Flavivirus envelope proteins (E proteins) have been shown to play a pivotal role in virus assembly, morphogenesis, and infection of host cells. Inhibition of flavivirus infection of a host cell by means of a small molecule envelope protein antagonist is an attractive strategy for the development of antiviral agents. Virtual screening of the NCI chemical database using the dengue virus envelope protein structure revealed several hypothetical hit compounds. Bioassay results identified a class of thiazole compounds with antiviral potency in cell-based assays. Modification of these lead compounds led to a series of analogues with improved antiviral activity and decreased cytotoxicity. The most active compounds 11 and 36 were effective in the low micromolar concentration range in a cellular assay system.

Introduction

Many flaviviruses are arthropod-borne, human pathogens that can cause encephalitis, hemorrhagic fever, shock syndrome, and jaundice. Examples of pathogenic flaviviruses include dengue virus, yellow fever virus, West Nile virus, tick-borne encephalitis virus, and Japanese encephalitis virus. A number of antiflaviviral agents with a wide variety of putative targets have been reported, including those that act on viral membranes, 1 translation, 2 c-Src protein kinase, 3 RNA polymerase, 4 α-glucosidase, 5,6 mRNA, 7 cellular IMP dehydrogenase, 8 2′-O-methyltransferase, 9 and RNA-dependent RNA polymerase. 10 In addition, peptide antiviral agents targeting West Nile virus envelope protein (E protein) have been reported to be active against both dengue virus and West Nile virus. 11 However, at the present time, there are no clinically useful antiflaviviral agents.

The flaviviruses infect host cells through receptor-mediated endocytosis to form a virus-containing endosome, followed by fusion of the viral envelope with the host cell membrane. 12 The fusion process is orchestrated by the viral E proteins, which are made up of three domains: domain I (DI), the middle domain, is attached at one end to domain II (DII), the fusion domain, and at the other end contacts domain III (DIII), the receptor binding domain (Figure 1). 13 Domain II contains the fusion peptide loop near its tip, which is inserted into the host cell membrane after the E protein undergoes a series of conformational changes.

Cryo-electron microscopy and X-ray crystallography studies of various flavivirus E protein fragments have revealed that domains I and II are connected by a mobile hinge that allows rotation of DII relative to DI—DIII during the processes of viral maturation and fusion of the viral envelope with the host cell membrane. 14—17 Mutations that affect the pH threshold for either fusion or virulence are clustered in this hinge region. 18—23 Furthermore, crystallization of a dengue virus type 2 E protein fragment in the presence of the detergent n-octyl-β-D-glucoside (β-OG) has led to structures with and without occupation of the hinge pocket by β-OG. 14 Occupation of the pocket by β-OG requires an altered conformation of the k loop toward an “open” position, and the angle that DII makes with DIII also differs between two crystal structures with and without β-OG. 14 The β-OG pocket may therefore represent an ideal target for structure-based design of potential antiviral agents because ligands that bind there could alter the conformational equilibrium associated with the hinge angle and inhibit virus maturation. 14,15 Moreover, the effect on hinge equilibrium could interfere with the fusion of the viral envelope with the host cell membrane.

Computational screening of an NCI library using the β-OG binding pocket recently identified a small set of compounds that were predicted to bind in the pocket. An array of 23 compounds were selected for antiviral testing vs yellow fever virus replication. Of these, nine compounds inhibited viral growth and several of them had EC50 values in the micromolar range and were potent enough to warrant further consideration. These
three hit compounds, M02, P02, and D03 (Figure 2), provided a starting point for lead optimization in the present study.

Design

The binding models derived from docking results indicate that compounds M02 and P02 might bind to the E protein β-OG binding site in a similar way. An overlay of their docked models suggested that these molecules occupy a pocket that is located in the hinge region between domains I and II (Figure 1). Computational analysis revealed that the middle part of the pocket is the most hydrophobic and the entrance part is the most hydrophilic (Figure 3). The bottom region of the pocket contains some hydrophilic amino acid residues that could interact with ligands through electrostatically favorable interactions as in the case of P02. One end of the ligand can reach into the pocket, and electrostatically favorable interactions could be formed between the aminoguanidine groups with Thr280 in domain I and Asp203, Gln200, Gln271, and Ser274 in domain II (Figure 4B). Topologically, the entrance of the pocket shows a rather wide space in which the ends of the hit compounds assume various positions, whereas the middle part of the pocket has the most limited space.

An analysis of the protein–ligand interaction suggests that tight-binding inhibitors might be obtained by grafting half of the structure of P02 onto half of the structure of M02. Driven by the hypothesis that the aminoguanidine group in P02 can potentially form electrostatically favorable interactions with the residues in the receptor pocket, one-half of the structure of P02 was incorporated by connecting it to M02’s thiazole ring, which is a common structural feature that many of the hits contain. This contributed to the design of the first two target compounds 1 and 2 (Figure 5). The double bond in the α,β-unsaturated ketone present in inhibitor M02 is electrophilic and could react nondiscriminately with biological nucleophiles, thus resulting in toxicity. Accordingly, in the design of the first two potential inhibitors, this moiety was replaced with a bioisosteric amide linkage. The Figure 5 depicts compounds 1, 2, and their analogues 3–8, which have been synthesized and bioassayed.

According to the docked models of the hit M02, one of the two phenyl rings attached to the 3,5-diphenyl-1H-pyrazole was calculated to point to an independent hydrophobic region. This phenyl ring has been thought to be redundant and was deleted in our first generation drug design because no comparable partial structures in the other hits can overlay with this group in the docked models. However, considering the fact that this phenyl ring may nevertheless play an important role in the hit’s antiviral activity, a class of compounds based on the structure of the hit M02 has been designed, synthesized, and bioassayed (Figure 6). The only modification in structure 11 is that the α,β-unsaturated ketone in M02 has been replaced by a bioisosteric amide, which was based on the same considerations with regard to toxicity as described above. To clarify the role of the 3,5-diphenyl-1H-pyrazole moiety of M02 in its antiviral activity, 3,5-diphenyl-1H-pyrazole (17) itself was tested for antiviral activity.

