28, 32.71; ESIMS m/z (rel intensity) 485/483/481 (MH^+, 17/46/41); HPLC purity 99.56%.

4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*-(ribityl)thiazole-5-carboxamide (24). White solid (11.9 mg, 86%), mp 217−219 °C. ^{1}H NMR (CD_3OD) δ 8.03 (d, J = 7.5 Hz, 2 H), 7.77 (s, 1 H), 7.52 (d, J = 7.8 Hz, 2 H), 2.98 (s, 3 H), 2.85 (s, 3 H), 2.80 (s, 1 H); ^{13}C NMR (CD_3OD) δ 170.96, 164.87, 159.65, 138.83, 132.26, 130.59, 129.47, 123.25, 74.10, 74.04, 69.97, 64.58, 36.98, 31.90; ESIMS m/z (rel intensity) 548/546/544/542 (MH^+, 17/81/31/53); HRMS (ESI), m/z 542.8996 MH^+, calcd for $C_{16}H_{18}Br_2ClN_2O_5S$ 542.8986; HPLC purity 98.98%.

4-(Dibromomethyl)-2-(4-chlorophenyl)-*N*-(D-glucosyl)thiazole-5-carboxamide (25). White solid (10 mg, 18%), mp 190−191 °C. ^{1}H NMR (CD_3OD) δ 8.24 (d, J = 7.8 Hz, 2 H), 7.82 (s, 1 H), 7.75 (d, J = 7.8 Hz, 2 H), 5.24 (s, J = 3.4 Hz, 1 H), 4.04 (dd, J = 3.5 and 10 Hz, 1 H), 3.89−3.70 (m, 5 H); ^{13}C NMR (CD_3OD) δ 169.07, 163.20, 154.42, 143.82, 136.25, 132.24, 130.56, 129.35, 91.10, 73.34, 72.19, 62.69, 62.19, 56.76. 31.93; ESIMS m/z (rel intensity) 597/595/593 (MNa^+, 87/100/55); HRMS (ESI), m/z 592.8757 MNa^+, calcd for $C_{17}H_{17}Br_2ClN_2O_6SNa$ 592.8755; HPLC purity 98.59%.

Ethyl 2-Chloro-3-(furan-2-yl)-3-oxopropanoate (27c). Sulfuryl chloride (3.3 mmol) was added dropwise to a stirred solution of diketo compounds **26c** (3 mmol) in dry methylene chloride (10 mL). The mixture was vigorously stirred for 1 h. The solvent was evaporated.[20] The product was collected as a colorless to faint-yellow oil and purified by column chromatography, using 6:4 hexane−ethyl acetate. Yellow oil (621 mg, 96%). ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 1 H), 7.47 (s, 1 H), 6.82 (s, 1 H), 5.20 (s, 1 H), 4.85 (q, J = 7.2 Hz, 2 H), 1.26 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 182.80, 164.86, 149.09, 144.38, 109.02, 63.25, 59.75, 13.97; ESIMS m/z (rel intensity) 218/216 (MH^+, 23/100); HRMS (ESI), m/z 216.0184 MH^+, calcd for $C_9H_9ClO_4$ 216.0189; HPLC purity 96.81%.

Preparation of Alkyl 2-(4-Chlorophenyl)-4-alkylthiazole-5-carboxylates (28−31). General Procedure. 4-Chlorothiobenzamide (171 mg, 1 mmol) and α-chlorodiketo derivatives **27a−d** (1.2 mmol) were added to absolute ethanol (15 mL). The reaction mixture

was heated to reflux for 24 h. After removal of solvent under reduced pressure, the residue was purified by silica gel chromatography (hexanes−ethyl acetate 7:3) to provide the desired compounds. The physical and spectral data of products are listed below.