By comparing the docked models of P02 and M02, a class of branched compounds (18, 19, and 20, Figure 7) was designed, synthesized, and bioassayed. These compounds can be considered as analogues in which a derivative of the substituted thiazole moiety of M02 has been attached to a fragmented analogue of P02.

Another class of compounds was designed to mimic the hit D03, which provides a convenient template for diversification. An array of analogues of D03 shown in Figure 8 has therefore been synthesized and bioassayed. It was expected that these structurally related compounds could provide useful SAR information.

A final set of compounds was derived from fragments of M02 and D03 (Figure 9). The phenylthiazole moiety of D03 was attached to an analogue of the 3,4-dichlorostyryl moiety of M02, resulting in 34. Introduction of bromine into the methyl group of 32 led to the dibromosubstituted compound 36. This compound, showing unexpected stability, presented an interesting chemical probe to test the influence of electronic and steric effects on antiviral activity.

Synthesis

The synthetic route to compounds 1–8 is outlined in Scheme 1. Radical bromination of methyl 3-oxobutanoate (37) was used to construct methyl 2-bromo-3-oxobutanoate (38), which reacted with thiourea at room temperature to give rise to the thiazole intermediate. 2-amino-4-methylthiazole-5-carboxylic acid methyl ester (39). Reaction of this intermediate with 1-(4-isocyanatophenyl)ethanone in pyridine afforded compound 3. Hydrolysis of 3 with sodium hydroxide to the corresponding acid 4 and then amidation with 2,4-dichloro- or 3,4-dichlorobenzylamine mediated by 1-ethyl-3-(3’-dimethylaminopropyl)carbodiimide (EDCI) readily provided the desired compounds 7 and 8. Target hydrazones 1 and 2 were synthesized by reacting 7 with semicarbazide hydrochloride or aminoguanidine hydrochloride, respectively. Compounds 5 and 6 were prepared from 3 similarly.

Compounds 9–16 were synthesized through the route shown in Scheme 2, in which a copper-catalyzed N-arylation reaction of the pyrazole 17 is the key step. The reaction is highly sensitive to air. In our experiments, the reaction was conducted in a sealed pressure vessel filled with argon gas. Conversions of 10 to 11–16 were performed smoothly.

The branched potential inhibitors 18–20 were made as outlined in Scheme 3. Bisalkylation of 2-amino-4-methylthiaz-
ole-5-carboxylic acid methyl ester (39) with 1-(4-(bromomethyl)-phenyl)ethanone (41) was performed with sodium hydride as the base to afford 18. In the presence of ethyl acetate, the major product of the reaction was 20. Compound 18 was further converted to 19 through the acid 42 via a hydrolysis–amidation sequence similar to the synthetic route to compounds 10 and 11.

Analogues of D03 were prepared following the routes depicted in Scheme 4. Heterocyclic ring closure of 4-chlorothiobenzamide (43) with 1-chloroacetone (44) afforded compound 21. Reaction of 1,3-dichloroacetone (45) with aromatic thioamides, followed by nucleophilic substitution of the chloride intermediates with thiophenols, readily provided 23–30 in good yield.28,29

Potential inhibitors 31–36 were obtained by the route detailed in Scheme 5. Heterocyclic ring closure of arylthioamides with 2-chloro-3-oxo-butyric acid methyl ester (46)30,31 furnished compounds 31–33. Hydrolysis of 32 to the corresponding acid and then amidation or esterification afforded compounds 34 and 35. Bromination of 32 with NBS provided the dibromosubstituted product 36.

Biological Results and Discussion

All of the compounds were evaluated in a yellow fever virus inhibition assay (see Experimental Section). The compounds that showed over 50% inhibitory activity on viral replication at a concentration of 50 µM were considered to be active and were tested to determine their EC50 and CC50 values. The biological data are summarized in Table 1.

Bioassay results showed that compounds 2, 3, and 8 exhibited better antiviral activity against yellow fever virus replication in a cellular bioassay system than the parent compounds M02, but they were not more potent than P02. The results suggest that a core structure consisting of a thiazole ring and an aromatic ring linked by a urea moiety could be a promising structural template for potential antiviral agents. The activity of 3 appears to be structurally specific. For instance, compound 3 lost its activity upon being hydrolyzed to the corresponding acid 4. In addition, compounds 1 and 7 are inactive vs the active compounds 2 and 8, even though the structures are very similar. Although the bioassay results revealed several active compounds in this class, their potencies are still moderate.

Although most of the compounds in the 9–16 array did not show over 50% inhibition of virus production at a concentration of 50 µM, the target molecule 11 showed enhanced antiviral activity and decreased cytotoxicity compared with the parent compound M02. The antiviral activity of 11 (EC50 5 ± 1.7 µM) is 10 times that of parent compound and cytotoxicity is 2.7 times less, resulting in a therapeutic index of 37. These data validate

Figure 3. Lipophilic potential analysis of the E protein β-OG binding site showing the entrance region is relatively more hydrophilic and the middle region is the most lipophilic region. The lipophilic potential ranges from −0.241 (blue, hydrophilic) to 0.147 (brown, hydrophobic). The left picture views the pocket from entrance side, and the right picture views the pocket from the bottom side. The red stick model is P02 and the stick model colored by atom type is M02.