Methyl 2-(4-Chlorophenyl)-4-ethylthiazole-5-carboxylate (28). White solid (277.6 mg, 98%), mp 86−87 °C. ^{1}H NMR ($CDCl_3$) δ 7.93 (d, J = 8.4 Hz, 2 H), 7.20 (d, J = 8.4 Hz, 2 H), 3.96 (q, J = 7.5 Hz, 2 H), 3.89 (s, 3 H), 1.36 (t, J = 7.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 172.21, 166.97, 160.32, 136.90, 131.44, 129.17, 127.94, 120.00, 52.10, 24.31, 13.52; ESIMS m/z (rel intensity) 284/282 (MH^+, 35/100); HRMS (ESI), m/z 282.0360 MH^+, calcd for $C_{13}H_{13}ClNO_2S$ 282.0350; HPLC purity 97.80%.

Methyl 2-(4-Chlorophenyl)-4-isopropylthiazole-5-carboxylate (29). White solid (186 mg, 63%), mp 70−71 °C. ^{1}H NMR ($CDCl_3$) δ 7.92 (d, J = 8.4 Hz, 2 H), 7.39 (d, J = 8.4 Hz, 2 H), 3.98 (m, 1 H), 3.88 (s, 1 H), 1.345 (d, J = 6.9 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 171.00, 168.55, 162.20, 138.09, 133.67, 129.09, 127.95, 120.10, 52.04, 29.10, 21.99; ESIMS m/z (rel intensity) 298/296 (MH^+, 34/100); HRMS (ESI), m/z 296.0509 MH^+, calcd for $C_{14}H_{15}ClNO_2S$ 296.0506; HPLC purity 97.78%.

Methyl 4-*tert*-Butyl-2-(4-chlorophenyl)thiazole-5-carboxylate (30). White solid (270 mg, 87%), mp 105−106 °C. ^{1}H NMR ($CDCl_3$) δ 7.91 (d, J = 8.4 Hz, 2 H), 7.41 (d, J = 8.4 Hz, 2 H), 3.87 (s, 3 H), 1.53 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 171.62, 165.84, 162.00, 136.61, 131.63, 129.10, 127.82, 121.34, 52.27, 36.61, 29.36; ESIMS m/z (rel intensity) 312/310 (MH^+, 27/100); HRMS (ESI), m/z 310.0672 MH^+, calcd for $C_{15}H_{17}ClNO_2S$ 310.0663; HPLC purity 95.63%.

Ethyl 2-(4-Chlorophenyl)-4-(furan-2-yl)thiazole-5-carboxylate (31). 4-Chlorothiobenzamide (256.5 mg, 1.5 mmol) and α-chlorodiketo derivative **27c** (4.33 mg, 2 mmol) were added to absolute ethanol (15 mL). The reaction mixture was heated at reflux for 24 h. After removal of solvent under reduced pressure, the residue was purified by silica gel chromatography (hexanes−ethyl acetate 7:3, then 9:1) to provide the desired compound as a white solid (481 mg, 96%): mp 82−83 °C. ^{1}H NMR ($CDCl_3$) δ 8.59 (s, 1 H), 7.98 (d, J = 8.4 Hz, 2 H), 7.83 (d, J = 8.4 Hz, 2 H), 7.44 (s, 1 H), 7.23 (s, 1 H), 2.47 (q, J = 7.2 Hz, 2 H), 1.40 (t, J = 7.2 Hz, 3 H); ESIMS m/z (rel intensity) 336/334 (MH^+, 19/100); HRMS (ESI), m/z 334.0309 MH^+, calcd for $C_{16}H_{13}ClNO_3S$ 334.0229; HPLC purity 95.33%.