Figure 4. (A) Two active compounds docked in the β-OG pocket of dengue E protein. The structure colored magenta is P02, and the structure colored blue is M02. (B) Potential electrostatically favorable interactions formed between residues Asp203, Gln200, Gln271, Ser274, Thr48, and Thr280 with P02. Part of the protein is shown in cartoon representation with red color for domain I and yellow color for domain II.
the hypothesis that replacing the Michael acceptor in M02 with a bioisosteric amide would reduce the undesired cytotoxicity. The weak antiviral activities of the other analogues may indicate that an aromatic substituent attached to the thiazole ring through a stable linker such as an amide is necessary for optimal antiviral activity. The hydrolytic instability of the ester linker could explain why the ester analogues, such as compounds 12–16, did not display antiviral activity. Obviously, the amide 11 should be more stable than the esters to enzymatic hydrolysis. Testing the contribution of the 3,5-diphenyl-1H-pyrazole moiety to antiviral activity of M02 revealed that the 3,5-diphenyl-1H-pyrazole (17) fragment is at least as active as the parent M02 (EC50 44 vs 51 µM).

Bioassay results showed that the branched compound 18 is active against yellow fever virus, with an EC50 value of 25 µM. The results suggest that replacement of the diphenylpyrazole moiety of structure M02 with tertiary amines could be a possible strategy to develop more potent flavivirus inhibitors.

Compounds 23–27 mimicking D03 exhibited antiviral activity at 30–50 µM range, which is comparable to the activity of D03 itself (EC50 31 µM). It seems that the overall physical–chemical properties of the compounds are the determining factor for their biological activity. Slight modifications in structure do not cause a significant change in antiviral activity. A preliminary SAR analysis suggested that an electron-rich aromatic ring attached to a thiazole ring that is linked to a 2- or 4-substituted aniline group may represent a template for antiviral agents. The presence of a pyridine

Figure 5. The first generation of designed molecules 1 and 2 and analogues.

Figure 6. Analogues of M02.
ring, such as in compounds 28 and 29, or the absence of the aniline group, such as in compound 30, causes these analogues to lose antiviral activity.

The most active compound was 36, which showed 99% inhibition of yellow fever virus and dengue virus replication at the initial 50 \( \mu \)M concentration tested. The EC\(_{50}\) value of the compound is 0.9 \( \mu \)M vs yellow fever virus replication. Encouragingly, although this compound contains a dibromomethyl substituent on the thiazole ring that was considered to be likely to cause toxicity by alkylating biological nucleophiles, it was in fact not very cytotoxic (CC\(_{50}\) 153 \( \mu \)M), resulting in a good therapeutic index of 170. Obviously, this compound provides an intriguing lead that deserves further study. Comparing the structure of 36 with the others in this class, it seems that the dibromomethyl substituent is important for antiviral activity. Compound 32, the synthetic precursor of 36, is inactive against yellow fever virus and it does not contain the dibromomethyl substituent.

The most promising compounds 11 and 36 were docked in the dengue virus E protein/\( \beta\)-OG binding pocket in order to

\[ \text{Scheme 1} \]

\[
\begin{align*}
\text{37} & \xrightarrow{a} \text{38} \\
\text{b} & \rightarrow \text{39} \\
\text{c} & \rightarrow \text{3} \xrightarrow{d} \text{5 or 6} \\
\text{e} & \rightarrow \text{7} \xrightarrow{f} \text{7 or 8} \\
\text{7} & \rightarrow \text{1 or 2}
\end{align*}
\]

\( ^a \) Reagents and conditions: (a) NBS, benzene, AIBN, RT for 2 h, 100%; (b) thiourea, ethanol, RT for 3 h, 81%; (c) 1-(4-isocyanatophenyl)ethanone, pyridine, RT for 12 h, 83%; (d) semicarbazide hydrochloride for 5 or aminoguanidine hydrochloride for 6, ethanol, triethylamine for 5, AcOH, heated to reflux for 6 h, 70% for 5, and 65% for 6; (e) NaOH, EtOH, MeOH, heated to reflux for 3 h, 73%; (f) 2,4-dichlorobenzylamine for 7 or 3,4-dichlorobenzylamine for 8, EDCI, pyridine, RT for 12 h, 60–70%; (g) semicarbazide hydrochloride for 1 or aminoguanidine hydrochloride for 2, ethanol, triethylamine for 1, AcOH, heated to reflux for 6 h, 73% for 1, and 67% for 2.

The most active compound was 36, which showed 99% inhibition of yellow fever virus and dengue virus replication at the initial 50 \( \mu \)M concentration tested. The EC\(_{50}\) value of the compound is 0.9 \( \mu \)M vs yellow fever virus replication. Encouragingly, although this compound contains a dibromomethyl substituent on the thiazole ring that was considered to be likely to cause toxicity by alkylating biological nucleophiles, it was in fact not very cytotoxic (CC\(_{50}\) 153 \( \mu \)M), resulting in a good therapeutic index of 170. Obviously, this compound provides an intriguing lead that deserves further study. Comparing the structure of 36 with the others in this class, it seems that the dibromomethyl substituent is important for antiviral activity. Compound 32, the synthetic precursor of 36, is inactive against yellow fever virus and it does not contain the dibromomethyl substituent.

The most promising compounds 11 and 36 were docked in the dengue virus E protein/\( \beta\)-OG binding pocket in order to
gain insight into their probable binding modes (Figure 10). These two compounds may bind in the ∞-OG binding pocket in an area surrounded by amino acid residues including Thr280, Val130, Leu198, Gln200, Ala205, Ile270, Ser274, Gln271, Leu277, Lys47, Thr48, Glu49, Leu135, Ala50, and Thr137. The entire binding site forms a channel. Compound 11 places its 3,5-diphenyl-1H-pyrazole group in the middle region, leaving its amide tail positioned in the entrance region. It is still unclear what role the second phenyl ring of the 3,5-diphenyl-1H-pyrazole in compound 11 plays in its antiviral activity. The docked model suggests that this phenyl ring, with its bulky size, could block the compound from docking deeply into the channel. The smaller compound 36 has been suggested by docking simulation to bind deeply into the channel. The binding could be tighter than other compounds binding to the entrance and middle regions, thus resulting in stronger potency. The dibromomethyl substituent of compound 36, as the biological data indicate, is likely to be a key structural element for the antiviral activity. One bromine atom may hinder nucleophilic displacement of the other bromine atom by biological nucleophiles, thus lowering the cytotoxicity. On the other hand, the docked model suggests that one of the bromine atoms may fit a small region between the entrance region and the middle region. The nonbonded interactions obtained from this extra binding may result in stronger biological activity.