2-(4-Chlorophenyl)-4-(methoxymethyl)-*N*-methylthiazole-5-carboxamide (33). NaOH (4.00 g, 100 mmol) was added to a solution of methyl carboxylate **32** (6.237 g, 21 mmol) in ethanol (40 mL) and water (5 mL). The reaction mixture was heated under reflux for 5 h and then allowed to cool to room temperature. The reaction mixture was filtered, and the pH value of the liquid phase was adjusted to 2 with hydrochloride acid. The solid was filtered and dried to provide an off-white solid (4.980 g, 84%). The crude obtained solid material (4.88 g, 17.2 mmol) was heated under reflux with thionyl chloride (25 mL) for 6 h. The solvent was evaporated under reduced pressure. The brown residue was collected and purified by silica gel flash chromatography, using hexane−ethyl acetate (7:3), to yield the corresponding acid chloride as a white solid (2.507 g, 96%). Methylamine hydrochloride (88 mg, 1.3 mmol) and potassium carbonate (27 mg, 0.5 mmol) were dissolved in water (10 mL), and then the corresponding acid chloride (380 mg, 1.26 mmol) was added after 5 min. The reaction mixture was stirred at room temperature for 1 h. The white flocculant solid was collected by filtration and washed with HCl (0.1 M, 5 mL) and then water (3 × 10 mL). The white solid was further purified by crystallization from EtOAc to yield a white solid (339.4 mg, 91%): mp 113−114 °C. ^{1}H NMR ($CDCl_3$) δ 8.07 (brs, 1 H), 7.86 (d, J = 8.7 Hz, 2 H), 7.41 (d, J = 8.7 Hz, 2 H), 4.78 (s, 2 H), 3.46 (s, 3 H), 2.99 (d, J = 4.8 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 166.05, 161.56, 151.46, 136.78, 135.11, 131.33, 129.25, 127.80, 69.32, 58.04, 26.90; ESIMS m/z (rel intensity) 298/297 (MH^+, 38/100); HRMS (ESI), m/z 297.0464 MH^+, calcd for $C_{13}H_{14}ClN_2O_2S$ 297.0459; HPLC purity 95.00%.

2-(4-Chlorophenyl)-4-formyl-*N*-methylthiazole-5-carboxamide (34). Methyl ether **33** (300 mg, 1 mmol) and NBS (178 mg, 1 mmol) were added to CCl_4 (15 mL). The reaction mixture was heated at reflux for 1 h and was illuminated 4 times by a 60 W bulb for 5 min with time intervals of 15 min. The solvent was removed under reduced pressure. The residue was partitioned between EtOAc (10 mL) and NaOH (0.1 M NaOH, 5 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, and removed under reduced pressure. The semisolid residue was purified by silica gel flash chromatography using hexane−ethyl acetate (3:1) to afford the product as a white solid (56%): mp 231−232 °C. ^{1}H NMR ($CDCl_3$) δ 10.15 (s, 1 H), 9.78 (brs, 1 H), 7.93 (d, J = 8.4 Hz, 2 H), 7.46 (d, J = 8.4 Hz, 2 H), 3.04 (d, J = 4.8 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 188.92, 168.13, 159.29, 147.60, 145.36, 137.68, 130.49, 129.53, 127.96, 29.65; MS m/z (rel intensity) 283/281 (MH^+, 51/100); HRMS (ESI), m/z 281.0157 MH^+, calcd for $C_{12}H_{10}ClN_2O_2S$ 281.0146; HPLC purity 95.13%.

Methyl 2-(4-Chlorophenyl)-4-cyanothiazole-5-carboxylate (36). *Method A.*[23] To a stirred solution of $AlCl_3$ (40 mg. 0.3 mmol) in THF (3 mL) was added NaN_3 (61 mg, 0.94 mmol) followed by aldehyde **35** (44 mg, 0.15 mmol). The mixture was heated to gentle reflux. After 12 h the reaction mixture was diluted with 10% HCl (3 mL) and THF was removed under reduced pressure. The organic layer was extracted with ethyl acetate (3 × 5 mL), dried, and evaporated. The residue was purified by column chromatography on silica gel using a mixture of hexane−ethyl acetate (4:1) to obtain the product (9 mg, 21%).

Method B.[24] A solution of aldehyde **35** (20 mg, 0.07 mmol) and iodine (12.7 mg, 0.1 mmol) in ammonia solution (3 mL of 28% solution) and THF (0.5 mL) was stirred at room temperature for 1 h (until the solution became colorless). Then the reaction mixture was charged with aqueous $Na_2S_2O_3$ (5% solution) followed by extraction with ethyl acetate (2 × 5 mL) to give the crude nitrile **36**, which was purified by column chromatography on silica gel using a mixture of hexane−ethyl acetate (7:3) to yield a white solid (18.5 mg, 99%): mp 142−143 °C. ^{1}H NMR ($CDCl_3$) δ 7.92 (d, J = 8.7 Hz, 2 H), 7.48 (d, J = 8.7 Hz, 2 H), 4.02 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 171.24, 159.01, 138.63, 133.28, 129.65, 129.27, 128.23, 112.49, 53.43; CIMS m/z (rel intensity) 280/278 (M^+, 30/100); HRMS (CI), m/z 278.9992 MH^+, calcd for $C_{12}H_9ClN_2O_2S$ 278.9989; HPLC purity 100%.

Methyl 4-(Dichloromethyl)-2-(4-chlorophenyl)thiazole-5-carboxylate (37)[25]. The aldehyde derivative **35** (25 mg, 0.09 mmol), acetyl chloride (13 μL, 0.178 mmol), and anhydrous aluminum chloride (3.8 mg, 0.028 mmol) were added to dichlorobenzene (3 mL). The reaction mixture was heated at reflux for 24 h. The solvent was taken off under reduced pressure and the orange oily residue was purified by silica gel flash chromatography using hexane−ethyl acetate (7:3) to yield **37** as an off-white solid (29 mg, 99%): mp 133 °C. ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 3 H), 7.98 (d, J = 8.7 Hz, 2 H), 7.46 (d, J = 8.7 Hz, 2 H), 3.95 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 168.07, 160.64, 155.78, 137.79, 130.66, 129.33, 128.30, 124.30, 75.49, 52.96; ESIMS m/z (rel intensity) 304/302/300 (MH^+ − HCl, 25/62.8/100); HRMS (ESI), m/z 299.9650 (M^+ − HCl), calcd for $C_{12}H_7Cl_2NO_2S$ 299.9647; HPLC purity 99.78%.

Methyl 4-(Bromomethyl)-2-(4-chlorophenyl)thiazole-5-carboxylate (38). Methyl ester **2** (1.045 g, 3.9 mmol), NBS (767 mg, 4.3 mmol), and benzoyl peroxide (10 mg) were added to CCl_4 (25 mL). The reaction mixture was heated at reflux for 24 h. After removal of solvent under reduced pressure, the residual NBS was removed by adding saturated aqueous NaOH (20 mL), filtering, and washing with distilled water. The collected yellowish-white precipitate was purified by silica gel chromatography (ethyl acetate−hexanes 1:4) to provide the desired compound as a white solid (676 mg, 50%): mp 151−152 °C.^{1}H

NMR ($CDCl_3$) δ 7.92 (d, J = 8.7 Hz, 2 H), 7.43 (d, J = 8.7 Hz, 2 H), 4.97 (s, 2 H), 3.93 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 169.45, 161.37, 158.71, 137.55, 130.86, 129.36, 128.12, 124.05, 52.68, 24.80; ESIMS m/z (rel intensity) 349/347/345 (MH^+, 11/38/29); HRMS (ESI), m/z 344.9229 M^+, calcd for $C_{12}H_9BrClNO_2S$ 344.9226; HPLC purity 100%.

Methyl 2-(4-Chlorophenyl)-4-[(methylsulfonyl)methyl]thiazole-5-carboxylate (39). Compound **38** (46 mg, 0.133 mmol) and sodium methylsulfinate (24 mg, 0.26 mmol) were added to absolute ethanol (3 mL). The reaction mixture was heated at reflux for 12 h. After removal of solvent under reduced pressure, the off-white solid was partitioned between EtOAc (5 mL) and water (10 mL). The organic layer was separated, dried over $MgSO_4$, and evaporated under reduced pressure. The collected solid material was purified by crystallization from MeOH−EtOAc to provide the product as a white solid (38.1 mg, 83%): mp 178−179 °C. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 8.4 Hz, 2 H), 7.44 (d, J = 8.4 Hz, 2 H), 5.01 (s, 2 H), 3.93 (s, 3 H), 3.09 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 169.80, 161.41, 150.42, 137.83, 130.62, 129.48, 128.06, 126.68, 55.24, 52.88, 41.33; CIMS m/z (rel intensity) 347/345 (M^+, 4/19), 268/266 ($M^+ - CH_3SO_2$, 39/100); HRMS (CI), m/z 344.9902 M^+, calcd for $C_{13}H_{12}ClNO_4S$ 344.9896; HPLC purity 96.02%.