**Conclusion**

Although the active compounds have variable structures, many of them share the common feature of a central thiazole ring. SAR analysis suggests that the hits M02, P02, and D03 indeed provide promising structural templates for potential antiflaviviral agents. Replacing the Michael acceptor in M02 with an amide in compound 11 significantly enhanced antiviral activity and diminished the undesired cytotoxicity. Alteration of the chloro and amino substitution pattern of the lead compound D03 provided equally potent derivatives 23-27.

The biological testing results indicate that compounds 11 and 36 inhibit yellow fever virus replication with EC50 values in the low micromolar range. Clearly, the preliminary antiviral potency displayed by these thiazole derivatives warrants further investigation. Future work will focus on modification of two
or nitrogen atmosphere, unless otherwise specified. All reactions were conducted under argon (triplet), and m (multiplet). All reagents and dry solvents were therapeutically useful antiviral agents.

Supporting Information. Proton (1H NMR) and carbon (13C NMR) spectra were recorded in CDCl3 or DMSO-d6. The EC50 is the concentration of the compound resulting in a 50% growth inhibition of uninfected BHK cells. The CC50 is the concentration of the compound resulting in a 50% growth inhibition of infected BHK cells. The TI is the therapeutic index and is equal to CC50/EC50.

Table 1. Antiviral Activities vs Yellow Fever Virus and Cytotoxicities of the Original Hits and Second Generation Compounds

<table>
<thead>
<tr>
<th>compound</th>
<th>EC50 (μM)a</th>
<th>CC50 (μM)b</th>
<th>TIc</th>
</tr>
</thead>
<tbody>
<tr>
<td>M02</td>
<td>51 ± 7.2</td>
<td>70 ± 12</td>
<td>1.37</td>
</tr>
<tr>
<td>P02</td>
<td>13 ± 0.3</td>
<td>398 ± 0.1</td>
<td>30.6</td>
</tr>
<tr>
<td>D03</td>
<td>31 ± 0.3</td>
<td>61 ± 3</td>
<td>4.1</td>
</tr>
</tbody>
</table>

- a The EC50 is the concentration of the compound resulting in a 50% inhibition in virus production.
- b The CC50 is the concentration of the compound causing a 50% growth inhibition of uninfected BHK cells.
- c TI is the therapeutic index and is equal to CC50/EC50.

of these structural templates, as well as NMR and crystallographic studies of drug–receptor complexes, testing vs drug-resistant mutant viruses, and evaluation of efficacy in animal models. On the basis of the data in hand, there is every reason to hope that derivatives of 11 or 36 could be developed into therapeutically useful antiviral agents.

Experimental Section

Experimental procedures for the syntheses of compounds 12–16, 23–31, 33, and 34, including analytical data, are reported in Supporting Information. Proton (1H NMR) and carbon (13C NMR) spectra were recorded in CDCl3 or DMSO-d6 on a Bruker ARX-300 or on a Bruker AVANCE 500 spectrometer. The NMR solvents are specified individually for each compound. Chemical shifts are given in parts per million (ppm) on the delta (δ) scale. The solvent peak or the internal standard tetramethylsilane were used as reference values. For 1H NMR: CDCl3 = 7.27 ppm, TMS = 0.00 ppm. Multiplicities were recorded as s (singlet), d (doublet), t (triplet), and m (multiplet). All reagents and dry solvents were purchased from Aldrich. All reactions were conducted under argon or nitrogen atmosphere, unless otherwise specified.

(E)-2-(3-(4-(1-(2-Carbamoylhydrazono)ethyl)-phenyl)-ureido)-N-(2,4-dichlorobenzyl)-4-methylthiazole-5-carboxamide (11). Semi-carbazide hydrochloride salt (20 mg, 0.22 mmol) was added to ethanol (5 mL). Et3N was added dropwise to this mixture until it became a clear solution. A solution of 2-(3-(4-acetylphenyl)-ureido)-N-(2,4-dichlorobenzyl)-4-methylthiazole-5-carboxamide (1) (100 mg, 0.21 mmol) in ethanol (5 mL) was added to the reaction solution followed by acetic acid (0.1 mL). The reaction mixture was heated to reflux for 6 h. The reaction mixture was allowed to cool to room temperature. After removal of solvent under reduced pressure, the resulting residue was washed with cold ethyl acetate (1 mL × 3) and then recrystallized from ethanol to provide the desired compound as a white solid (80 mg, 73%): mp 184 184°C. 1H NMR (500 MHz, DMSO-d6): δ 2.46 (s, 3 H), 2.12 (s, 3 H). 13C NMR (125 MHz, DMSO-d6): δ 162.3, 159.0, 157.7, 143.9, 139.6, 130.2, 131.2, 130.3, 132.0, 134.0, 128.7, 127.7, 127.0 × 2, 118.2 × 2, 40.5, 17.1, 13.4. IR (KBr) 3271, 2922, 2851, 1667, 1531, 1454, 1373, 1204, 1185 cm-1. MS (ES+) calcd for C22H23Cl2N8O2S 533.10, found 532.88. HRMS (ES+) calced for C22H23Cl2N8O2S 533.1042, found 533.1040.

(E)-2-(3-(4-(1-(2-Carbamimidoylhydrazono)ethyl)-phenyl)-ureido)-N-(2,4-dichlorobenzyl)-4-methylthiazole-5-carboxamide (2). Aminoguanidine hydrochloride salt (20 mg, 0.22 mmol), 2-(3-(4-acetylphenyl)-ureido)-N-(2,4-dichlorobenzyl)-4-methylthiazole-5-carboxamide (7) (100 mg, 0.21 mmol), and acetic acid (0.1 mL) were added to ethanol (5 mL). The reaction mixture was heated to reflux for 6 h and allowed to cool to room temperature. After removal of solvent under reduced pressure, the resulting residue was washed with cold ethyl acetate (1 mL × 3) and then recrystallized from ethanol to provide the desired compound as a white solid (60 mg, 62%): mp 177°C. 1H NMR (500 MHz, DMSO-d6): δ 11.4 (s, 1 H), 107.2 (s, 1 H), 8.52 (s, 1 H), 7.90 (d, J = 9.0 Hz, 2 H), 7.89 (s, 4 H), 7.56 (d, J = 2.0 Hz, 1 H), 7.49 (d, J = 9.0 Hz, 2 H), 7.39 (d, J = 8.5 Hz, 1 H), 7.33 (d, J = 8.5 Hz, 1 H), 4.38 (d, J = 5.5 Hz, 2 H), 2.46 (s, 3 H), 2.30 (s, 3 H). 13C NMR (125 MHz, DMSO-d6): δ 162.4, 158.5, 156.5, 152.1, 151.4, 140.5, 136.0, 133.1, 132.4, 130.4, 128.8, 127.9 × 2, 127.6, 117.7 × 2, 117.4, 40.5, 17.2, 14.9. IR (KBr) 1329, 1270, 913, 700 cm-1. MS (ES+) calcd for C22H23Cl2N8O2S 533.10, found 532.88. HRMS (ES+) calced for C22H23Cl2N8O2S 533.1042, found 533.1040.

Methyl 2-(3-(4-Acetylphenyl)ureido)-4-methylthiazole-5-carboxylate (3). 1-(4-Isocyanatophenyl)ethanone (500 mg, 3.1 mmol) was added to a solution of methyl 2-amino-4-methylthiazole-5-carboxylate (4) (550 mg, 3.1 mmol) in pyridine (15 mL). The reaction mixture was stirred at room temperature for 12 h. The solid was filtered and washed with cold methanol (5 mL × 3) to provide the desired compound as a white solid (876 mg, 83%): mp 232°C. 1H NMR (500 MHz, DMSO-d6): δ 9.51 (s, 1 H), 9.24 (s, 1 H), 8.12 (d, J = 8.5 Hz, 1 H), 7.49 (d, J = 8.5 Hz, 2 H), 7.39 (d, J = 8.5 Hz, 1 H), 7.33 (d, J = 8.5 Hz, 1 H), 4.38 (d, J = 5.5 Hz, 2 H), 2.46 (s, 3 H), 2.30 (s, 3 H). 13C NMR (125 MHz, DMSO-d6): δ 162.4, 158.5, 156.5, 152.1, 151.4, 140.5, 136.0, 133.1, 132.4, 130.4, 128.8, 127.9 × 2, 127.6, 117.7 × 2, 117.4, 40.5, 17.2, 14.9. IR (KBr) 1329, 1270, 913, 700 cm-1. MS (ES+) calcd for C22H23Cl2N8O2S 533.10, found 532.88. HRMS (ES+) calced for C22H23Cl2N8O2S 533.1042, found 533.1040.

(E)-Methyl 2-(3-(4-(1-(2-Carboxamidomethyl)hydrazono)-ethyl)-phenyl)-ureido)-4-methylthiazole-5-carboxylate (5). Semicarbazide hydrochloride salt (111 mg, 1 mmol) was added to ethanol (5 mL). Et3N was added dropwise to this mixture until it became a clear solution. A solution of methyl 2-(3-(4-acetylamino)-ureido)-4-methylthiazole-5-carboxylate (3) (333 mg, 1 mmol) in ethanol (10 mL) was added to the reaction mixture, followed by acetic acid (0.5 mL). The reaction mixture was heated to reflux for 3 h and allowed to cool to room temperature. After removal of solvent under reduced pressure, the resulting residue was washed with cold ethyl acetate (1 mL × 3) and then recrystallized from ethanol to provide the desired compound as a white solid (323 mg, 83%): mp 265–267 °C. 1H NMR (500 MHz, DMSO-d6): δ 9.82 (s, 1 H), 9.21 (s, 1 H), 7.77 (d, J = 8.5 Hz, 2 H), 7.44 (d, J = 8.5 Hz, 2 H), 6.45 (s, 2 H), 3.72 (s, 3 H), 2.48 (s, 3 H), 2.11 (s, 3 H). 13C NMR (125 MHz, DMSO-d6): δ 162.9, 161.0, 157.7, 152.0, 144.3, 139.0, 133.1, 130.4, 127.0 × 2, 118.3 × 2, 117.9, 52.0, 17.2, 13.4. IR (KBr) 2382, 2300, 1698, 1535, 1493, 1376, 1328, 1259, 1219, 1070 cm⁻¹. MS (ES⁺) calc for C16H18N6O4S found 428.80; H, 4.70; N, 20.65.

Figure 10. The docked models of compounds 11 (left) and 36 (right). The compounds are colored by atom type and the dengue β-OG binding pocket is displayed as a Connolly surface model.

(E)-Methyl 2-(3-(4-(1-(2-Carboxamidomethyl)hydrazono)-ethyl)-phenyl)-ureido)-4-methylthiazole-5-carboxylate (6). Aminoguanidine hydrochloride salt (111 mg, 1 mmol) and methyl 2-(3-(4-acetylamino)-ureido)-4-methylthiazole-5-carboxylate (3) (333 mg, 1 mmol) were added to ethanol (10 mL), followed by acetic acid (0.5 mL). The reaction solution was heated to reflux for 3 h and allowed to cool to room temperature. After removal of solvent under reduced pressure, the resulting residue was washed with cold ethyl acetate (1 mL × 3) and then recrystallized from ethanol to provide the desired compound as a white solid (291 mg, 75%): mp 212–214 °C. 1H NMR (500 MHz, DMSO-d6): δ 11.0 (s, 2 H), 10.4 (s, 1 H), 7.90 (d, J = 8.5 Hz, 2 H), 7.50 (d, J = 8.5 Hz, 2 H), 3.73 (s, 3 H), 2.49 (s, 3 H), 2.26 (s, 3 H). IR (KBr) 2382, 1319 cm⁻¹. MS (ES⁺) calc for C16H19N7O3S 390.1348, found 390.1340. Anal. (C16H19N7O3S) C, H, N.