Bioassay Methods. *BHK Cells.* BHK-15 cells obtained from the American Type Culture Collection (ATCC, Rockville, MD) were maintained in MEM (Invitrogen, Carlsbad, CA) containing 10% FBS. Cells were grown in incubators at 37 °C in the presence of 5% CO_2.

YFV-IRES-Luc. A firefly luciferase reporter gene was inserted into pYF23, a derivative of pACNR that is the full-length cDNA clone of YFV 17D, to construct YFV-IRES-Luc, a luciferase-reporting full-length virus. To facilitate this construction, an NsiI restriction site was introduced at the beginning of the 3′NTR immediately following the UGA termination codon of NS5 in pYF23 using standard overlapping PCR mutagenesis. To construct YFV-IRES-Luc, an IRES-FF.Luc (EMCV IRES-fire fly luciferase) cassette was amplified by PCR from YFRP-IRES-Luc, a YFV replicon, and inserted into the NsiI restriction site.[26]

Generation of YFV-IRES-Luc Virus. In vitro transcribed YFV-IRES-Luc RNA was transfected into BHK-15 cells using Lipofectamine (Invitrogen, Carlsbad,CA). At 4 days post-transfection, the resulting YFV-IRES-Luc virus was harvested and the titer of the virus determined by a standard plaque assay. The infectivity of the virus could be assayed directly as a measure of the luciferase amounts produced in infected cells over a period of time.

Inhibition of YFV-IRES-Luc Virus Growth. BHK cells were plated in a 96-well plate and grown at 37 °C. At confluency, cells were infected with YF-IRES-Luc virus at a multiplicity of infection (MOI) of 0.1. A low MOI was utilized to ensure that fewer cells were infected so that the spread of released virus could be monitored. Cells were then overlaid with culture medium containing serial dilutions of compounds at concentrations below the GI_{50} values. Controls included uninfected cells, infected cells and DMSO-treated infected cells. Cells were incubated at 37 °C, 5% CO_2 for ∼36 h and lysed using 50 μL of cell culture lysis buffer (Promega Inc., Madison, WI), and 10 μL of cell extracts was placed into a 96-well opaque plate. Luciferase activity was determined from the luminescence generated with firefly luciferase substrate (Promega Inc., Madison, WI). Luminescense was measured in a 96-well-plate luminometer, LMax II (Molecular Devices, Sunnyvale, CA). A reduction in luciferase activity indicates inhibition of YFV-IRES-Luc virus growth. The luciferase luminescence as a function of compound concentration was analyzed by nonlinear regression analysis using GraphPadPrizm to estimate the IC_{50} of each compound. The IC_{50} was defined as the concentration of the compound that causes 50% reduction of luciferase activity in infected cells compared to the DMSO-treated cells.

Cell Viability Assay. BHK cells were plated in a 96-well plate and grown at 37 °C. At confluency, cells were overlaid with culture medium containing serial dilutions of compounds (compound stocks were generated by dissolving compounds in DMSO). Untreated and DMSO-treated cells served as positive controls. Cells were then incubated at 37 °C, 5% CO_2 for ∼36 h. At ∼36 h post-treatment, medium on cells was replaced with fresh medium to remove the compounds. Then 10 μL of XTT-substrate from the Quick Cell Proliferation Kit (Biovision Inc., CA) was added to each well. Cells were incubated at 37 °C for a further 2 h. Plates were then removed, and OD_{450} was measured using a 96-well plate reader (Molecular Devices, Sunnyvale, CA). The OD_{450} value for cells treated with a compound was compared to that obtained from cells treated with 1% DMSO, and the GI_{50} for each compound was calculated.