2-(3-(4-Acetylphenyl)-ureido)-N-(2,4-dichlorobenzyl)-4-methylthiazole-5-carboxamide (7). 2,4-Dichlorobenzylamine (90 mg, 0.5 mmol) and EDCI (100 mg, 0.52 mmol) were added to a solution of 2-(3-(4-acetylphenyl)-ureido)-4-methylthiazole-5-carboxylic acid (4) (150 mg, 0.46 mmol) in pyridine (5 mL). The reaction mixture was stirred at room temperature overnight. After removal of solvent under reduced pressure, the resulting residue was purified by chromatography (ethyl acetate—hexanes = 2:3) to provide the desired compound as a white solid (153 mg, 70%): mp 262–264 °C. 1H NMR (300 MHz, DMSO-d6): δ 9.53 (s, 1 H), 8.57 (dd, J = 5.7, 5.7 Hz, 1 H), 7.93 (d, J = 8.7 Hz, 2 H), 7.63 (d, J = 8.7 Hz, 2 H), 7.60 (d, J = 8.4 Hz, 1 H), 7.55 (d, J = 1.8 Hz, 1 H), 7.30 (dd, J = 8.4, 1.8 Hz, 1 H), 4.36 (d, J = 6.0 Hz, 2 H), 2.52 (s, 3 H), 2.50 (s, 3 H). 13C NMR (75 MHz, DMSO-d6): δ 197.3, 162.7, 141.9, 133.1, 132.4, 131.7, 131.4, 130.6 × 2, 133.0, 130.2, 128.6, 118.6 × 2, 42.6, 27.3, 17.4. IR (KBr) 3252, 1534, 1177 cm⁻¹. MS (ES⁺) calc for C12H10Cl2N2O4S 477.05, found 477.00. Anal. (C12H10Cl2N2O4S·1/2H2O) C, H, N.

Ethyl 2-(3,5-Diphenyl-1H-pyrazol-1-yl)-4-methylthiazole-5-carboxylate (9). 3,5-Diphenyl-1H-pyrazole (17) (80 mg, 0.4 mmol), 2-bromo-4-methylthiazole-5-carboxylic acid ethyl ester (40) (125 mg, 0.5 mmol), Cu2O (10 mg, 0.07 mmol), and 2-hydroxybenzaldehyde oxime (10 mg, 0.07 mmol) were added to a solution of cesium carbonate (325 mg, 1 mmol) in predegassed anhydrous CH2CN (5 mL) in a pressure reaction vessel. Argon gas was induced to reflux for 3 h. After removal of solvent under reduced pressure, the resulting residue was dissolved in methanol and purified by chromatography (ethyl acetate—hexanes = 2:3) to provide the desired compound as a white solid (100 mg, 65%): mp 114–116 °C. 1H NMR (300 MHz, CDCl3): δ 7.92 (d, J = 6.9 Hz, 2 H), 7.55 (m, 2 H), 7.44 (m, 4 H), 6.79 (s, 1 H), 4.31 (ddd, J = 6.9, 6.9, 6.9 Hz, 2 H), 2.51 (s, 3 H), 1.34 (dd, J = 7.2, 7.2 Hz, 3 H). 13C NMR (75 MHz, CDCl3): δ 162.1, 162.0, 158.2, 153.8, 145.9, 131.4, 129.6 × 2, 129.5, 129.0, 128.9, 128.6 × 2, 127.7 × 2, 126.0 × 2 × 2, 119.2, 108.4, 61.0, 17.4, 14.2. IR (KBr) 1712, 1499, 1256, 1095, 759 cm⁻¹. MS (ES⁺) calc for C22H18Cl2N4O3S, 477.05. Anal. (C22H18Cl2N4O3S) C, H, N.

2-(3,5-Diphenyl-1H-pyrazol-1-yl)-4-methylthiazole-5-carboxylic Acid (10). Lithium hydroxide (120 mg, 5 mmol) was added to a solution of ethyl 2-(3,5-diphenyl-1H-pyrazol-1-yl)-4-methylthiazole-5-carboxylate (9) (70 mg, 0.18 mmol) in methanol (5 mL), ethanol (10 mL), and water (5 mL). The reaction mixture was heated to reflux for 3 h. After removal of solvent under reduced pressure, the residue was dissolved in water (10 mL) and the resulting solution was filtered. The solution was acidified to pH 2–3 with hydrochloric acid and the resulting solid was filtered. The crude product was recrystallized from methanol (10 mL) to provide the desired compound as a white solid (51 mg, 80%): mp 232–234


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° C. 1H NMR (500 MHz, DMSO-d6): 7.93 (d, J = 7.5 Hz, 2 H), 7.57 (br, s, 2 H), 7.48 (m, 6 H), 7.23 (s, 1 H), 2.35 (s, 3 H). 13C NMR (75 MHz, CDCl3): 163.7, 161.9, 154.7, 154.6, 146.6, 131.8, 130.4 × 2, 130.2, 130.1, 129.8 × 2, 128.7 × 2, 126.8 × 2, 126.0, 121.3, 110.0, 17.4. IR (KBr) 2925, 1675, 1493, 1316, 759 cm−1. MS (ES+) calc'd for C24H25N2O4S: 360.09, found 359.87. Anal. (C24H25N2O4S) C, H, N.

1-(4-(Dichlorobenzyl)-2-(3,5-diphenyl-1H-pyrazol-1-yl)-4-methylthiazole-5-carboxamide (11). 3,4-Dichlorobenzylamine (25 mg, 0.13 mmol) was added to a solution of 2-(3,5-diphenyl-1H-pyrazol-1-yl)-4-methylthiazole-5-carboxylic acid (10) (45 mg, 0.12 mmol) in anhydrous pyridine (3 mL). EDCI (20 mg, 0.13 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature overnight. After removal of solvent under reduced pressure, the residue was purified by silica gel chromatography (ethyl acetate–hexanes = 1:4) to provide the desired compound as a white solid (47 mg, 70%): mp 144 – 176 °C. Anal. (C24H25N2O4S) C, H, N.

Methyl 1-(2-(4-acylbenzyl)amino)-4-methylthiazole-5-carboxylate (18). Sodium hydride (95%, 30 mg, 1 mmol) was added to a solution of methyl 2-amino-4-methylthiazole-5-carboxylate (21) (211 mg, 1 mmol) in hexanes (10 mL). The reaction mixture was stirred at room temperature overnight. After removal of solvent under reduced pressure, the residue was recrystallized from hexane to provide the desired compound as a white solid (260 mg, 74%): mp 162 – 184 °C. 1H NMR (500 MHz, CDCl3): 7.93 (d, J = 7.5 Hz, 2 H), 7.57 (br, s, 2 H), 7.48 (m, 6 H), 7.23 (s, 1 H), 2.35 (s, 3 H). 13C NMR (125 MHz, CDCl3): 163.7, 161.9, 154.7, 154.6, 146.6, 131.8, 130.4 × 2, 130.2, 130.1, 129.8 × 2, 128.7 × 2, 126.8 × 2, 126.1, 110.0, 17.4. IR (KBr) 2925, 1675, 1493, 1316, 759 cm−1. MS (ES+) calc'd for C24H25N2O4S: 360.09, found 359.87. Anal. (C24H25N2O4S) C, H, N.
IR (KBr) 1717, 1519, 1435, 1398, 1328, 1277, 1090, 830, 758 cm⁻¹. MS (ES⁺) calcd for C₁₂H₁₆ClNO₄S 268.01, found 267.92. Anal. (C₁₂H₁₆ClNO₄S) C, H, N.

2-(4-Chloro-phenyl)-4-methyl-thiazole-5-carboxylic Acid Adaman-tan-1-ylmethyl Ester (35). 2-(4-Chloro-phenyl)-4-methyl-thiazole-5-carboxylic acid methyl ester (32, 80 mg, 0.3 mmol) and LiOH (45 mg, 1.8 mmol) were added to ethanol—methanol—H₂O (3 mL, 1:1:1). The reaction mixture was heated at reflux for 1 h and allowed to cool to room temperature. The reaction mixture was filtered. After removal of solvent under reduced pressure, water (5 mL) was added. After adjusting pH to 3–4 with HCl, the solid was filtered and washed with water and a small amount of ethanol. The solid was dried to provide 2-(4-chloro-phenyl)-4-methyl-thiazole-5-carboxylic acid as a white solid (47, 60 mg, 80%) that was used directly in the next step without further purification. 2-(4-Chlorophenyl)-4-dibromomethyl-thiazole-5-carboxylic acid (47, 60 mg, 0.25 mmol) and EDCI (50 mg, 0.25 mmol) were added to pyridine (5 mL). The reaction mixture was stirred at room temperature for 2 h. After removal of solvent under reduced pressure, the residue was purified by silica gel chromatography (ethyl acetate–hexanes 1:9) to provide the desired compound as a colorless oil (20 g, 100%). 1H NMR (300 MHz, CDCl₃): δ 7.93 (d, J = 8.7 Hz, 2 H), 7.43 (d, J = 8.7 Hz, 2 H), 3.89 (s, 2 H), 2.79 (s, 3 H), 2.02 (s, 3 H), 1.70 (m, 12 H).

13C NMR (75 MHz, CDCl₃): δ 170.0, 160.8, 158.8, 137.8, 130.5, 129.3, 127.9 × 2, 122.2, 74.7, 39.2, 36.8, 32.2, 29.6, 27.0, 17.5, 0.9. IR (KBr) 2930, 2848, 1714, 1437, 1287, 1091, 767 cm⁻¹. MS (ES⁺) calcd for C₁₂H₁₆ClNO₄S 402.12, found 402.02. Anal. (C₁₂H₁₆ClNO₄S) C, H, N.

2-(4-Chloro-phenyl)-4-dibromomethyl-thiazole-5-carboxylic Acid Methyl Ester (36). 2-(4-Chlorophenyl)-4-methyl-thiazole-5-carboxylic acid methyl ester (32, 133 mg, 0.5 mmol) and NBS (531 mg, 3 mmol) were added to CCl₄ (10 mL). The reaction mixture was heated at reflux for 12 h. After removal of solvent under reduced pressure, the residue was purified by silica gel chromatography (ethyl acetate–hexanes = 1:9) to provide the desired compound as a white solid (127 mg, 60%): mp 154–156 °C.

1H NMR (300 MHz, CDCl₃): δ 7.93 (d, J = 8.7 Hz, 2 H), 7.43 (d, J = 8.7 Hz, 2 H), 3.89 (s, 2 H), 2.79 (s, 3 H), 2.02 (s, 3 H), 1.70 (m, 12 H).

13C NMR (75 MHz, CDCl₃): δ 168.4, 162.2, 160.8, 136.9, 131.3, 129.2 × 2, 127.9 × 2, 122.2, 74.7, 39.2, 36.8, 32.2, 29.6, 27.0, 17.5, 0.9. IR (KBr) 2930, 2848, 1714, 1437, 1287, 1092 cm⁻¹. MS (ES⁺) calcd for C₁₂H₁₆ClNO₄S 402.12, found 402.02. Anal. (C₁₂H₁₆ClNO₄S) C, H, N.