Molecular Modeling. *Molecule Construction and Energy Optimization.* Compounds **1**, **23**−**25**, and **39** were built with Sybyl 7.1 software and minimized to 0.01 kcal/mol by the Powell method, using Gasteiger−Hückel charges and the Tripos force field. To save calculation time, solvents were not taken into account, and instead the dielectric constant was set to a value of 4 to mimic the aqueous environment. The minimized molecules underwent 10 rounds of simulated annealing to search for the optimized conformation. During the simulation process, the starting conformation in each round was heated to 700 K within 1000 fs and then cooled to 200 K in the same period. Conformations were recorded at each temperature level (700 and 200 K). The conformers located at the starting point at the each round of simulation were selected for further energy refinement using the same parameter set as the ones in molecular construction. The minimized conformer with the lowest energy was selected as the optimized conformation of the molecule which was docked into the β-OG binding pocket of the yellow fever virus E-protein (PDB code 1OKE).

Docking Simulation. The energy-optimized compounds were docked into the β-OG binding domain in the E-protein of the dengue virus after removal of the *n*-octyl-β-D-glucoside (β-OG). The parameters were set as the default values for GOLD. The maximum distance between hydrogen bond donors and acceptors for hydrogen bonding was set to 3.5 Å. After docking, the first pose conformation of compounds **1**, **23**−**25**, and **39** were merged into the ligand-free protein. The new ligand−protein complex was subsequently subjected to energy minimization using the Amber force field with Amber charges. During the energy minimization, the structure of the compounds of interest and only chain A of the viral E protein were allowed to move. Chain B was kept frozen. The energy minimization was performed using the Powell method with a 0.05 kcal/(mol Å) energy gradient convergence criterion and a distance dependent dielectric function.

In Vitro Hydrolytic Stability Assay Utilizing Rat Plasma. Compounds **1** and **16** were tested for their hydrolytic stability in solutions of reconstituted rat plasma. Compounds **1** and **16** (15−5 μmol) and 7 μmol of 4-bromopyrazole as an internal standard were dissolved in DMSO (1.0 mL). This solution was filtered through a 0.45 μM filter (Millex-HN). Lyophilized rat plasma (1.0 mL) (lot no. 048K7420, Sigma Chemical Co., St. Louis, MO) was reconstituted with water of HPLC (1.0 mL). The plasma solution was incubated at 37 °C for 15 min and was then diluted with 0.01 M saline (0.250 mL) to afford an 80% plasma solution. The plasma solution was incubated again at 37 °C for an additional 5 min. An aliquot of the compounds **1** and **16** in DMSO (100 μL) was added to the rat plasma (0.75 mL), and the mixture was incubated at 37 °C throughout the course of the experiment. Aliquots (10 μL) of the compound−plasma mixture were collected at various time intervals and diluted with methanol (90 μL) to precipitate any proteins present. The aliquots were mixed and centrifuged at 10 000 rpm for 5−10 min to pellet the precipitated proteins. After centrifugation, the supernatants (20 μL) of the aliquots were analyzed by HPLC to determine the residual amount of tested compounds present in the sample. The aliquot supernatants were analyzed using a Waters binary HPLC system (model 1525, 10 μL injection loop) and a Waters dual wavelength absorbance UV detector (model 2487) set for 254 nm. Data were collected and

processed using the Breeze software (version 3.3) on a Dell Optiplex GX280 personal computer. The mobile phase consisted of 85:15 (v/v) methanol/water, and the Sunrise HPLC column (4.6 mm × 150 mm) was packed with C18 silica from Waters. The column was maintained at room temperature during the analyses. The half-lives of **1** and **16** were calculated from regression curves fitted to plots of the compound concentration versus time.

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ABBREVIATIONS USED

BHK, baby hamster kidney cells; β-OG, *n*-octyl-β-D-glucoside; DMF-DMA, *N*,*N*-dimethylformoxide dimethyl acetal; EMCV, encephalomyocarditis virus; E-protein, Envelop-protein; FBS, fetal bovine serum; Luc, luciferase; MEM, minimal essential medium; NBS, *N*-bromosuccinimide; NCSe, *N*-chlorosuccinimide; PCR, polymerase chain reaction; SAR, structure−activity relationship; TI, therapeutic index; YFV, yellow fever virus; IRES, internal ribosome entry site

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