2-Chloro-3-oxo-butyric Acid Methyl Ester (38). Sufuryl chloride (18 g, 110 mmol) and a catalytic amount of AIBN were added to a solution of methyl 3-oxobutanoate (12 g, 100 mmol) in anhydrous benzene (50 mL). The reaction mixture was stirred at room temperature for 2 h. After removal of solvent under reduced pressure, the residue was purified by silica gel chromatography (ethyl acetate–hexanes = 1:9) to provide the desired compound as a colorless oil (20 g, 100%). 1H NMR (300 MHz, CDCl₃): δ 4.73 (s, 1 H), 3.37 (s, 3 H), 2.38 (s, 3 H). Molecular Modeling. Compounds 1–36 were built within Sybyl 7.1 and minimized to 0.01 kcal/mol by the Powell method, using Gasteiger–Huckel charges and the Tripos force field. To save calculation time, solvent was not explicitly included and instead the dielectric constant was set to a value of 4. The minimized molecules underwent 10 rounds of simulated annealing to further relax the conformations of these manually drawn molecules. Conformations resulting from each simulation were selected and the energies were minimized using the same parameter set as the one used during molecule construction. The conformer with the lowest energy was used as the initial structure for each molecule to be docked into the β-OG binding pocket of the dengue virus E protein.

Docking Simulation. The energy-optimized compounds were docked into the β-OG binding domain in E protein of the dengue virus that was obtained by removal of the n-octyl-β-D-glucoside (BOG). The parameters were set as the default value that the GOLD software suggested.

Bioassay Methods. BHK cells. BHK-15 cells (baby hamster kidney cells) obtained from the American Type Culture Collection (ATCC, Rockville, MD) were maintained in minimal essential medium (MEM) (Invitrogen, Carlsbad, CA) containing 7.5% fetal bovine serum (FBS). Cells were grown in incubators at 37 °C in the presence of 5% CO₂.

YFV-IRES-Luc. A fire-fly luciferase reporter gene was inserted into pYF23, a derivative of pACNR, which 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-FF-Luc (EMCV-IRES-fire fly luciferase) cassette was amplified by PCR from YFRP-IRES-Luc, a YFV replication, and inserted into the NsiI restriction site.

Generation of YFV-IRES-Luc Virus. In vitro transcribed YFV-IRES-Luc RNA was transfected into BHK-15 cells via electroporation. Subconfluent monolayers of cells were grown in T-75 culture flasks and were harvested by trypsinization, then subsequently washed twice with phosphate-buffered saline (PBS) before being resuspended in 400 µL PBS. These cells were then combined with 40 µL of in vitro transcribed RNA, and then placed in a 2-mm gap cuvette (BioRad, Hercules, CA). The cells were subjected to electroperoration by being pulsed two times at settings of 1.5 kV, 25 µF, and 200 Ω using a GenePulser II apparatus (BioRad). After the two pulses, the cells were given a 5 min recovery time at room temperature. Following recovery, cells were resuspended inMEM supplemented with 10% FBS. 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 YFV-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 media containing serial dilutions of compounds at concentrations below the CC50 values. Controls included uninfected cells, infected cells, and DMSO-treated infected cells. Cells were incubated at 37 °C, 5% CO2 for ∼36 h, lysed using 50 μL of cell culture lysis buffer (Promega Inc., Madison, WI), and 20 μL of cell extracts were placed into a 96-well opaque plate. Luciferase activity was determined from the luminescence generated with fire-fly luciferase substrate (Promega Inc., Madison, WI). Luminescence was measured in a 96-well plate luminometer, LMax II (Molecular Devices, Sunnyvale, CA). A reduction in luciferase activity indicates inhibition of YFV-RES-Luc virus growth. The luciferase luminescence as a function of compound concentration was analyzed by nonlinear regression analysis using GraphPad Prism to estimate the EC50 of each compound. The EC50 was defined as the concentration of the compound to cause 50% reduction of luciferase activity in infected cells as compared to the DMSO treated cells.

**Dengue Immunofluorescence Assay.** In brief, Den-2 (strain 16681) was added to monolayers of BHK cells in a 24-well plate at an moi of 0.025 for 1 h at room temperature. Following infection, the excess/unbound virus was removed by washing with PBS. Cells were then overlaid with MEM + 2.5% FBS containing the compound at 50 μM concentration. The cells were then incubated at 34 °C for 48 h before performing immunofluorescence assay. Cell monolayers were fixed and incubated with the secondary antibody conjugated with TRITC. Three randomly selected fields per well were captured in both the TRITC (tetramethylrhodamine-5 (and 6)-isothiocyanate) and DAPI (4',6-diamidino-2-phenylindole dihydrochloride) channels. All infected cells were compared to total cells in all three fields and percent inhibition was determined.

**Cell Viability Assay.** Cellular viability was measured spectrophotometrically by monitoring the reduction of a tetrazolium salt to a formazan dye by mitochondrial dehydrogenase in living cells. BHK cells were plated in a 96-well plate and grown at 37 °C. At confluence, cells were overlaid with culture media 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% CO2 for ∼36 h. At ∼36 h post-treatment, media on cells was replaced with fresh media to remove the compounds. Then 100 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 OD450 measured using a 96-well plate reader (Molecular Devices, Sunnyvale, CA). The OD450 values for cells treated with a compound were compared to those obtained for cells treated with the control (1% DMSO) to determine the CC50 of each compound. The 1% DMSO in the control was necessary to solubilize some of the compounds, and it did not affect cell viability.

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**Supporting Information Available:** Results from elemental analyses of intermediates and target compounds, and full experimental details, including spectral data, for the syntheses of compounds 12−16, 23−31, 33, and 34. This material is available free of charge via the Internet at http://pubs.acs.org.


